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Thermal differences in *Daphnia magna* populations across a latitudinal gradient

Quantifying population dynamics with Integral Projection Models

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Table of contents

Summary

Temperature is expected to increase over the coming years as a result of climate change, affecting both abiotic and biotic factors in freshwater ecosystems such as ponds and shallow lakes. *Daphnia magna* is a keystone species in this ecosystem and therefore, the impact of temperature on *D. magna* is important to study. We conducted a thermal tolerance experiment to investigate genetic differentiation of thermal responses in natural populations. Two Norwegian populations and two Belgian populations, with each three clones, were put under four possible thermal regimes: 14°C, 18°C, 22°C or 26°C. We collected life-history data, using survival, somatic growth, offspring number and size as vital rates. We modelled the data with Integral Projection Models.

The effect of temperature on the population specific vital rates varied: it was positive for growth and offspring size, negative for survival and fluctuated for offspring number. The effect of density was negative on all vital rates, except offspring size. The northern and southern populations differed significantly in density under colder thermal regimes, but this difference disappeared as temperature increased. All populations, except one Belgian population, had their highest intrinsic population growth rate at 26°C, so we could not provide evidence for thermal adaptation. Overall the Belgian populations did better than the Norwegian ones. The populations and their clones did employ different life-history strategies, indicating that there is genetic variation within population and consequently evolutionary potential. Finally, we found that the variation in intrinsic growth rate could be explained more by within-population variation than across-population variation.

Samenvatting

Er wordt verwacht dat temperatuur de komende jaren zal stijgen als gevolg van klimaatsverandering, dat zowel abiotische als biotische factoren in zoetwater-ecosystemen zoals vijvers en ondiepe meren aantasten. *Daphnia magna* is een keystone-soort in dit ecosysteem en daarom is de invloed van temperatuur op *D. magna* belangrijk om te bestuderen. We verrichtte een thermische tolerantie experiment om genetische differentiatie van thermische reacties in natuurlijke populaties te onderzoeken. Twee Noorse populaties en twee Belgische populaties, met elk drie klonen, werden onder vier mogelijke thermische regimes gebracht: 14 ° C, 18 ° C, 22 ° C of 26 ° C. We verzamelden gegevens over de levensgeschiedenis, waarbij overleving, somatische groei, nakomelingenaantal en -grootte de vitale cijfers waren. We modelleerde de data met Integrale Projectie Modellen.

Het effect van temperatuur op de populatie-specifieke vitale cijfers varieerde: het was positief voor de groei en de nakomelinggrootte, negatief voor overleving en fluctueerde voor het aantal nakomelingen. Het effect van dichtheid was negatief op alle vitale cijfers, behalve de nakomelingsgrootte. De noordelijke en zuidelijke populaties verschilden aanzienlijk in dichtheid onder koudere temperaturen, maar dit verschil verdween als de temperatuur steeg. Alle populaties, behalve één Belgische bevolking, hadden hun hoogste intrinsieke bevolkingsgroei bij 26°C, dus we konden geen bewijs leveren voor thermische adaptatie. Over het algemeen deden de Belgische populaties het beter dan de Noorse. De populaties en hun klonen gebruikten verschillende levensgeschiedenisstrategieën, wat aangeeft dat er genetische variatie is binnen de populatie en bijgevolg ook evolutionair potentieel. Ten slotte vonden we dat de variatie in intrinsieke groei meer kon worden verklaard door variatie binnen de populatie dan door variatie tussen de populaties.

1. Introduction

1.1. Climate change

Climate change is one of the big five threats to biodiversity along with pollution, overexploitation, habitat loss and invasive species. It entails changes in climatic conditions caused by humans. During the last decades there has been an exponential increase in the use of fossil fuels and deforestation (Scheffers et al., 2016). This has led to an increase in the atmospheric concentrations of greenhouse gases to unforeseen levels compared to the past 20 million years (Beerling & Royer, 2011; Scheffers et al., 2016) (Fig. 1a). High greenhouse gas concentrations have altered the chemical composition of the Earth's atmosphere, but also of oceans and fresh water systems (Ciais et al., 2013). These alterations have raised temperatures in the upper ocean and on land with ~1°C and have led to more variable and extreme temperatures, wind, and precipitation around the world (IPCC, 2014) (Fig. 1b). Increased ambient temperature results in more evaporation from standing water which changes the water vapor transport and hydrologic cycle, which on its turn impacts both marine and freshwater systems (Solomon et al., 2009). This is but one example of how climate change can disrupt ecosystems and biological processes on a global scale. However, already on short and long time scale there are effects of climate change enabled that are irreversible (Solomon et al., 2009). Implementing a full emissions stop might not be effective enough to reverse the increase in atmospheric CO_2 concentration inducing these effects (Solomon et al., 2009). This means that natural ecosystems and its populations will eventually have to deal with the effects of climate change.

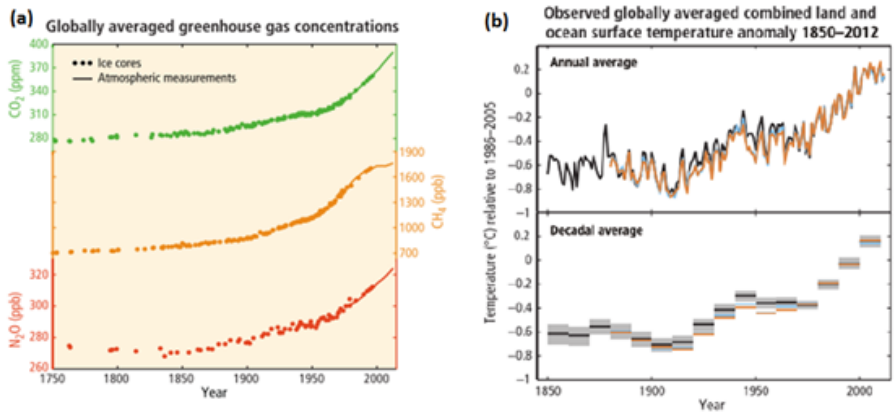

Figure 1: Graph showing a) the increase in greenhouse gas concentration (CO_2, CH_4 and N_2O) starting in 1750, intensifying around 1950 and b) annual and decadal average temperature increases for land and ocean combined over the past decades, starting in 1850. (Retrieved from IPCC, 2014).

Predicting the consequences of climate change is challenging, because it is not yet completely understood. Therefore, the Intergovernmental Panel on Climate Change (IPCC) mapped out different scenarios based on varying Representative Concentration Pathways (RCPs). These RCPs quantify the total radiative forcing, including human and natural causes, in 2100 relative to 1750 (IPCC, 2013). In total four scenarios are created: RCP 2.6, RCP 4.5, RCP 6.0 and RCP 8.5, with the number indicating the radiative forcing in Wm^{-2}. Different RCPs result in different predicted temperature increases (Fig. 2).

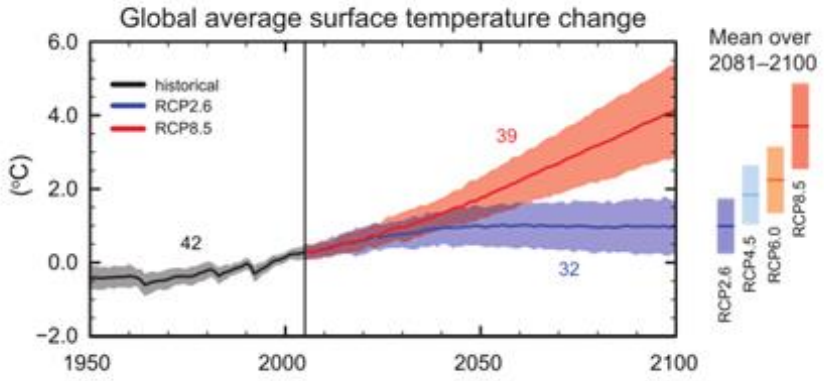

Figure 2: Projection of temperature increase under different Representative Concentration Pathways (RCPs). These vary from 2.6 to 8.5 and correlate to the magnitude of the increase in temperature. The indicated numbers are the number of models used in order to calculate the multi-model mean. (Retrieved from IPCC, 2013).

Providing solid evidence for climate change has proven to be challenging because it is difficult to quantify long term effects of climate change. Several methods can be used to counter the issues of long scale experiments (Anderson et al., 2012). A first method is reconstruction of the historical situation via paleo-ecology or paleo-genetics which allows comparison between the pre- and post-change state. An example of this is resurrection ecology using resting eggs of e.g. *Daphnia* to reconstruct a time series of genotypes, phenotypes and even the evolution of phenotypically plastic characters (e.g. see Geerts et al., 2015; Hairston & De Meester, 2008). Another viable option is the creation of long term datasets that span multiple years and even decades. This ensures that even the impact of long term biological processes is mapped. Next to a large temporal scale, large spatial scale experiments are also being applied. The advantage of this approach is that specific environments can be mimicked in which controlled climatic changes can be tested. Sites that differ spatially can be used in a space-for-time substitution by reconstructing a temporal gradient based on a spatial gradient. Thus the future thermal projections are substituted with current spatial patterns (Blois et al., 2013). Space-for-time substitutions have been used to understand the responses to climate change (Merilä & Hendry, 2014; Stoks et al., 2014). Locations are matched with each other based on either latitudinal or

altitudinal gradient. For example, in a study by Janssens et al. (2014), populations from two different latitudes were used. By doing so, the researchers tried to mimic the projected temperature of the northern latitude with the temperature of the southern latitude. The two populations per latitude were put under different thermal regimes and experienced different levels of stress due to Zinc exposure. This design allows to test is the populations were locally adapted to temperature and to predict if under increased temperature, the population can deal with predator and pollution stress (Janssens et al., 2014). Space-for-time substitution can also be used as a tool to asses succession of vegetation (Pickett, 1989) or the development of recessional moraine slopes (Welsh, 1970).

1.1.1. Impact of climate change on ecosystems

Effects of climate change impact diverse ecosystems in varying ways and can occur on every biological level of organization ranging from gene to organisms, populations, species, communities and ecosystems. The same effect can act on different levels, simultaneous influencing biological processes positively via adaptation or negatively via stress induction (Scheffers et al., 2016). The individual impacts of climate change on each level can multiply among the levels and lead to ecological regime shifts, i.e. the discrete shift of an ecosystem from one state to another (Scheffers et al., 2016). Abrupt climate changes can be caused naturally or triggered by humans (Alley et al., 2003). The most common example of such a shift is the switch from a clear to a turbid system in ponds and shallow lakes (Scheffer et al., 1993) (Fig. 3). Such a shift changes the composition, structure and function of the entire ecosystem (IPCC, 2014).

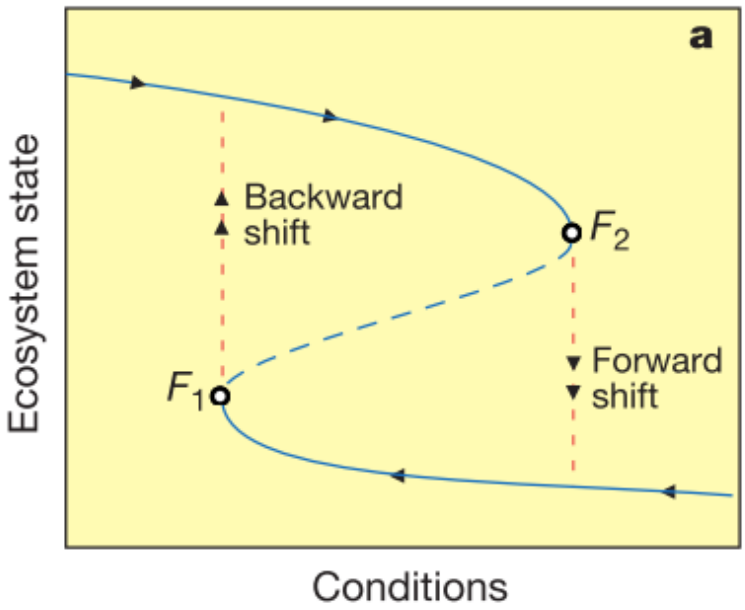

Figure 3: Alternative stable states for ecosystems. Perturbation cause shifts in these equilibria which are difficult to reverse. (Retrieved from Scheffer et al., 2001)

Terrestrial and aquatic ecosystems are exposed to multiple stressors that could be driven by climate change, such as increased temperature, hydrological changes, changes in precipitation regime and sea level rise (IPCC, 2013). For example, warmer temperatures cause melting of permafrost resulting in the release of the stored CO_2 and CH_4 (Lawrence & Slater, 2005). Permafrost melt finds its way to the ocean, adding to the freshwater discharge. Increased temperatures also cause melting of glaciers alternating the phenology of freshwater influx into rivers (Barnett et al., 2005). Furthermore, warming causes melting ice sheets at both poles (Pritchard et al., 2009), limiting the sea ice extent at the North Pole in both summer and winter (Durner et al., 2009). Less sea ice means habitat fragmentation for the polar bear, *Ursus maritimus*, and other polar animals (Moline et al., 2008). Peatlands, affected by the changing water table levels, release CH_4 into the atmosphere (Gorham, 1991), generating a positive feedback loop. Excess influx of freshwater into the ocean and the thermal expansion of the ocean itself both contribute to an elevated sea level. Sea level rise increases coastal erosion and the risk of inundation (Mimura, 2013). Other terrestrial ecosystems like habitats adapted to fires are likely to experience more fires due to longer and more intense periods of drought (Salguero-Gomez et al., 2012). Species inhabiting these areas are highly specialized which could result in an inability to adapt when facing changing environmental conditions.

Shifts in environmental conditions due to climate change forces animals and plants to respond to these shifts. The impact of climate change include changes in genetics, physiology, morphology, phenology, population dynamics, species distribution, interspecific relations and productivity (Scheffers et al., 2016). It is expected that climate change will elevate the extinction risk for certain species and that it will drive species to extinction in the future (Thomas et al., 2004). At the moment, only some extinctions can be attributed with certainty to climate change, for example anurans (Pounds et al., 1999) or more recently the Bramble Cay melomys *Melomys rubicola* (Gynther et al., 2016). Of all bird species on Earth, it was estimated that 25% have been driven to extinction due to human impact and currently many more animal and plant species are threatened with extinction (Vitousek et al., 1997).

1.1.2. Population responses to climate change

Changes in environmental conditions simultaneously affect population dynamics, life history traits, gene frequencies and morphology (Coulson et al., 2011). The way species can respond to ongoing, anthropogenic climatic change depends on the magnitude of the change, the state of the ecosystem and characteristics of the confronted individual or species, such as thermal sensitivity, dispersal capacity and generation time (Boeye et al., 2013; Scheffers et al., 2016). Already in 1990, Holt stated that ‘There is almost no species for which we know enough relevant ecology, physiology, and genetics to predict its evolutionary response to climate change’ (Holt, 1990, p 311). Populations experiencing a change in selection pressure face three

options to avoid extinction: they either need to migrate, respond plastically, or adapt (Gienapp et al., 2008; Holt, 1990).

Migration

The first strategy that species can employ to deal with climate change is shifting their geographical distribution. Many species have been found to shift their range in either latitude northwards or altitude upwards (meta-analysis by Chen et al. (2011)). Shifts in geographical ranges result in species redistribution and consequently new community compositions and new species interactions within and among communities (Scheffers et al., 2016). Redistribution can also lead to hybridization zones which results into a loss in biodiversity as the genome of the hybrid is mixed and its fitness declines due to outbreeding (Hoffmann & Sgro, 2011). However, hybridization can also facilitate evolutionary adaptation because it allows ranges to be expanded and it introduces new genetic variation to the population (Anderson et al., 2012; Hoffmann & Sgro, 2011). Unfortunately, migration does not guarantee survival of the species, it only delays the consequences of long-term directional change (Nunney, 2016). Additionally, not all species are capable of shifting their geographical ranges (IPCC, 2014). These species cope with changes in ways other than migration, such as phenotypic plasticity.

Phenotypic plasticity

Phenotypic plasticity is the potential of a single genotype to produce a range of different phenotypes dependent on the environmental condition and is thus a strategy employed to better match the phenotype with the local environment, resulting in higher fitness (DeWitt et al., 1998; Scheiner & Berrigan, 1998). Phenotypic plasticity can involve alternative forms of morphological and physiological state, and/or behavior due to different environmental conditions (West-Eberhard, 1989), and the range of its expression may vary among traits and populations (Scheiner, 1993). Varying expression of phenotypic plasticity might reflect differences in the relative costs and benefits of plasticity among traits (DeWitt et al., 1998; Van Buskirk & Steiner, 2009). Costs of phenotypic plasticity may involve maintenance costs, production costs, information acquisition costs, developmental instability, and genetic costs (DeWitt et al., 1998). It has been shown that phenotypic plasticity might provide a short-term solution for a population to respond to climate change but it is unable to produce extreme phenotypes, which is possible via micro-evolutionary responses (Gienapp et al., 2008). For instance, long term research on the great tit, *Parus major*, revealed similar plastic behavior in individuals mirroring variation in the environment (Charmantier et al., 2008). An example of plastic response is the change in body size in red deer, *Cervus elaphus*, under thermal variation. Under higher temperatures, the males grew faster and larger, while the female's body size became smaller, furthering the sexual dimorphism (Post et al., 1999).

While phenotypic plasticity is an important mechanism to cope with changing environments, it is also limited its responses to changing conditions (DeWitt et al., 1998) and therefore cannot

provide long term solutions under persisting directional environmental change (Gienapp et al., 2008).

Genetic adaptation

The third possible strategy to deal with climate change is genetic adaptation, which might provide long term solutions via micro-evolutionary adaptations (Gienapp et al., 2008; Jansen et al., 2017). Species can adapt to the new environmental conditions if sufficient genetic variation is available. High levels of genetic variation is assumed to be associated with large effective population sizes (Frankham, 1996). The reaction of organisms to climate change can be categorized in two general responses: (i) organisms evolve to exploit potentially favorable conditions to their advantage and (ii) organisms evolve to tolerate the new conditions (Franks & Hoffmann, 2012). An example for (i) are small mammalian species, such as rodents and shrews, inhabiting low elevations that expand their range to higher altitudes in Yosemite National park due to increased temperature (Moritz et al., 2008). An example for (ii) is that an increase in temperature for species with temperature dependent sex ratios is harmful because it disturbs the sex ratios in the population (Neuwald & Valenzuela, 2011).

Failure in an evolutionary response of a species to climate change could be due to a low level of heritable variation, or because the environmental changes occurred too fast or because the selection pressure was too strong (Hoffmann & Sgro, 2011). Additionally, trade-offs between traits and other forms of genetic interaction may also slow down the evolutionary responses to shifting selection pressures caused by climate change (Hoffmann & Sgro, 2011).

A specific type of genetic adaptation is thermal adaptation (Carvalho, 1987). Species are adapting to temperature extremes and these adaptations include changes in species physiology to tolerate higher temperatures (Yampolsky et al., 2014a). Increased temperature imposes thermal stress and this leads to directional selection for heat resistance (Hoffmann & Sgro, 2011). In addition to physiological changes, changes in morphology are also possible. For example, species under warmer thermal regimes have found to shrink in body size to optimize their surface/volume ratio (Scheffers et al., 2016). Not only species traits are impacted by increasing temperatures, but also population dynamics are indirectly influenced by the warming climate (Scheffers et al., 2016). For example, the shrinking amount of available habitat for polar bears could lead to shifted ranges, forcing more individuals into a smaller area. This results in decreased individual reproductive fitness due to density-dependent effects. Furthermore, reduced habitat causes food shortage which is detrimental to survive the winter sleep. This then could compromise reproductive success because the uptake of energy is used for survival and not reproduction (Durner et al., 2009).

1.2. Study system: ponds and shallow lakes

Freshwater ecosystems contain only 0.01% of the Earth's total water and cover merely 0.8% of Earth's total surface (Dudgeon et al., 2006). Surprisingly, this tiny fraction holds almost 6% of all described species, marking an extraordinary level of endemism. Freshwater bodies can either contain flowing water or standing water. Pools, ponds and lakes all contain standing water, but can differ in the scale of their magnitude and in the degree of hydroperiod ranging from temporary to permanent (De Meester et al., 2005; Wellborn et al., 1996). As the size and hydroperiod vary, so do the impacting factors on these freshwater systems and this creates a diverse range of species that are able to colonize and survive in these various freshwater bodies. Pools, ponds and lakes are well defined ecosystems that are characterized by a high degree of endemicity and high β diversity among the ponds. Furthermore, freshwater systems provide multiple ecosystem services, such as water supply, food production and waste treatment. Additionally they are also recreationally and esthetically important (Costanza et al., 1998).

Ponds are ideal model systems to test a diverse array of hypotheses concerning ecological, evolutionary and conservational research questions (De Meester et al., 2005). We next provide a short list on why ponds are good model systems.

- Ponds occur on a broad latitudinal, longitudinal and altitudinal gradient which might be characterized by different environmental properties causing variation in hydroregime, nutrient level,… (De Meester et al., 2005). This ensures that field experiments can be done on a variety of environmental conditions, but also that different sampling sites can be compared to each other.
- The aquatic community is well-defined, which makes it easier to take representative samples and to replicate quasi natural conditions in experimental settings, e.g. in mesocosms (De Meester et al., 2005).
- Because their boundaries are well-defined, freshwater bodies are very useful in the context of metacommunity studies, in which they can be seen as 'aquatic islands' surrounded by terrestrial landscapes (De Meester et al., 2005; Miner et al., 2012).
- Last, ponds can be considered as proxy for larger freshwater bodies due to their size (De Meester et al., 2005). Long term effects can be researched in these small ponds and pools so these effects can be better understood before they act upon large water systems.

1.2.1. Impact of climate change on freshwater ecosystems

Changes in temperature and precipitation caused by global change will impact the characteristics and occurrence of freshwater bodies (Bauder, 2005; Oertli et al., 2009). The temperature in freshwater systems is rising and this influences the seasonal patterns of mixing and flows (Scheffers et al., 2016) and the metabolic rate of the ectothermic inhabitants (Van Doorslaer et al., 2010). Warmer water limits mixing and nutrient recycling due to thermal stratification and thus decreasing primary productivity and phytoplankton growth. This

decrease then influences the rest of the food web, resulting in a decrease in fish abundance. Warming increases the oxygen demand of individuals because their metabolic rate is higher, but also simultaneously depletes the oxygen content of the water because it solubility decreases (Yampolsky et al., 2014b). This enlarged gap in oxygen demand and supply results in metabolic costs for the inhabitants. Higher temperatures amplify the effects of eutrophication resulting in increased productivity and phytoplankton blooms, including blooms of toxic cyanobacteria, which induce bottom up-effects on the food web (Scheffers et al., 2016). The impact of global change on freshwater bodies causes many freshwater species to shift in their spatial characteristics (ranges and migration patterns), their phenology (seasonal activity) and their population dynamics (abundances and species interactions) (IPCC, 2014).

1.3. Study species: *Daphnia magna*

Daphnia magna (Straus, 1820) is a species of Cladocera that inhabits freshwater bodies, such as lakes and ponds. Their common name is water flea, named aptly after the way they move through the water. They are small, translucent animals that use filter feeding to retrieve nutrients from the surrounding water. *Daphnia* have a high phosphor concentration in their body, which allows fast somatic growth but also makes them a preferred prey species for planktivorous fish and macro-invertebrates. This P-demand differs genetically among populations and is found to be highest for populations with short growing seasons (Miner et al., 2012). *Daphnia* are short lived organisms, reaching maturation within a week from birth (Alekseev & Lampert, 2001). The body of *D. magna* consists of three main zones: head, thorax and tail. Attached to the head, there are two pairs of antennae and the feeding apparatus. On the head, we find the typical compound eye. The thorax contains five pairs of limbs and is connected to the telson, which makes up the tail part of *D. magna* (Mittmann et al., 2014) (Fig. 4).

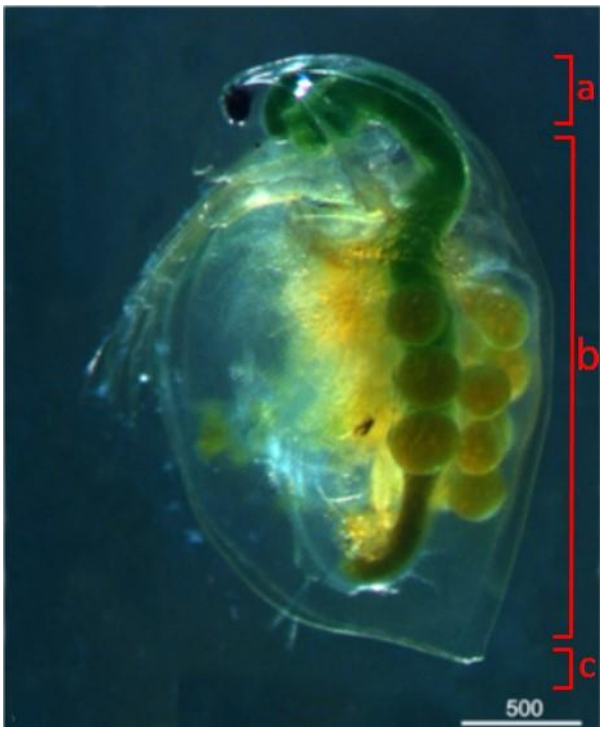

Figure 4: Picture of *Daphnia magna*. The head (a), thorax (b) and tail (c) are distinctive and eggs are visible in brood pouch. The scale is in µm. (Modified from Mittmann et al., 2014)

Due to their central position in freshwater food webs and their large effects on trophic levels above and below them, *Daphnia* is considered to be a key-stone species in their ecosystem (Jones et al., 1994) (Fig. 5). They control the trophic level below them, the autotrophs, by grazing mainly on phytoplankton which leads to seasonality in phytoplankton availability (Lampert, 2006) and consequently induce a clear state of water transparency when the phytoplankton is consumed the most (Miner et al., 2012). At the same time, however, *Daphnia* is a preferred prey species for planktivorous fish and invertebrate predators, both secondary consumers (Miner et al., 2012). Their role as keystone species is even further cemented by also consuming bacteria (Lampert, 2006) and protozoans (Miner et al., 2012). Next to trophic effects, micro-evolutionary changes in *Daphnia* species can also lead to changes in communities and, via cascading responses, in the ecosystem itself (De Meester et al., 2011; Miner et al., 2012).

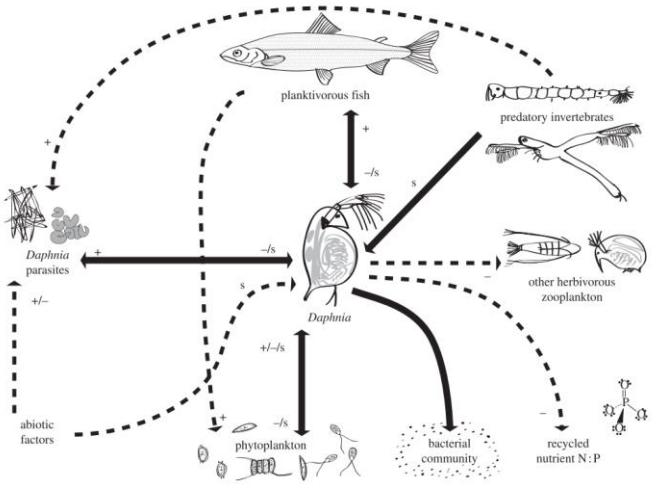

Figure 5: *D. magna* is considered a keystone species due to its central position in the food web. It consumes autotrophs and micro-organisms (bacteria and protozoans) and is consumed by secondary consumers (planktivorous fish and invertebrate predators) (Adapted from Miner et al., 2012).

1.3.1. Model organism

Daphnia magna is widely recognized for its capacity as model organism. The earliest publication dates from 1934: "*Über die Anwedung von Daphnia magna Straus als Versuchstier zur experimentellen Klarlegung der Lebensverhältnisse im Wasser*" (Naumann, 1934). More recently, organisms from the genus *Daphnia* are used in research concerning genetics, development, ecology, evolution, ecotoxicology and so on.

There are multiple reasons why *Daphnia* is such a suitable organism to use in scientific research and we next list the main reasons.

- They are small in size but large enough to identify separately (Lampert, 2006), so they can be kept easily without the need for large available space.
- They reproduce fast and asexually, which allows to set up large populations of several genetically identical generations.
- *Daphnia* species have a continuous global distribution ranging from South Africa to near artic sites, from which they are accessible and can be used for comparisons across latitudes (Lampert, 2006; Yampolsky et al., 2014a).
- Due to the production of resting eggs which are buried in the layered sediments, evolutionary timescales can be reconstructed (Hairston & De Meester, 2008; Jansen et al., 2011).
- They are capable of rapid evolutionary adaptation (Miner et al., 2012) and exhibit phenotypic plasticity in response to environmental cues. Responses to fish kairomones include the induction of morphological adaptions like helmets or neck teeth or a behavioral response involving their phototaxis (Hairston & De Meester, 2008).

- *D. magna* possesses the ability for epigenetic inheritance, e.g. its DNA can be methylated at certain locations (Vandegehuchte, 2009).
- Researchers are working to sequence the genome of *D. magna*. By using the fully sequenced genome of *Daphnia pulex* as reference for BLAST, it is already known that the *D. magna* genome includes sequences that are species specific (Vandegehuchte et al., 2009).

1.3.2. Reproductive cycle

Daphnia species are cyclical parthenogenetic organisms, meaning that they can reproduce both sexually and asexually (Fig. 6). During the asexual reproduction phase, female individuals produce asexual diploid eggs derived via mitosis without fertilization (Harris et al., 2012). The asexual eggs of the *D. magna* are encapsulated within a brooding pouch located dorsally on the egg carrying female. The embryonal development of these asexually produced offspring has recently been described in detail and 12 stages have been identified based on morphological indicators (Mittmann et al., 2014). After a few days, the embryos emerge from the brooding pouch as genetically identical versions of the mother individual during the shedding of the carapace (Mittmann et al., 2014).

Sexual reproduction occurs under the anticipation of stressful conditions, such as heat waves or the start of winter when food gets scarce and temperature decreases. Certain environmental cues, for example photoperiod, temperature, food abundance and crowding (Alekseev & Lampert, 2001; Harris et al., 2012; Walsh, 2013), provide the females with the information needed to trigger sexual reproduction (Caceres, 1998). A female mates with a male and via meiosis a haploid resting egg is produced (Harris et al., 2012; Miner et al., 2012). Resting eggs are encased per two within an ephippium (Alekseev & Lampert, 2001), which can be buried in the sediment to endure harsh conditions, comparable to seed banks (Caceres, 1998). The importance of dormancy varies across different environments according to their heterogeneous temporality (Walsh, 2013). The intensity of resting egg production differs between populations and even between clonal lines (Alekseev & Lampert, 2001). The production of resting eggs is a form of bed-hedging and is under maternal control (Alekseev & Lampert, 2001). Resting-egg production is seasonal and occurs towards the end of the growing season when environmental conditions become harsh (Alekseev & Lampert, 2001). When conditions are better again, often coinciding with the beginning of spring, the dormant eggs can hatch (Alekseev & Lampert, 2001). The dormant period can last several generations, thus greatly extending the generation time of the population. This mechanism also allows to hatch dormant eggs from different time periods, buried at different depths throughout the sediment. Increasing burying depth correlates with an increased age of buried resting eggs and thus hatching eggs from different sediment layers provides a natural historical time sequence (Miner et al., 2012). The hatched individuals can then be used to reconstruct the evolution of traits and the amount of plasticity present in the population (Hairston & De Meester, 2008). This is done by using both recent and ancestral

hatchlings in a common garden experiment (Cousyn et al., 2001; Stoks et al., 2016). With the recent advancement of genomic tools, dormant eggs even do not have to be viable, but their DNA can simply be extracted and used for analysis (Miner et al., 2012). This extensively broadens the scope of resurrection ecology.

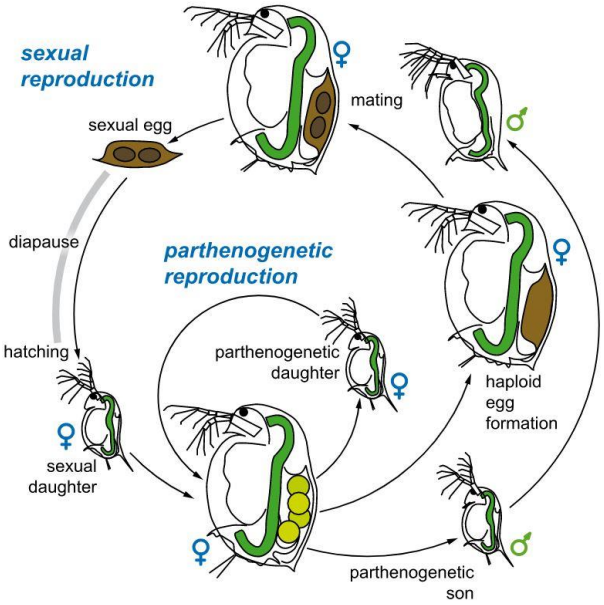

Figure 6: Schematic overview of a *Daphnia* reproductive cycle, which includes a sexual and an asexual phase. This is called cyclical parthenogenesis. (Retrieved from Vizoso, 2013)

The production of male daphnids is also environmentally controlled (Harris et al., 2012). The male hormonal pathway, indicating the start of the shift from asexual to sexual reproduction, is associated to the concentration of hemoglobin, which is triggered by increased temperatures (Yampolsky et al., 2014b).

1.4. Thermal adaptation in *D. magna*

Organisms of the genus *Daphnia* are poikilothermic and ectothermic, meaning that their body temperature, growth and metabolic rate are sensitive to the environmental temperature (Chopelet et al., 2008; Mckee & Ebert, 1996). Many studies have been investigating the effects of temperature on *D. magna* life history (e.g. Atkinson, 1994), intraspecific competitive strength (e.g. Van Doorslaer et al., 2009), plasticity (e.g. Jansen et al., 2017) and evolutionary response (e.g. Jansen et al., 2017). According to the Temperature-Size Rule, ectothermic individuals decrease in size at maturation with increasing temperature and this due to oxygen shortage at high temperature which constrains the metabolic and growth rate of the individual (Atkinson, 1994). Therefore, ectotherms in cold thermal regimes have a larger size at

maturation, but mature slower compared to ectotherms in warmer thermal regimes which are smaller in size at maturation (Atkinson, 1994). A negative correlation between body size and CT_{max}, a proxy for thermal tolerance, has been found, indicating that individuals under higher temperature become smaller in order to increase their thermal tolerance (Geerts et al., 2015). It has been shown that temperature can have a strong effect on the somatic and intrinsic growth rate (λ) of *D. magna* (Giebelhausen & Lampert, 2001; Van Doorslaer et al., 2010), but also on offspring and mother weight and the clutch size (Mckee & Ebert, 1996).

Due to its broad distribution, *Daphnia* occurs in diverse thermal regimes and populations adapted to these different regimes have found to differ in their optimal temperature for growth. While the optimal temperature for *D. magna* growth ranges from 23-26°C, it has been found that cold-adapted genotypes exhibit optimal growth rates around 15°C (Mitchell & Lampert, 2000). Under natural conditions these temperatures are only reached during parts of the growth season at which the population exhibits maximal growth rates. Moreover, *Daphnia* populations from different thermal regimes are found to have genes expressed in varying degrees, indicating local thermal adaptation (Jansen et al., 2017). Clones originating from warmer climatic conditions showed higher thermal tolerance than those from colder climates even though all individuals were acclimatized to the same temperature (Yampolsky et al., 2014a). Thermal tolerance is correlated to the average high temperature of the origin sites, again suggesting local adaptation (Yampolsky et al., 2014a). *D. magna* thus shows a remarkable capacity for adaptation to changes in temperature, specifically increasing temperature (Geerts et al., 2015). It was able to evolve a coping mechanism in order to deal with higher thermal extremes over a time span of merely 40 years (Scheffers et al., 2016). Intraspecific thermal adaptation under selection experiments is shown via rapid responses of their sensitivity to temperature (Mitchell & Lampert, 2000). *D. magna* have exhibited both phenotypic and genetic variation in response to temperature (Yampolsky et al., 2014a). Even among clones within population variation for growth rate under different thermal regimes has been shown (Mitchell & Lampert, 2000). Furthermore, *Daphnia* populations have the capacity to respond to varying thermal regimes during the duration of one growing season, dealing with daily and seasonal thermal variation (Van Doorslaer et al 2009; Yampolsky et al., 2014a).

So, *D. magna* can respond to temperature change via plastic or evolutionary responses in the form of phenotypic plasticity or genetic adaptation, respectively (Jansen et al., 2017).

1.5. Integral projection model

Integral projection models (IPMs) can be used to describe the dynamics of a population through time characterized by a continuous individual-level state variable that influences an individual's vital rates such as survival, growth, and reproduction (Easterling et al., 2000; Merow et al., 2014). The relationships between the state variable and the vital rates are obtained via

regression models and provide the building blocks of the IPM (Merow, Dahlgren, et al., 2014). The size distribution of the population is described by *n(y,t)* which denotes the number of individuals of a certain size *y* at time *t*. The core function to build an integral projection model is given by the kernel, which maps the size distribution at time *t* to a size distribution at time *t+1* (Easterling et al., 2000; Merow, Dahlgren, et al., 2014).

$$n(y, t+1) = \int_L^U [P(x,y) + F(x,y)]\, n(x,t)dx.$$

In this integral L and U is the lower and upper limit, respectively, for the range of a continuous variable, e.g. body size. *P(x,y)+F(x,y)* represent the kernel, where *P* describes the survival-growth kernel and *F* describes the fecundity kernel. The survival-growth kernel contains both growth and mortality functions, describes the survival probability of an individual to the next time step and if it survives, the probability of what size it will become. Because not all the individuals survive to the next time step, the integral of this function is typically less than one (Easterling et al., 2000). The fecundity kernel comprises the number of offspring produced by reproductive individuals and the size distribution of these offspring. In practice, describing the kernel of an IPM is often done by constructing regression models linking the vital rates to body size of individuals, reflecting four life history parameters: survival, reproduction, growth and inheritance (Easterling et al., 2000; Jongejans et al., 2017; Merow et al., 2014) (Fig. 7).

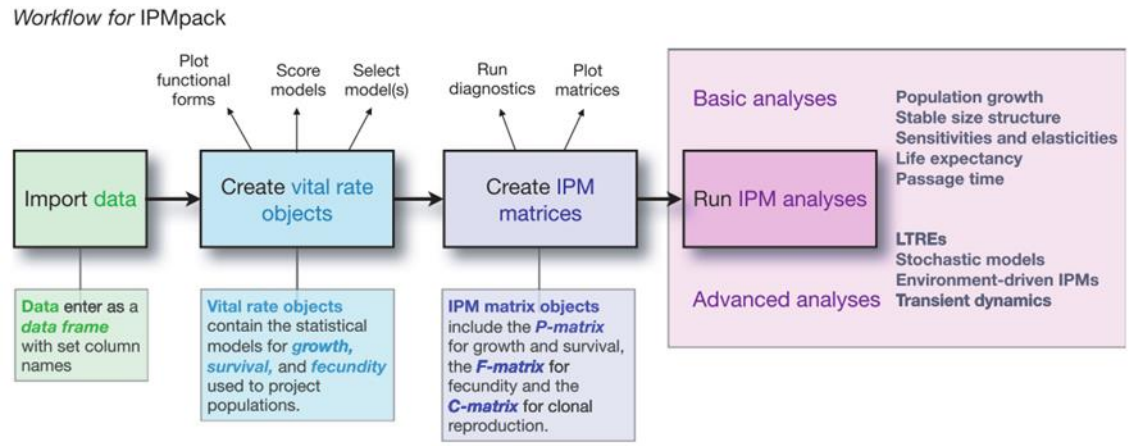

Figure 7: Workflow of IPM building. After the data is imported, vital rate objects are created with regressions of growth, survival and fecundity. Based on these regressions, the P and F kernels can be made and combined into the full IPM matrix. On the IPM matrix, the usual matrix analyses can be performed. Retrieved from Metcalf et al. (2013).

The advantage of IPMs is that they naturally accommodate both discrete and continuous state variables (e.g. Childs et al., 2003; Coulson et al., 2011; Jacquemyn et al., 2010; Yule et al., 2013) and they are usually parametrized with simple regressions which require fewer parameters compared to most matrix projection models (Ellner & Rees, 2006). By modelling vital rates via regressions, continuous estimations of the state variable are generated and the

state transitions can be described at a very high resolution (Merow, Dahlgren, et al., 2014). Another advantage of IPMs is that the same analyses as for matrix population models can be performed, i.e. calculation of the intrinsic growth rate (λ), stable state distribution (ω) and reproductive value distribution (ν), prospective and retrospective perturbation analysis such as sensitivity and elasticity analysis and life table response experiments, respectively (Fig. 7). In IPMs, elasticities and sensitivities can be broken down into a survival-growth component and a reproduction component. Contributions of each of these components can be calculated as well (Ellner & Rees, 2006). IPMs are used to examine simultaneously the ecological and evolutionary responses to environmental changes and these responses depend on which component of the kernel is affected (Coulson et al., 2011).

Although IPMs are very useful, a major assumption is that dispersal rates are small enough so they can be ignored (Coulson et al., 2010). This assumption results in the exclusion of some populations where dispersal is a contributor to the population structure and size. Another assumption for IPMs is that the population size should be large enough so that demographic stochasticity can be excluded. It is important to account for between-individual variation in the quality of the variable to avoid overestimation of variability, miscalculation of extinction risk, underestimation of uncertainty, estimation biases for demographic rates and incorrect predictions within perturbation analysis (Ellner & Rees, 2006). Another drawback is that none of the modelling framework captures the natural contribution of evolution, plasticity and demography to the population dynamics (Jongejans et al., 2017).

1.6. Thermal adaptation examined with IPMs

Studies combining the statistical power of IPMs with thermal adaptation are scarce, mainly because IPMs itself were only recently developed. One study by Vindenes et al. (2014) investigated the impact of temperature on the dynamics of a top predator in freshwater ecosystems, i.e. the pike, *Esox lucius*, using IPMs. The study had a clear focus on vital rates, population dynamics and body size, with the latter being directly impacted by temperature via the Temperature-Size Rule. They found that all vital rates responded differently to temperature and that this response depended on the body size of the individual. Temperature had a negative effect on fecundity in small individuals, but a positive effect in large individuals, while for survival, this was the other way around: temperature had a positive impact for small individuals and a negative effect for large individuals. Overall, they observed a positive net effect of increased temperature on population growth rate, indicating that the negative effects on the vital rates are overruled by the positive effects and that the thermal sensitivity of the growth rate depends on the body size and vital rates of the individual. This implies that the sensitivity for temperature can change throughout the life cycle. Warming does induce a shift in stable body

size structure towards more medium sized individuals and less small and large individuals. This study can be a helpful guideline for this thesis.

2. Objectives

The general aim of this thesis is to test the degree of genetic differentiation in thermal response of natural populations. We therefore perform a population-level experiment using the keystone freshwater species *D. magna* as a model organism. Collecting data on the effects of different environmental changes on *D. magna* is useful because these effects will likely impact, in a direct or indirect way, the other species in the ecosystem via the keystone species. The main objective of this thesis translates into three specific research questions:

1. What is the effect of density and temperature on the population dynamics?
   Temperature and density are known to have effects on the population dynamics. Increasing temperature leads to smaller size at maturation but faster somatic growth (Atkinson, 1994). Furthermore, it also influences the metabolic rate of ectothermic species and can cause hypoxia in the environment (Van Doorslaer et al., 2010). Higher temperature has a positive effect on intrinsic growth rate (Heugens et al., 2006), so we expect a positive influence of temperature on the population dynamics.
   Increased population density leads, under natural conditions, to an increased competition for food. But crowding itself, even under abundant food availability, can trigger a stress response via transferred chemicals released by individuals or via direct interference (Gergs et al., 2014). Therefore, under higher densities, *D. magna* responds by increasing their effort for offspring production, yielding less but bigger eggs that are more resistant to starvation (Glazier, 1992; Mckee & Ebert, 1996). In addition, the size of the individuals will also decrease (Boersma, De Meester, & Spaak, 1999). Based on these findings, we expect density to negatively influence growth, survival and offspring number, but to positively impact the offspring size. The response to crowding, however, is variable among populations (Boersma et al., 1999) and could even be clone-specific (Burns, 2000) leading to different observed responses than expected.

2. Do southern and northern populations differ in their thermal response?
   In earlier studies, it has been shown that *D. magna* clones collected from different latitudes exhibit different somatic growth rate and metabolic rate under similar thermal conditions (Chopelet et al., 2008). Seasonal clones have shown significant differences in their thermal response, recorded in previous research by Carvalho (1987). Based on these findings, we expect a difference in thermal response in the southern and northern populations. At the same temperature, we expect the northern clones to have a slower somatic growth, and reaching larger body sizes than the southern populations, after the Temperature-Size Rule (Atkinson, 1994). Furthermore, we also expect the southern populations to have a higher thermal tolerance (i.e. deal better with higher temperatures) than their northern counterparts. This expectation is based on the findings from the research of Yampolsky et al. (2014a).

If there is a difference found in thermal response, we will further investigate if the pattern indicates thermal adaptation. It is important to note, that a difference in thermal response does not necessarily indicate thermal adaptation. In the case of thermal adaptation, northern populations will perform best at their natural temperature (i.e. the lower experimental temperatures) and the same holds for the southern populations. The intrinsic growth rate has been found to correlate to the latitude of sample site (Mitchell & Lampert, 2000). We expect that the clonal lines derived from the northern latitudes will perform better at the lower temperatures, while the clonal lines from the southern latitudes will perform better at higher temperatures.

3. To what extent do the clonal lines within populations differ in their thermal response? Clones can differ in their thermal tolerance, which may allow for intra-population variation in thermal tolerance. Preceding research by Carvalho (1987) and Chopelet et al. (2008) already indicate that, under different thermal regimes, mean intrinsic growth rates can vary significantly among clones of the same population as well as between populations. We specifically investigate the difference between intra- and inter-population differences in thermal tolerance, by looking at the intra- population variation in the vital rates. We also asses the contributions of population and clone to the variation in lambda. We therefore hypothesize that differences in vital rates among clones of the same population could also occur in our experiment and that the variation between populations is bigger than the variation within population.

To answer these specific research questions, we use IPMs, described earlier. The use of IPMs will also allow us to evaluate the role of IPMs as tool for unravelling thermal tolerance, thermal adaptation and their consequences on population dynamics in *D. magna*.

3. Material and methods

3.1. Origin of the clones

Four *Daphnia magna* populations, collected from two different latitudes in Western Europe (Fig. 8a), were used for the experiment. These latitudes differ in their climatic conditions such as precipitation, average temperature and hours of sunlight. For the two populations corresponding to the more northern latitude, resting eggs were collected from sediment samples taken from the Asklund pond in Frosta, near Trondheim, and Ringve pond in Trondheim (Fig. 8b). These populations will further be referred to as the Asklund and Ringve population. From the more southern latitude, coinciding with Flanders, Belgium (Fig. 8c), resting eggs were collected from ponds in Zonhoven, Limburg and in Lapscheure, West Flanders. These two populations will further be referred to as the BNK and Blap population.

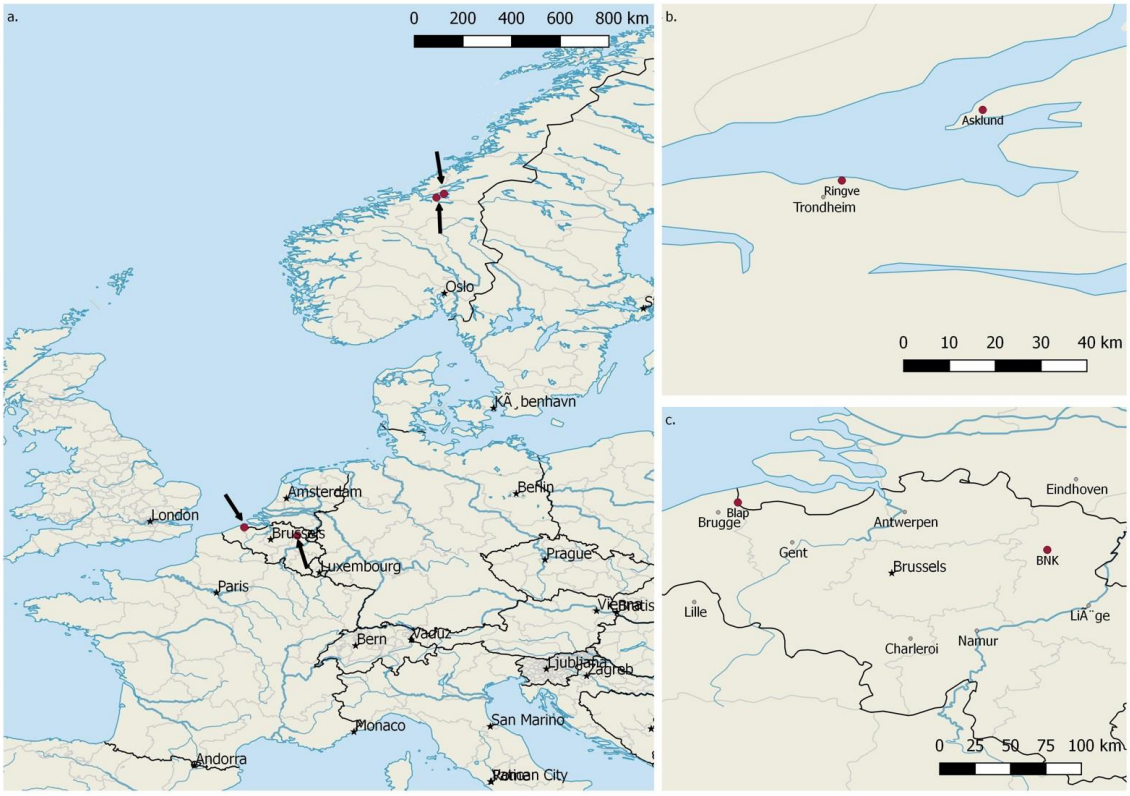

Figure 8: Maps locating a) the four locations in two countries, Norway and Belgium., b) Ringve and Asklund, both collected near Trondheim and c) Blap and BNK and their locations in Belgium (Created in QGIS)

The ponds from which the sediment was collected are similar in size and degree of vegetation. Furthermore, all the ponds were fishless in the present and in the recent past, so none of the populations recently encountered predation by fish. *D. magna* individuals of the Asklund, Ringve and BNK population were hatched at Norwegian University of Science and Technology (NTNU) in Trondheim, Norway while *D. magna* individuals of the Blap population were

already hatched for several years in the lab in Leuven. From each population five clonal lines were selected, further indexed with numbers 1 to 5. For each of these five clonal lines five replicates were kept, resulting into 4 x 5 x 5 = 100 individual jars of 500ml containing filtered water. The five replicates of each population were indicated with letters A to E (Fig. 9). This generated a unique code for each jar of water fleas.

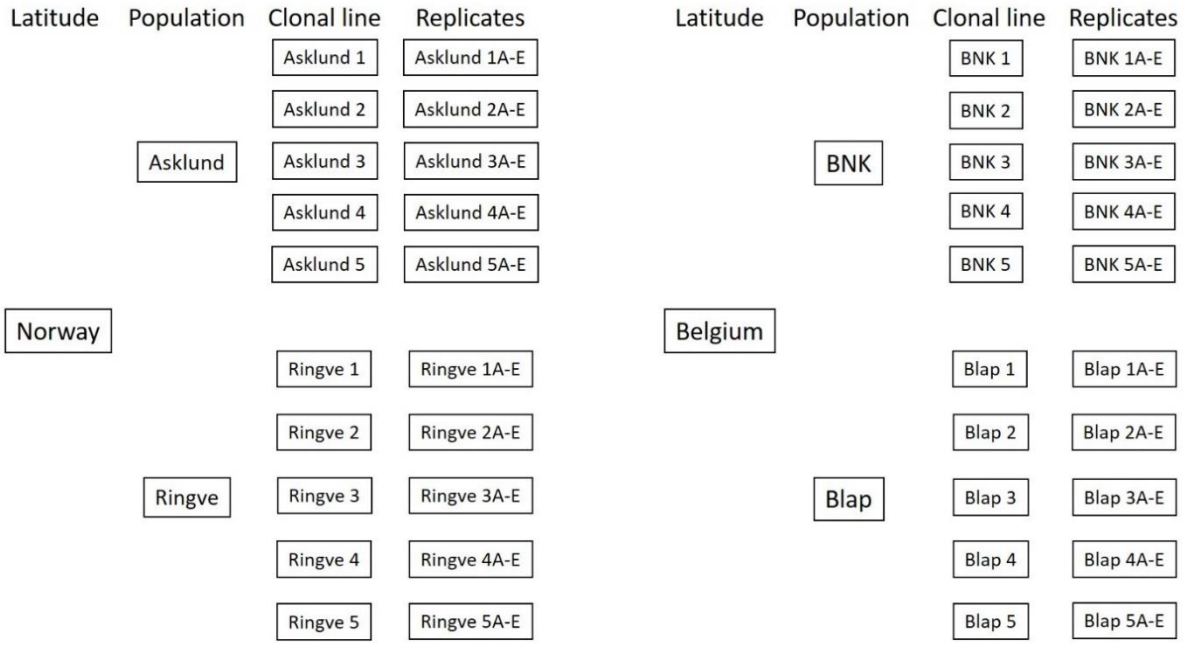

Figure 9: A diagram showing how the unique codes were created.

3.2. Culturing the clones

Before the start of and during the experiment, all *D. magna* individuals were fed a solution of dried shellfish, dissolved into water. The ratio to create this solution was 50ml of shellfish to 950ml of filtered water. The feeding concentration was 2ml L^{-1} day $^{-1}$. Later on in the experiment, tubes were placed inside the aquarium and we fed 1.5µl directly into each tube and the remaining 2.7ml in the surrounding aquarium. Using shellfish instead of the commonly used algae as food source eliminated any possible concerns or problems with a steady food supply and quality. All individual jars with water fleas were kept in bain-maries under a light regime of 14L:10D and with an ambient water temperature of 20°C. This temperature was chosen because 20°C is the mean temperature for the experimental temperatures used later on.

We cultured all four populations until the fourth generation by each time discarding the first clutch of each generation and using 10 individuals of the second clutch to start the next generation. Culturing the clones up until the fourth generation took 7 weeks to complete (10^{th} of July - 28^{th} of August). This approach aimed to eliminate possible maternal effects that otherwise could possibly interfere with the results.

All jars and aquaria were fed the same amount throughout the experiment to eliminate possible effects of food concentration on the response of the test subjects (Giebelhausen & Lampert, 2001; Trubetskova & Lampert, 1995).

3.3. Experimental conditions

When all the individuals of the fourth generation produced their second clutch, we started the experiment. The 4 populations were assigned letters A, B, C, D corresponding respectively with Asklund, Ringve, Blap and BNK populations. Due to time and space limits, we could only use three clonal lines from each population in the experiment instead of the pre-selected five. For each of the three clonal lines of a population, monocultures were set up at four different temperatures (14°C, 18°C, 22°C and 26°C) to examine thermal adaptation. To do so, we randomly selected 12 individuals from the five replicates of each clonal line and placed them in the assigned jar of 500ml. In addition to the monocultures, we also constructed mixed cultures by combining two clonal lines from the same population or from different populations to examine clonal competition within and between populations. This was done by randomly selecting six individuals from each clonal line and placing them into the assigned jar. Every possible clone-by-clone combination was made, however, due to space limits, not at every temperature. The 48 monocultures and the 66 mixed cultures add up to a total of 114 jars, each unique in aspect of clonal line, temperature treatment and the presence or absence of competition.

Distributing all individuals to the correct experimental jars took three days (29th of August - 31st of August) due to the slightly unsynchronized hatching and because some clonal lines did not produce enough individuals to complete all the jars in one go. Once the jar was filled, it was immediately transferred into the corresponding thermal treatment. The next day (1st of September), all jars were cleaned and we checked for mortality. After 3 days, all the jars were omitted and the individuals inside were transferred into aquaria of 2L with 1,5L of dechlorinated tap water (4th and 5th of September) (Fig. 10a). Within each aquarium, we placed two tubes that would serve to easily identify the daphnid whose lifetable characteristics would be measured that week (Fig. 10b). These tubes had holes in them and were covered with a semi-permeable mesh to allow the movement of water containing nutrients and density cues transmitted from the rest of the population, but that contained the individual daphnid nonetheless.

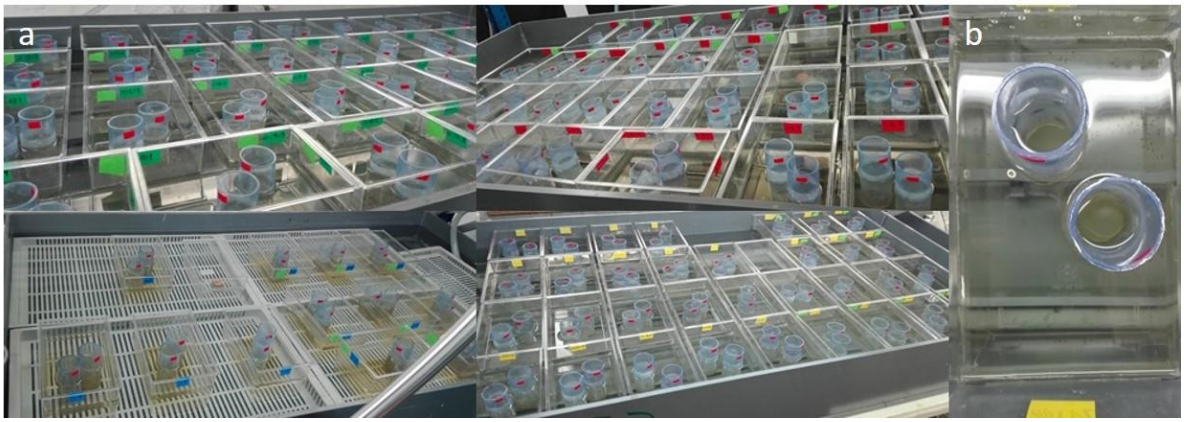

Figure 10: Photographs of the experimental set up. a) the four different temperature regimes: 14°C (blue), 18°C (green), 22°C (yellow) and 26 °C (red). b) Individuals tubes placed inside each aquarium.

Thermal tolerance experiment

The total experiment ran for 12 weeks (4th of September-21st of November). All the aquaria were randomly split into two groups for measurements. The first group, marked with a green sticker, were measured on Monday and Thursday while the unmarked second group were measured on Tuesday and Friday. During measurements, aquaria were taken out of the bain-maries to the working area one at the time. The tubes (arrow A in Fig. 11) were taken out of the aquarium (arrow B in Fig. 11) and set aside for measurements of the *D. magna* individuals inside (arrow C in Fig. 11). When individuals were taken out of the tube, the following measurements were noted down: size, survival, number and stage of eggs, number of offspring, size and sex of a randomly selected offspring if applicable. These measurements were taken by using a binocular microscope. The magnitude of this microscope was set to 2 or 3 and the conversion of the measured length under the microscope to the actual length was done afterwards. The length of the individual was measured from the middle of the eye to the base of the tail. Following the recent description of egg development by Mittmann et al. (2014), eggs were classified belonging to one of the three stages: stage 1 was characterized by spherical eggs, stage 2 by elongated eggs and in stage 3 the eye of the individual developing inside the egg became visible. After the measurements, individuals were either returned into their aquarium or were sampled to be used later for genotyping purposes. In the meanwhile, the population density of the remaining population per aquarium was filmed using a Canon Eos 550D camera for 5 to 10 seconds for later analysis (arrow D in Fig. 11). Using the remote life view in the Eos utility program, we obtained movie clips that could be stored and easily accessed for later analysis using the 'trackdem' package in R (Bruijning et al., 2018, https://github.com/marjoleinbruijning/trackdem). This package counts the number of moving objects per movie clip, tracks their movement and can estimate the population density per aquarium. During the filming, the aquarium was cleaned and the medium was refreshed by keeping 500ml of the original medium and adding 1L of filtered water. Afterwards, the tubes

were put back into the cleaned aquarium (arrow E in Fig. 11) and two new individuals were randomly picked and placed inside the tubes after the same measurements, as described earlier, were taken (arrow F in Fig. 11). At the second day of measurements, respectively Thursday and Friday for group 1 and 2, these individuals in the tubes were taken out, measurements were taken again and the process started anew.

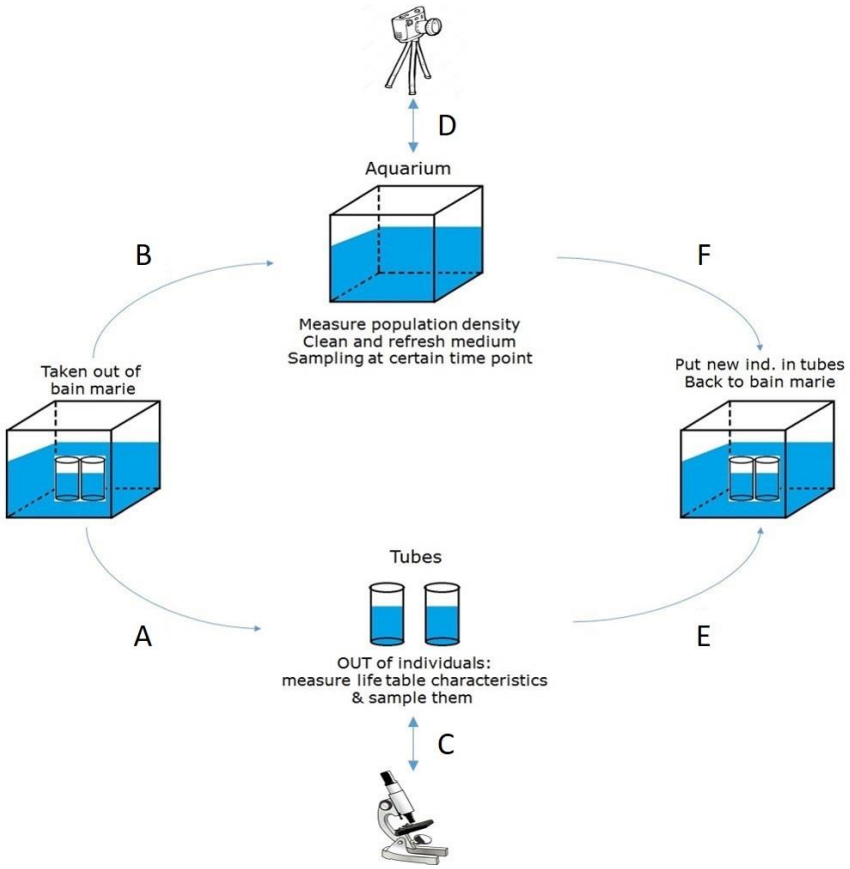

Figure 11: Visualization of the work flow of the daily measurements. **A**: Both tubes are taken from the aquarium. **B**: Aquarium without tubes is transferred into the filming installation. **C**: *D. magna* individual from tube is measured under the microscope. Measurements are size, survival, number and stage of eggs, number of offspring, size and sex of a randomly selected offspring. **D**: Meanwhile, the population density of the remaining daphnids is measured by filming it. **E**: The individuals from the tubes are either sampled or returned to the aquarium. **F**: Tubes are put back into cleaned aquarium and 2 randomly selected *D. magna* individuals are put in the tubes.

Complementary to this approach, sampling was also done to provide information about the genotype of the individual. We sampled the individuals that were kept in the tubes after their final measuring. Additional sampling was done once during the experiment and once after the experiment was completed. Whether sampling was done during the experiment depended on the density of the population. If there were less than 50 individuals, none of the individuals were sampled. When the population did not look well, sampling was also not done or in case that there were few adults and many individuals, we preferred to sample individuals. In the

mixed aquaria, multiple samples were taken when the experiment was running 4 weeks (25th and 26th of September) when each time 20 individuals per population were sampled. This has as purpose to determine the genotypic composition per aquaria during the time period of the experiment. These samples were put in Eppendorf tubes of 1.5ml filled with 100% ethanol and stored in the freezer to guarantee optimal DNA conservation for further analysis. To impose the same interference, 20 individuals from each monoculture were also taken out of the population.

3.4. Data analysis

For the scope of this thesis, we only used the data collected from the monocultures to investigate thermal adaptation and tolerance of the northern and southern populations. In addition, we limited the dataset until 8 weeks after the start of the experiment, instead of the full 12 weeks, because from the 9th week onward, we observed the presence of an infection in all our populations.

Exploratory analyses

We first performed exploratory analyses by visualizing population densities from the raw data over time reached by the clones of each population using ggplots from the 'ggplot2' R package (Wickham, 2009). These plots give an idea of the observed population dynamics at play, and reveal potential differences among the clonal lines within the populations.

Vital rates

We then constructed regression models to determine whether the populations differed in somatic growth, survival and fecundity for the different temperatures. Somatic growth was quantified as the size ($t+1$) – size (t). Survival was indicated whether the individual survived the period between t and $t+1$. Fecundity consisted of the number of offspring produced between t and $t+1$, counted at $t+1$ and the average size of offspring. All vital rates are on the same scale, namely the time period between t and $t+1$, which varied from 3 to 4 days. The results of these regressions for each vital rate were plotted for each population at each temperature. This descriptive analysis, comparing the populations' vital rates, allowed to preliminary explore the patterns that will emerge later on. More specifically, the following explanatory variables were included in all models: size at time t, temperature and density as continuous variables, population as fixed factor (four levels for the four populations A, B, C and D) and clone nested in population as a random factor. The base model looked as follows:

response variable ~ size (t) + temperature + population + density + (1|population:clone)

Temperature and density were both scaled to mean 0 and standard deviation 1. For each vital rate, we identified the model that best fits the data, using Akaike's Information Criterion (AIC). Because forwards step AIC model selection doesn't include mixed models, we manually constructed a step-wise selection and compared the AIC value of each model. We looked in the

selection for which two-way interactions to add to the base model and to possibly add size²(t) as explanatory variable. We only considered a possible quadratic effect for size and assumed a linear effect for temperature and density.

The regression model for growth used the difference in body size over the 3,5 days (i.e. the average duration of a time step) as response variable (noted as GR). Because, the variable GR was considered to have a Gaussian distribution, we used a regression model with a normal distribution implemented via the 'lmer' function from the R package 'lme4' (Bates et al., 2015). Based on the AIC values (Table S1 Appendix), the optimal model for growth included a quadratic effect of size, $size^2(t)$ and the interaction between temperature and density. For the regression model of survival, individual survival (i.e. the individual survived (1) or it did not (0)) was used as response variable (noted as surv). We used a regression model with a binomial distribution and logit link function implemented via the 'glmer' function from the R package 'lme4'. All survival regressions were accompanied by a glmer control to ensure convergence. We tested for the best optimizer and decided to use 'bobyqa' as control. The following control was added to each regression: glmerControl (optimizer="bobyqa", optCtrl=list(maxfun=2e5)). The model that fitted best to the survival data based on the AIC values (Table S2 Appendix) included the interactions between density and population and between temperature and population. Because fecundity consisted of the number of offspring produced and the average size of offspring, two regression models were constructed for fecundity. First, we constructed a regression model for fecundity using the number of offspring as response variable (noted as offspring). The number of offspring followed a Poisson distribution. Therefore, we used a 'glmer' function with a Poisson distribution and log link function. The model selected as the best fit included a quadratic effect of size and the interactions between density and population and between temperature and population (Table S3 Appendix). Second, we constructed a regression model using the offspring size as response variable (noted as sizeoff). Using a 'lmer' function, we constructed a regression model with normal distribution. Based on the AIC values, we selected the best model, which included a quadratic effect of size and no interactions (Table S4 Appendix).

Based on the regression models we could estimate which factor influenced each vital rate the most. For the general linear mixed models, we could not calculate p-values, so we made models in which one factor was dropped. Then we assess the difference between the full model and the model without that factor to see the p-value of that specific factor. To explicitly assess the effect of temperature and density on the vital rates, we extracted the slopes of temperature and density from the regressions' summary for each vital rate. We also did the same for population-specific regressions to assess the effect of temperature and density on the population-specific vital rates. We did this to better understand our data before we started to build IPMs

Differences among populations

The research into the differences among the populations was split into two parts: general and thermal differences. The general differences in density of the populations were investigated via an ANOVA test and a Tukey-HSD test to explore between which groups the difference was significant. The trend in differences was further examined with a linear model that incorporated the same factors as the ANOVA.

IPM building

To address whether populations differed in their population dynamics depending on the temperature treatment, we constructed Integral Projection Models (IPMs; Section 1.5). In order to build an IPM for each population, we had to define functions for each of the vital rates (i.e. growth, survival, offspring number and offspring size). These vital rate functions combined, constituted the population dynamics. These functions were modelled using the selected regressions described above through the 'predict.merMod' function of the 'lme4' package (Bates et al., 2015). Based on each regression model (e.g. modGrowth), a predicting model (e.g. muGrowth) was build.

The survival vital function looked as follows:

$$S(x,t) = \frac{e^{\mu}}{1 + e^{\mu}}$$

The mean, μ, was defined as the predicted values for the survival in function of size based on the survival regressions, noted down as muSurv. The function for number of offspring was similar to the survival function with the exception that it was not limited to having values between 0 and 1:

$$OffN(x,t) = e^{\mu}$$

Here μ reflects the predicted values for offspring number, calculated in muOffN The functions for growth and offspring size were modelled using the 'dnorm' function in R, which generates the following function:

$$f(x|\mu,\sigma) = \frac{1}{\sigma\sqrt{2\pi}} e^{-\frac{(x-\mu)^2}{2\sigma^2}}$$

For the growth vital rate function, the standard deviation equaled the standard deviation of the residuals of the growth regression model and μ equaled the predicted values of the growth regression model muGrowth. In the offspring size vital rate function, μ equaled the predicted values calculated in muOffS and the standard deviation equaled the standard deviation of the residuals for the offspring size regression model modOffS.

The vital rates functions constructed here were then used to build the IPM for a defined range of body sizes. Individuals that grew to sizes outside the used body size range or offspring produced outside the size range were evicted from the model. Possible eviction reduces the

representation of the model. This was accounted for in the growth and offspring size function. In the growth function, individuals larger than the upper size limit were added to the largest size class. For the offspring size class, eviction occurred at the lower limit of the size range. These were added to the smallest size class.

Thermal differences

From the IPMs we calculated the intrinsic population growth rate, often referred to as lambda, of all populations across all temperatures over a range of densities using the 'popbio' R package (Stubben & Milligan, 2007). We then used these estimates of lambda to calculate the population sizes for each population at the different temperatures.

In order to compare populations from both latitudes with each other, we manually performed an analysis similar to a life-table response experiment, abbreviated to LTRE. By creating a baseline IPM and using vital rate kernels from each of the four populations in the baseline IPM, we could compare how populations differed from one another relative to this baseline. This approach also allowed to determine the influence of each vital rate on lambda. It differs from a traditional LTRE, because the difference is not multiplied by the sensitivity matrix. As baseline IPM, we constructed an average population. We did this by averaging each kernel for the four populations and combining these into an IPM. Lambda's were then calculated for this baseline population at each experimental temperature, and for each subsequent IPM for which a vital rate function was replaced by a vital rate function of one of the four populations. Populations from both latitudes were compared with each other at the same temperature. The resulting difference was positive when the vital rate was higher for the population than the baseline and negative when the baseline had higher values than the compared population. This calculation was done at five densities chosen to represent the range of observed densities: the minimal and maximal density, the total mean density and the density at the first and third quartile of the density distribution.

Thermal tolerance

Differences of thermal tolerance among clonal lines of the same population were examined by building IPMs for each clonal line within the framework of a population. This was done by accounting for the random effects of clone nested in population within the predict function from the 'lme4' R package (Bates et al., 2015). A similar method as described earlier was used to compare different clones of the same population. However, here, the baseline IPM was constructed using the population average. Finally, we conducted a linear mixed model with lambda as response variable and population (fixed effect) and clone (random effect nested in population) as predictor variables to determine whether the inter- or intra-population variance in lambda is largest.

All data analyses were executed in Rstudio version 1.1.383 and R version 3.4.3.

4. Results

4.1. Exploratory analyses

Generally, all populations show a rapid increase in their population size, followed by a decrease, either gradually or steep, and this independent from the temperature treatment they were reared in (Figs. 12-15). Depending which clone and which temperature treatment was used, population sizes varied at different temperature treatments. For example, for population A (Fig. 12), it can be observed that at both 14°C and 22°C, clone A3 goes extinct after reaching the highest density in the population. Clone A5 realizes the highest densities at 18°C and 26°C and at the other temperatures as well after clone A3 is gone extinct. Clone A2 has its highest density at 26°C, clone A3 at 18°C and clone A5 at 18°C.

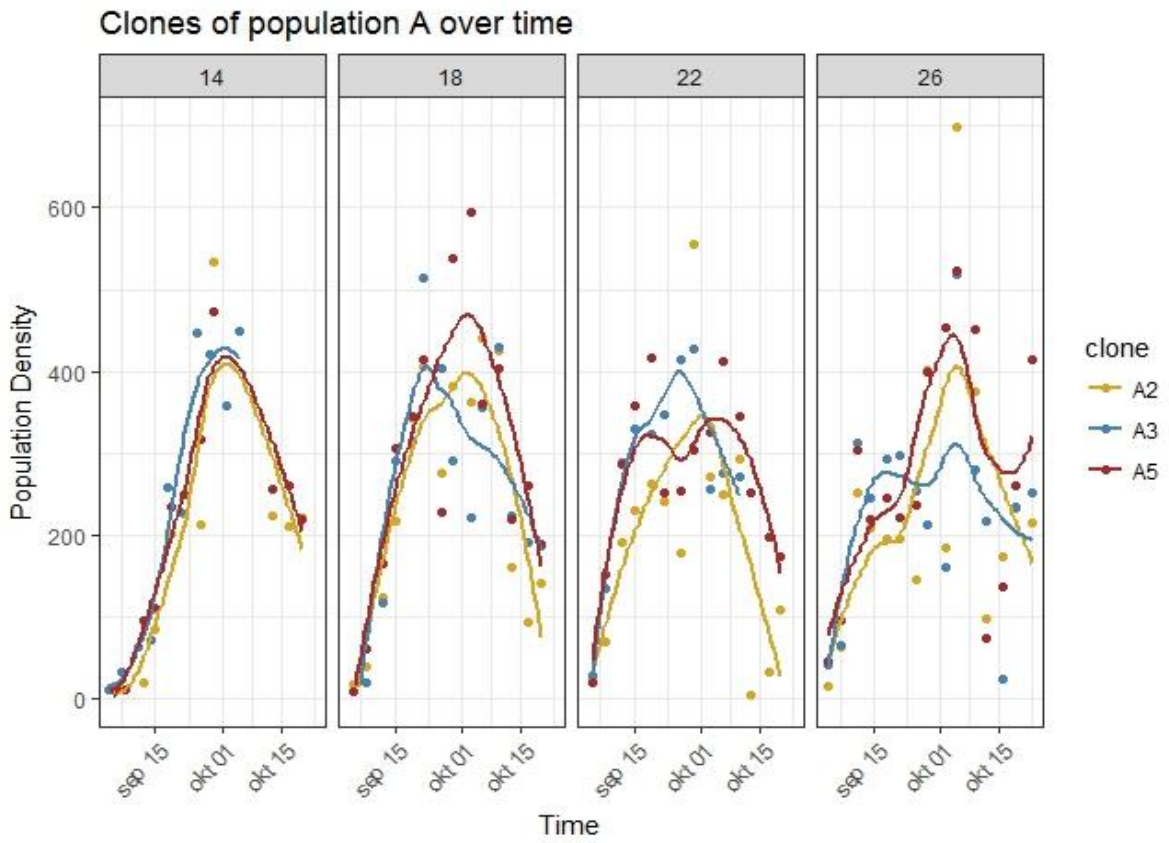

Figure 12: Population density over time for the clones A2 (yellow), A3 (blue), and A5 (red) of population A at the different thermal regimes of 14°C, 18°C, 22°C and 26°C. The dots represent the raw data, and the line gives the local average over time.

In population B, we see that all clonal lines survived after 8 weeks, except for clone B2 at 22°C (Fig. 13). At the end of this period, all clonal lines decreased in densities at all temperatures, except clones B2 and B4 at 26°C. At all temperatures, clone B2 has the lowest densities. Both clones B1 and B4 reached similar maximum densities at all temperatures, however, maximum densities were reached at different time points. Clone B1 reached their highest density at 14°C, clone B4 realized its highest density at 26°C and overall clone B2 had lowest densities at all temperatures, except 14°C.

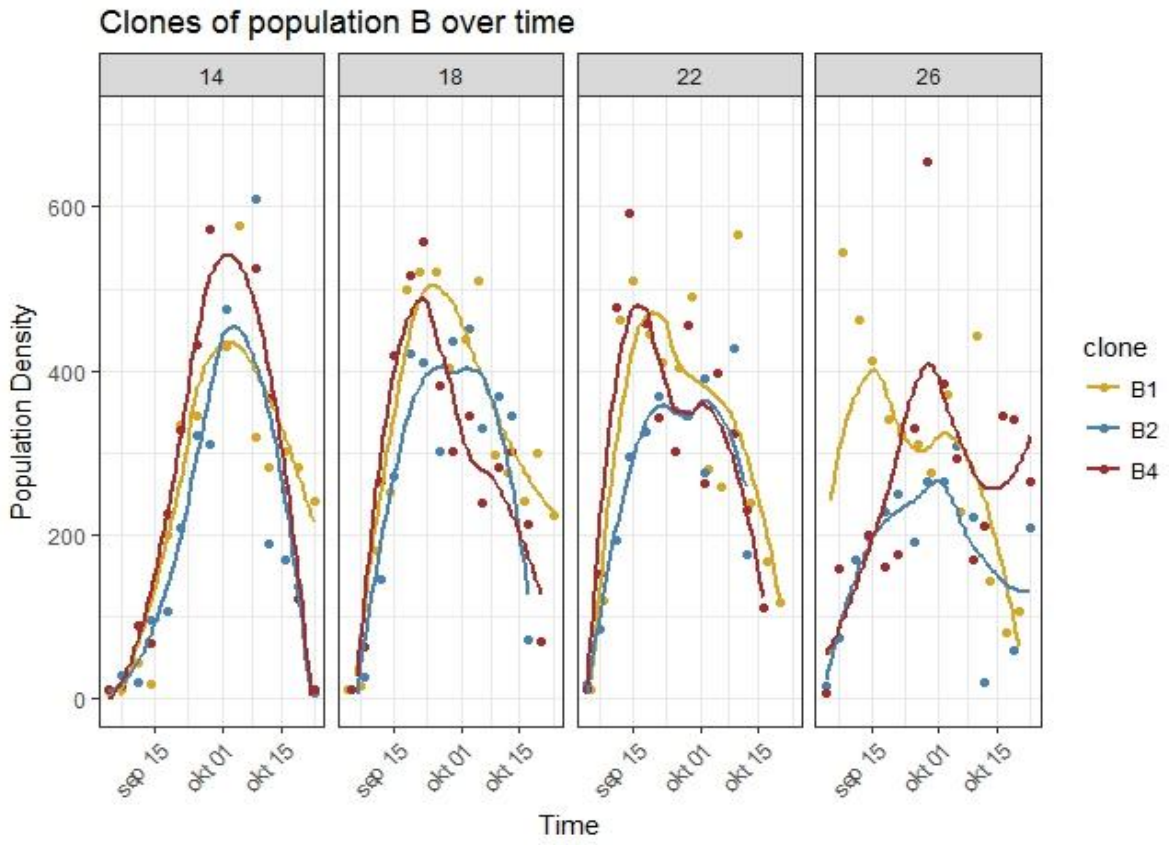

Figure 13: Population density over time for the clones of population B. These include clone B1 (yellow), clone B2 (blue) and clone B4 (red) at the different thermal regimes. The dots represent raw data, while the lines gives the local average over time.

All the clones of population C survived after 8 weeks. We observed that clone C4 had highest densities at 14°C, 18°C and 26°C, while clone C2 had highest densities at 22°C (Fig. 14). Clone C2 has lowest densities at the extreme temperatures (cold and warm), but reaches highest densities at the intermediate temperatures. Clones C4 and C5 reach their highest density at 14°C. All three clones recovered from their decline in population size at 18°C, while this was only the case for both clones C2 and C5 at 14°C, for clone C4 at 22°C , and for both clones C2 and C4 at 26°C.

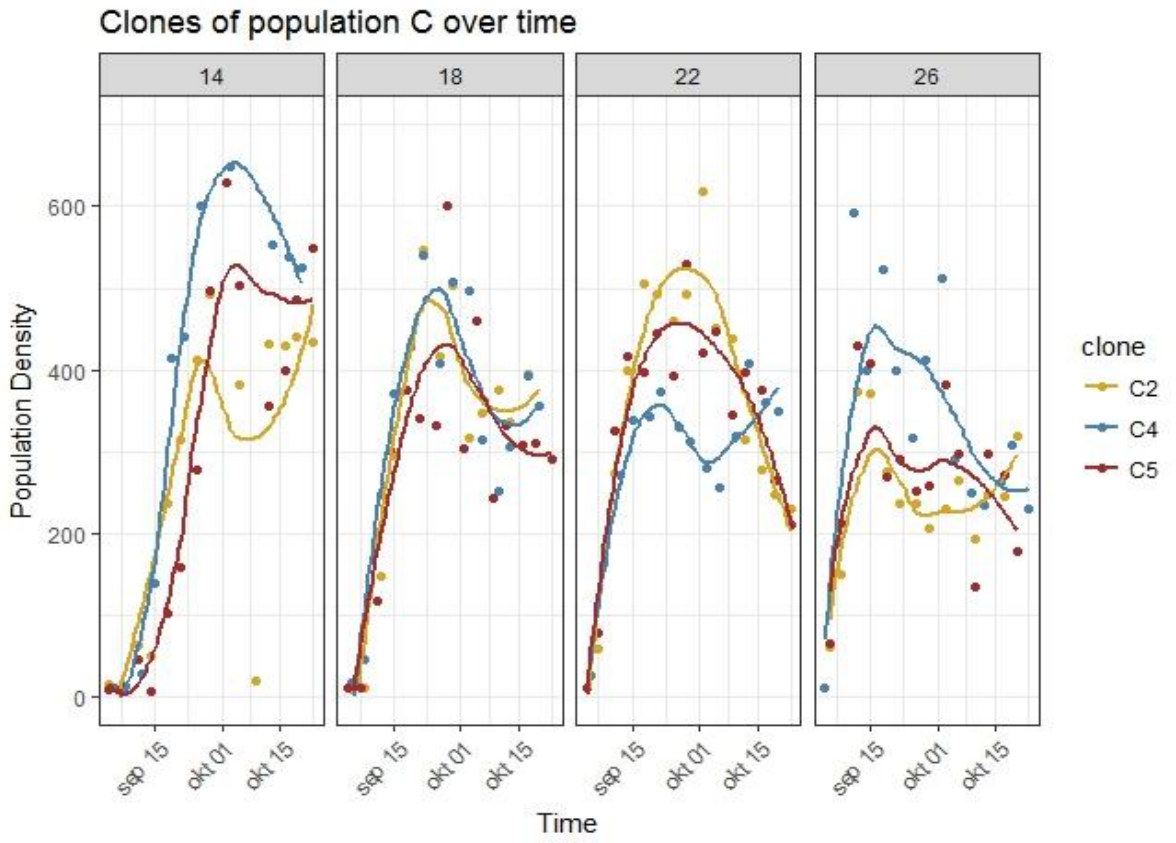

Figure 14: Population density over time for the clones of population C at the different thermal regimes. The clonal lines of population C are clone C2 (yellow), clone C4 (blue) and clone C5 (red). The dots depict raw data and the line gives the local average.

There were no extinctions during these 8 weeks in population D (Fig. 15). All clonal lines decrease in their densities toward warmer temperatures. While the clonal lines at the two colder temperatures show an increase in their population density at the end of the time period, they show a steep decrease in population size at the higher temperatures toward the end of the time period. In the latter temperatures, however, clone D3 and D1 slightly recovers, respectively at 22°C and 26°C. In population D, clone D1 reaches the highest density at the intermediate temperatures, although we do see a peak at 22°C for clone D2. Meanwhile, clone D2 reaches the highest density at 14°C, and clone D3 at 26°C.

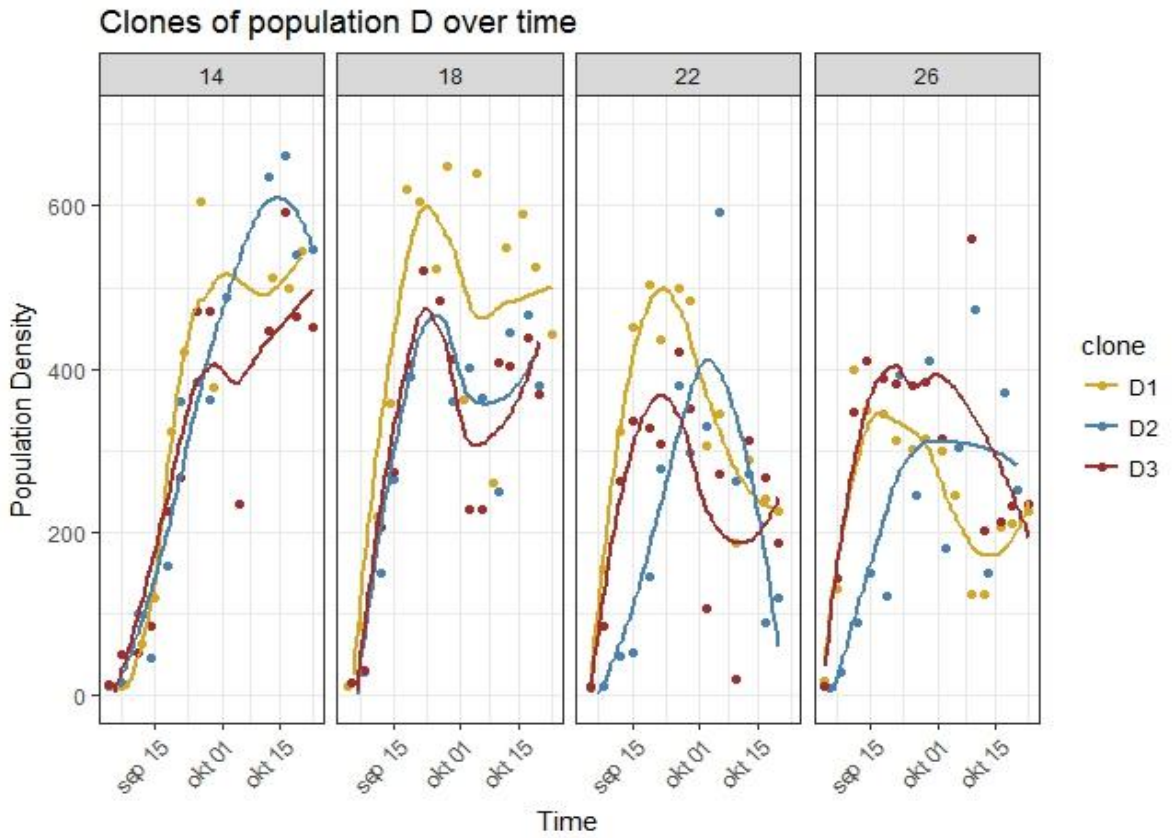

Figure 15: Population density over time for the clones of population D, clone D1 (yellow), clone D2 (blue) and clone D3 (red). This was done at the different thermal regimes. The dots are the raw data and the line gives the local average over time.

4.2. Effects of temperature and density on population dynamics

4.2.1. Vital rates under influence of temperature and population

Figures 16-19 show the vital rates for each population at each thermal regime at an average density. In general we observe, with the exception of offspring number, that the Belgian populations (i.e. populations C and D) have a higher somatic growth rate, a higher survival and produce larger offspring than the Norwegian populations (i.e. populations A and B). In terms of offspring number, it is population A that produces the least amount of offspring, and this across all temperature treatments. Across the various thermal regimes, the same pattern of dissimilarity emerges, yet at varying degrees. The survival regression looks similar throughout the different temperatures, yet a position switch occurs when the survival of population B drops below the survival of population A as the temperature increases. The growth regression remains the same across the temperatures. For both the offspring number and size the positions of the populations remain at the same place at all temperatures, but changes in slopes occur. The number of offspring increases for the Belgian populations at the higher temperatures, while this increase occurs in all populations for offspring size.

More specific, at 14°C, the Norwegian populations A and B have a lower somatic growth rate, a lower survival rate and smaller offspring (Fig. 16). The Belgian population D has for all four vital rates, except offspring number, the highest values, indicating faster somatic growth, higher survival and larger offspring. We observe similar patterns at 18°C (Fig.17). Here too, the Norwegian populations have a lower somatic growth rate, lower survival rate and smaller offspring.

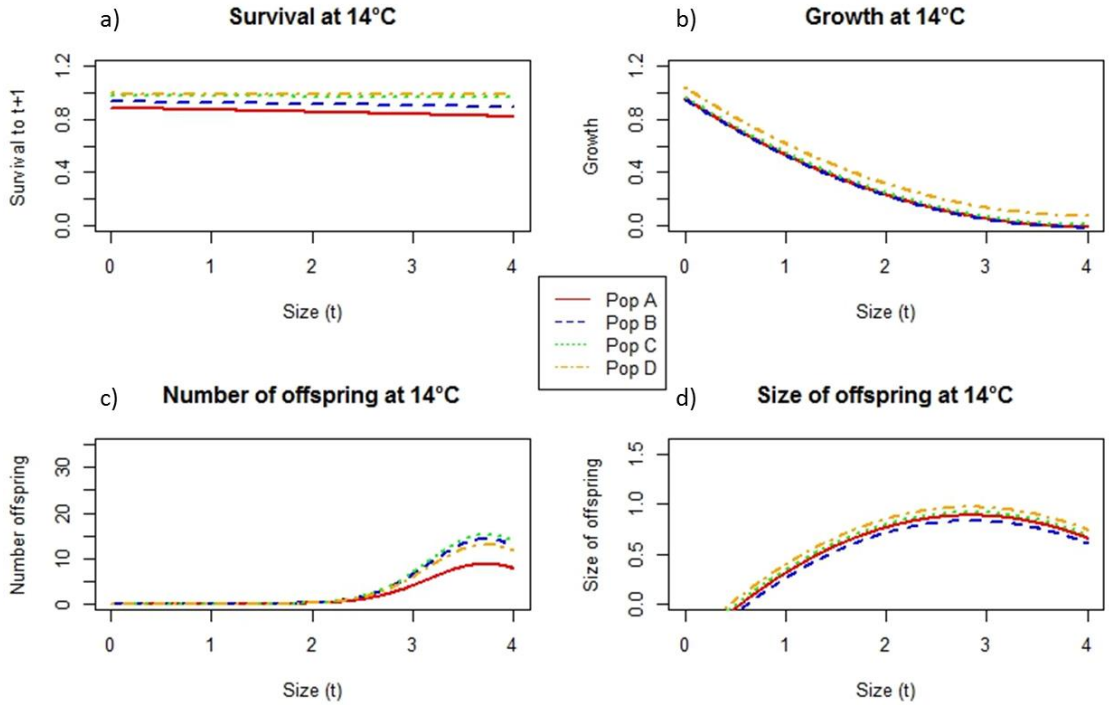

Figure 16: Visualization of the vital rates a) survival, b) somatic growth, c) number of offspring, and d) offspring size at 14°C for the Norwegian populations A (red) and B (blue) and Belgian populations C (green) and D (yellow).

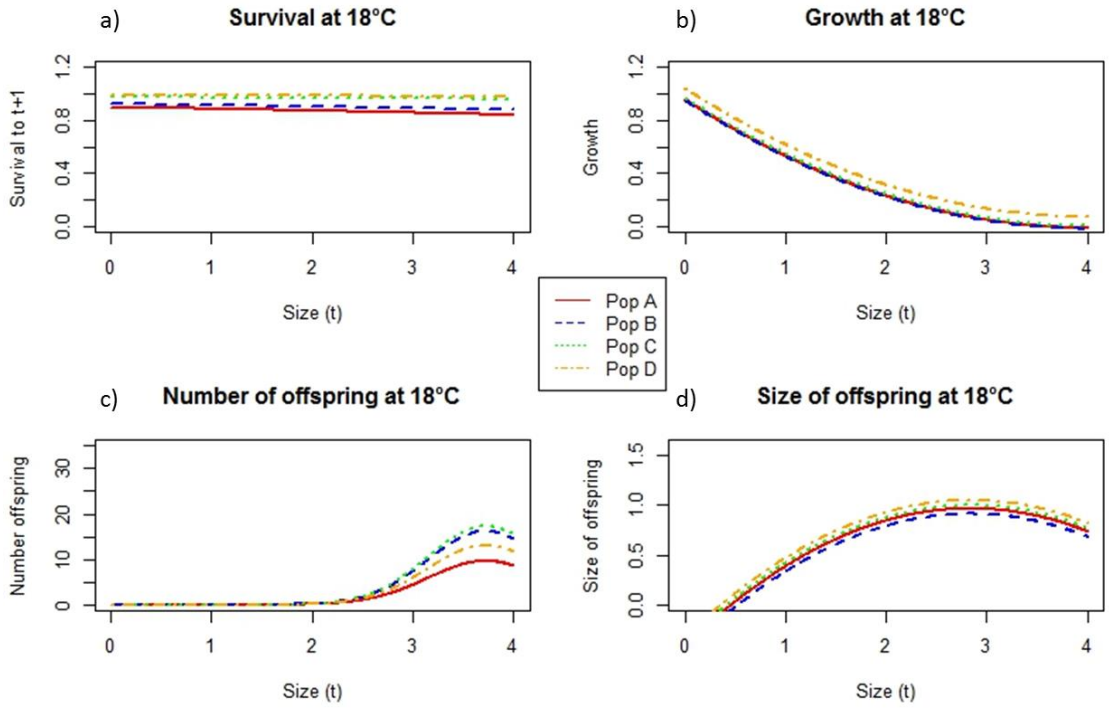

Figure 17: Visualization of the vital rates a) survival, b) somatic growth, c) number of offspring, and d) offspring size at 18°C for the Norwegian populations A (red) and B (blue) and Belgian populations C (green) and D (yellow).

At 22°C, the Norwegian populations A and B have similar survival rates, just as the Belgian populations C and D (Fig. 18). These latter ones, however, have higher survival rates. The number of offspring of the Belgian population D has declined compared to 18°C, and is now closer to the number of offspring vital function of the Norwegian population A. For all populations, except population D, the overall number of offspring is higher than at the lower temperatures.

At 26°C, the size of the offspring is increased in all populations (Fig. 19). Similarly, the number of offspring has also increased, but this only for the Belgian populations. A slight increase in offspring number can be detected in population A. The survival of Belgian population D has decreased below the survival of population C, while the survival of Norwegian population A has increased above the survival of population B. The survival of population D remains higher than that of population A.

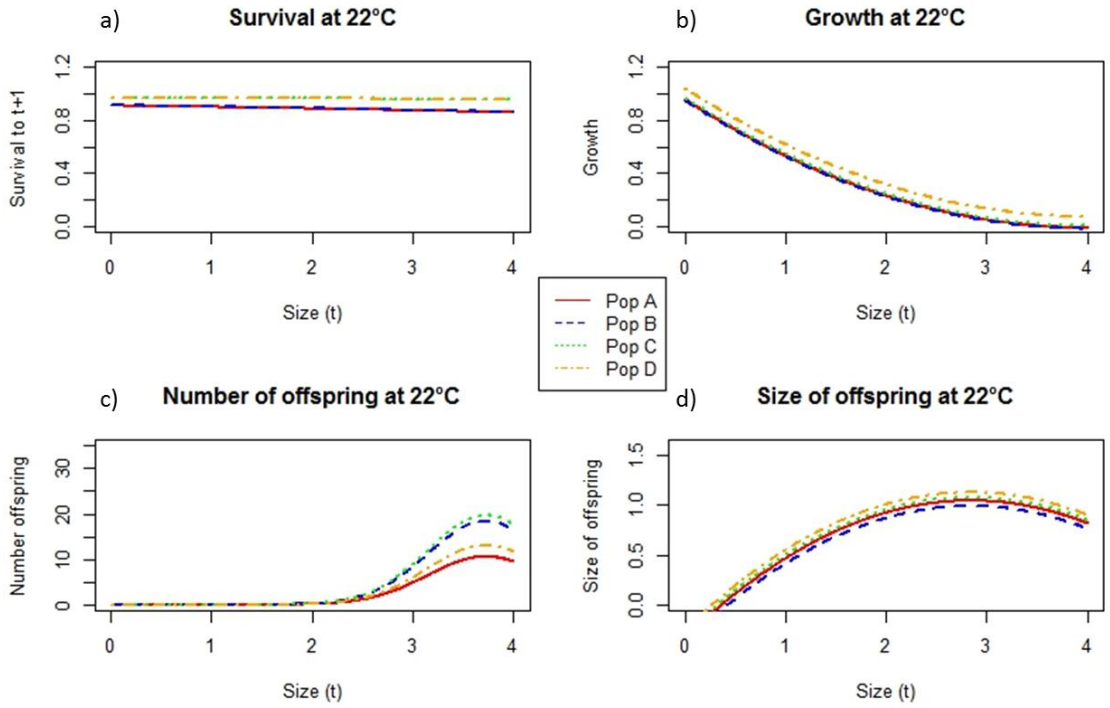

Figure 18: Visualization of the vital rates a) survival, b) somatic growth, c) number of offspring, and d) offspring size at 22°C for the Norwegian populations A (red) and B (blue) and Belgian populations C (green) and D (yellow).

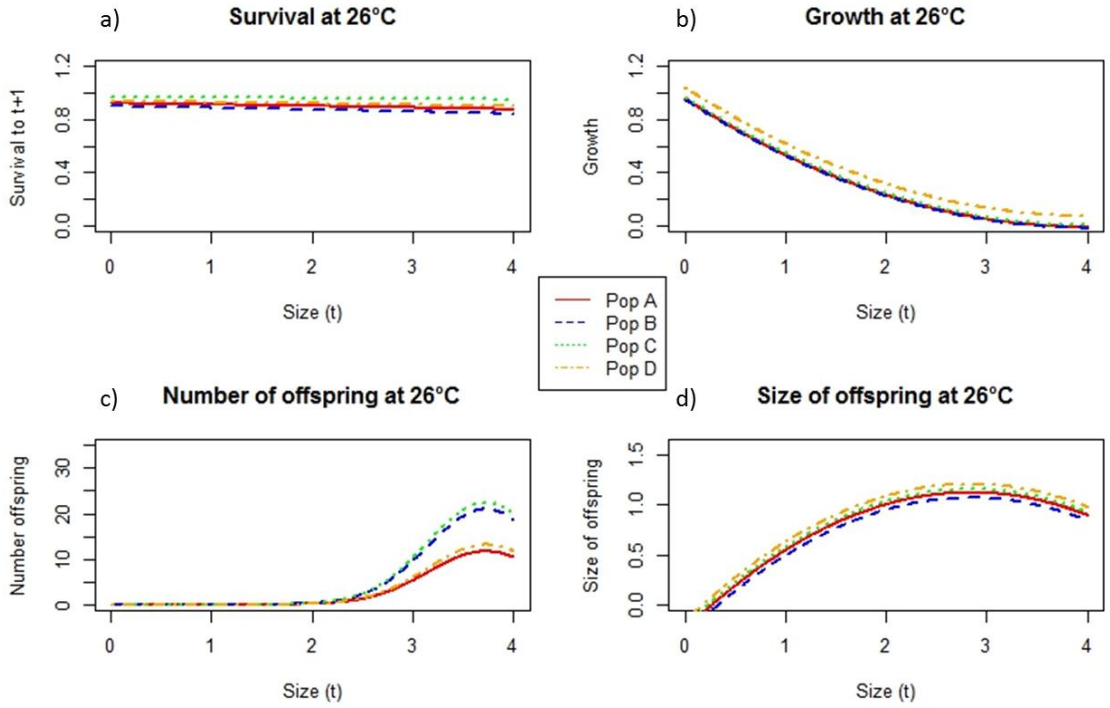

Figure 19: Visualization of the vital rates a) survival, b) somatic growth, c) number of offspring, and d) offspring size at 26°C for the Norwegian populations A (red) and B (blue) and Belgian populations C (green) and D (yellow).

4.2.2. Effects of factors on vital rates

Vital rates of pooled populations.

Survival differs significantly among populations ($p = 0.003$). Moreover, we also found a significant the interaction between density and population ($p < 0.001$) and between temperature and population ($p = 0.036$) indicating that survival is differently influenced by density and temperature for the different populations.

The somatic growth rate is significantly influenced by the linear ($p < 0.001$) and quadratic ($p < 0.001$) effect of size at time *t*, and is found to differ among population ($p = 0.016$). We also found a significant interaction effect of temperature and density ($p < 0.001$). Density ($p < 0.001$) negatively influences the somatic growth, meaning that with increasing density, the somatic growth rate will become lower.

Offspring number is mainly impacted by the linear ($p < 0.001$) and quadratic ($p < 0.001$) effect of size at time *t*, and significantly differs among populations ($p = 0.007$). We also found a significant effect of the random factor clone nested in population ($p < 0.001$). The interactions between temperature and density ($p < 0.001$), density and population ($p < 0.001$), and temperature and population ($p = 0.035$) are all significant. The significant temperature effect on offspring number ($p = 0.027$) is positive, so the clutch size under warmer thermal regimes is larger. The effect of density ($p < 0.001$) on offspring number is negative, so the amount of produced offspring will decrease when the population numbers rise.

Lastly, the offspring size is significantly influenced by the linear ($p = 0.009$) and quadratic effect of size ($p = 0.014$), and is found to differ among populations ($p = 0.022$). The significant effect of temperature ($p < 0.001$) in offspring size was positive, implying that the offspring will be larger under higher temperatures. Additionally, the effect of density ($p = 0.001$) was positive meaning that the offspring become larger under higher densities.

Population specific vital rates

We also performed regression analysis on the four vital rates for each population separately to detect whether these were influenced by temperature or density. For survival, we only found a significant effect of temperature in population D ($p = 0.015$), and this effect was negative. Temperature significantly influences the somatic growth rate of population A ($p = 0.013$) in a positive way, but no significant effect was found in other populations. Only for population C, a marginally non-significant ($p = 0.08$) effect of temperature for the somatic growth was found. The number of offspring is significantly affected by temperature in populations B, C and D (all $p < 0.001$). In populations B and C this effect was positive, while in population D there was a decrease in the number of offspring under higher temperatures. The size of the offspring is positively affected by temperature in all populations (A, B and C: $p < 0.001$, D: $p=0.02$).

Significant effects of density were found for survival of the northern populations A and B ($p = 0.042$ and $p < 0.001$, respectively). Density has a negative effect on survival in population A, whereas it has a positive effect on survival in population B. Both somatic growth rate and

offspring number of all populations are significantly influenced by density in a negative way ($p < 0.001$). The offspring size of all populations are positively influenced by density, but this effect is significant only in the southern populations C and D ($p = 0.04$ and $p = 0.02$ respectively).

A possible interaction effect of temperature and density was only included in the regression models of somatic growth and offspring number. For somatic growth, this interaction influences populations A, B and D in a significant and positive way ($p = 0.008$, $p = 0.003$ and $p < 0.001$, respectively). Only the offspring number of population D is significantly negative influenced by the interaction between temperature and density ($p < 0.001$).

4.3. Differences among populations

4.3.1. Differences in density

Using an ANOVA test, followed by a Tukey-HSD test, we explored whether northern and southern populations differed in their density (Table 1, full summary in Appendix, visually depicted in Figure 19). We only found significant differences at temperature 14°C and 18°C. At 14°C, there are significant differences between populations A and C, populations A and D, and populations B and D (Fig. 20a). Population A had the lowest densities, and population D the highest. At 18°C, it is only the difference between populations A and D that is significant (Fig. 20b). A marginally non-significant result was found between populations A and C at 22°C (Fig. 20c). At 26°C none of the populations differ from each other (Fig. 20d). Temperature-wise, the lowest densities are again recorded at 14°C and the highest are reached at 18°C. This difference is significant. Overall, we found that the populations of the same latitude (i.e. populations A & B and populations C & D) never differ significantly from each other in population density at any temperature. In addition, populations B and C also never differ significantly from each other, even though they belong to opposing latitudes.

Table 1: All p-values calculated by the Tukey-HSD test for differences in density between populations at the different temperature regimes. Significance levels are indicated in brackets and significant differences are shaded.

Combination	**14**	**18**	**22**	**26**
A-B	0.999	0.999	0.541	1.000
A-C	0.036 (*)	0.802	0.062 (·)	0.973
A-D	0.006 (**)	0.014 (*)	1.000	0.999
B-C	0.144	0.999	0.999	0.982
B-D	0.029 (*)	0.337	0.735	0.999
C-D	0.999	0.922	0.126	0.999

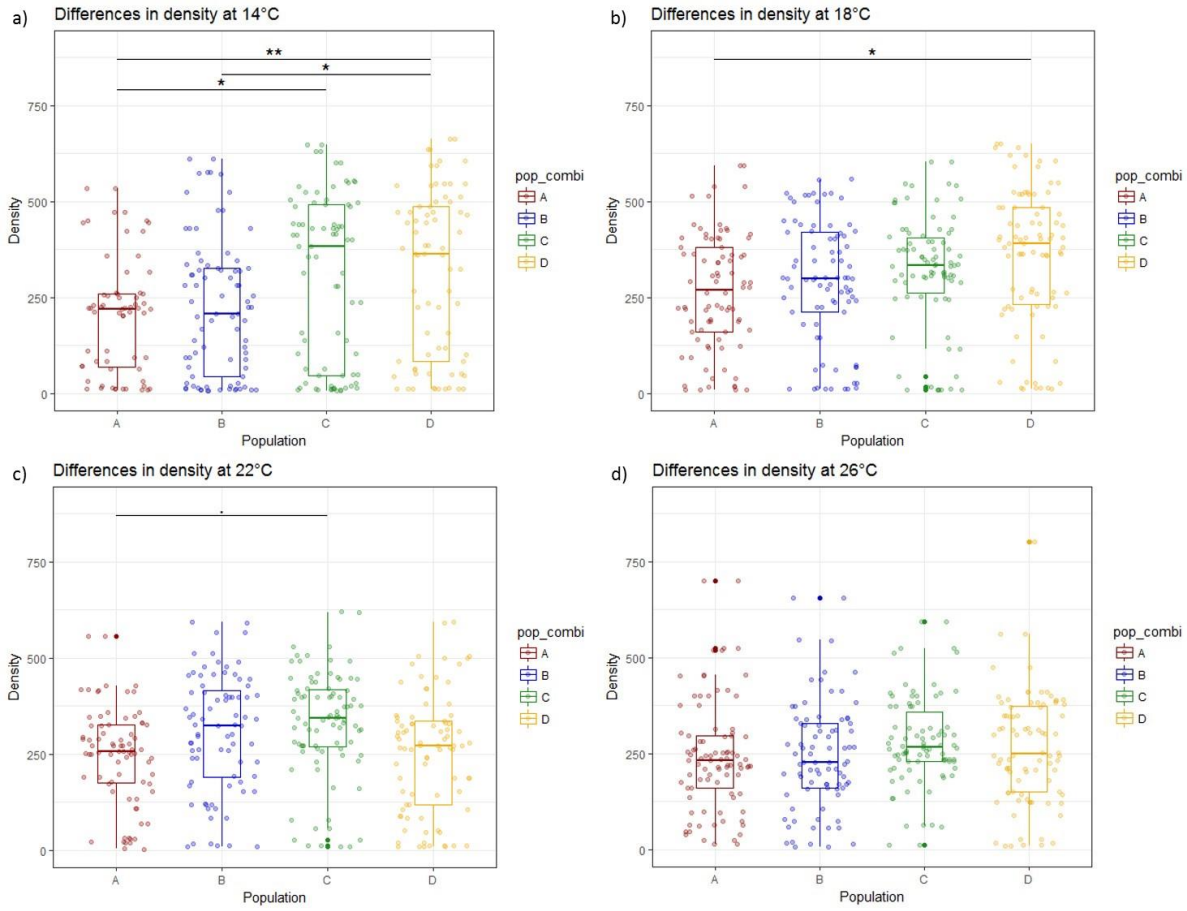

Figure 20: Boxplot representation of the density differences between populations A (red), B (blue), C (green) and D (yellow) for a) 14°C, b) 18°C, c) 22°C and d) 26°C. Dots represent the raw data. Horizontal lines and their associated asterisk indicate significant differences. · $p < 0.1$, * $p < 0.05$, ** $p < 0.01$

4.3.2. Thermal differences in lambda

From the previous analysis we found that populations differ in their density and in their vital rates. However, this difference depends on the thermal regime. While we found significant differences between some populations at the colder temperatures, none were found at the highest temperatures. To further explore thermal differences between populations, we used the intrinsic population growth rate, lambda, obtained from the IPMs constructed earlier for each population for all temperatures with varying densities (Figs. 21-24).

A general trend found for all populations, is that the intrinsic population growth rate decreases with increasing density, but the extent of this decrease varies across populations and thermal regimes (Figs. 21-24). The threshold density at which lambda becomes less than one varies for each population. For our experimentally observed range of density (i.e. from 0 to 800 individuals), we found that the Norwegian population A reaches the fastest a negative intrinsic growth rate (density threshold varying from 430 to 540 individuals going from 14°C to 26°C; Fig. 21a). The Belgian population C was the second one to reach fastest a negative population growth (density threshold varying from 610 to 660 individuals going from 14°C to 26°C; Fig.

23a), and at high densities intrinsic growth rates of the colder thermal regimes become larger than those of the warmer thermal regimes. For the Norwegian population B, we only found a negative intrinsic growth rate at 14°C for a density threshold of around 700 individuals (Fig. 22a). Last, for the Belgian population D, no negative intrinsic growth rate was reached for the experimentally observed density range (Fig. 24a). For this population at lowest densities highest intrinsic growth rate was found for the intermediate temperatures alike. This quickly switches to highest intrinsic growth rates for 14°C thermal regime at intermediate densities, followed by another switch around 400 individuals at which highest intrinsic growth rates were found for the 22°C thermal regime. A last position switch occurs around 550 individuals and from there on, the lambda at 14°C is the lowest and the lambda at 22°C is the highest. Except for population C at very high densities (>700), population D was the only population in which maximum intrinsic growth rates differed among thermal regimes depending on the density. For population A, B and C, lambda was always highest at the warmest thermal regime.

When using the density-dependent lambda's to project the expected population sizes starting from an initial of 12 individuals, we found that both the Norwegian population A and Belgian population C show similar patterns (Figs. 21b and 23b). Both populations show increasing population size with increasing densities and temperatures. In populations A and C, changing thermal regimes at time step 20 from 14°C to 26°C leads to a 1.32% and 1.16% increase of population size, respectively. Both populations show a rapid diversion in population size between thermal regimes, with the warmest thermal regime having highest population sizes across the entire density range. The main difference between these populations lies in the time period needed to grow. Population C reaches high densities faster than population A. For population B, population sizes remained similar until time step 17 (Fig. 22b). At time step 20, population size only increases 1.05% from the coldest to the warmest thermal regime. Last, population D is the only population for which the warmest thermal regime has the lowest population size, and the coldest thermal regime the highest population size (Fig. 24b). This, however, changes towards time step 25 in which the coldest thermal regime quickly drops in population size.

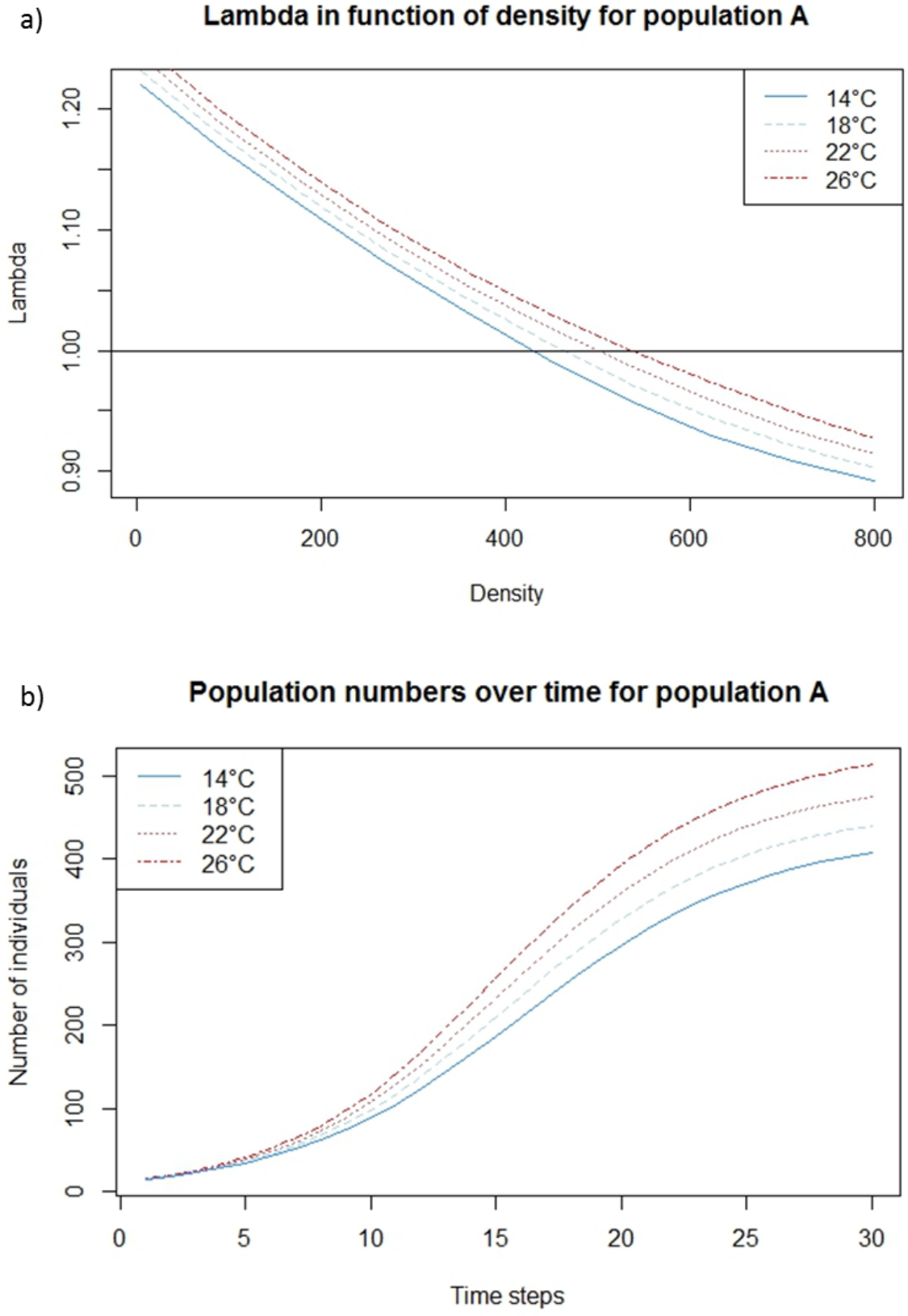

Figure 21: a) Graphical visualization of lambda of population A in function of density for different temperatures: 14°C (full blue line), 18°C (striped blue line), 22°C (dotted red line), 26°C (striped-dotted red line). b) Projection of the population sizes using the density-dependent lambda's given in a. for the different temperature regimes.

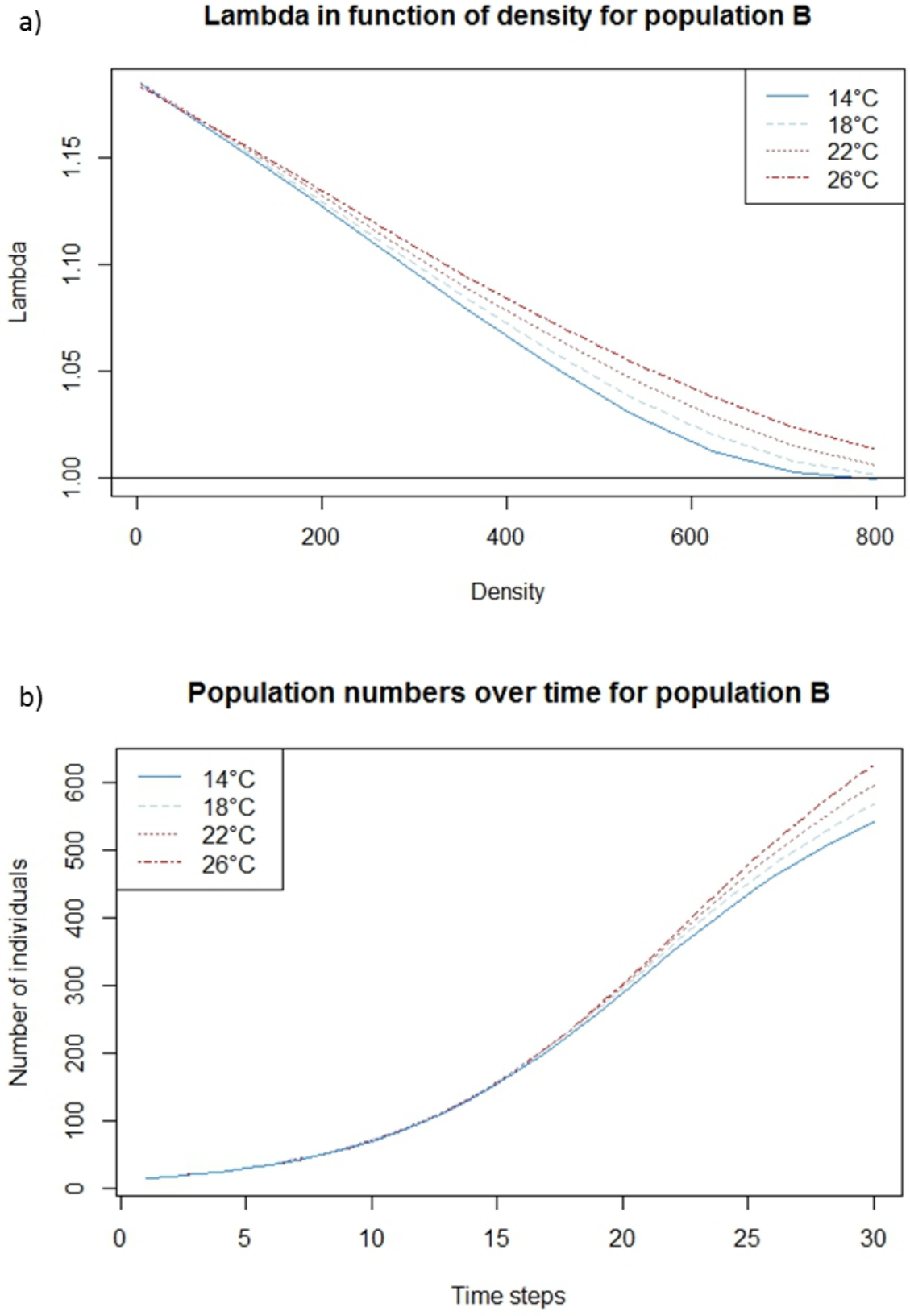

Figure 22: a) Graphical visualization of lambda of population B in function of density for different temperatures: 14°C (full blue line), 18°C (striped blue line), 22°C (dotted red line), 26°C (striped-dotted red line). B) Projection of the population sizes using the density-dependent lambda's given in a. for the different temperature regimes.

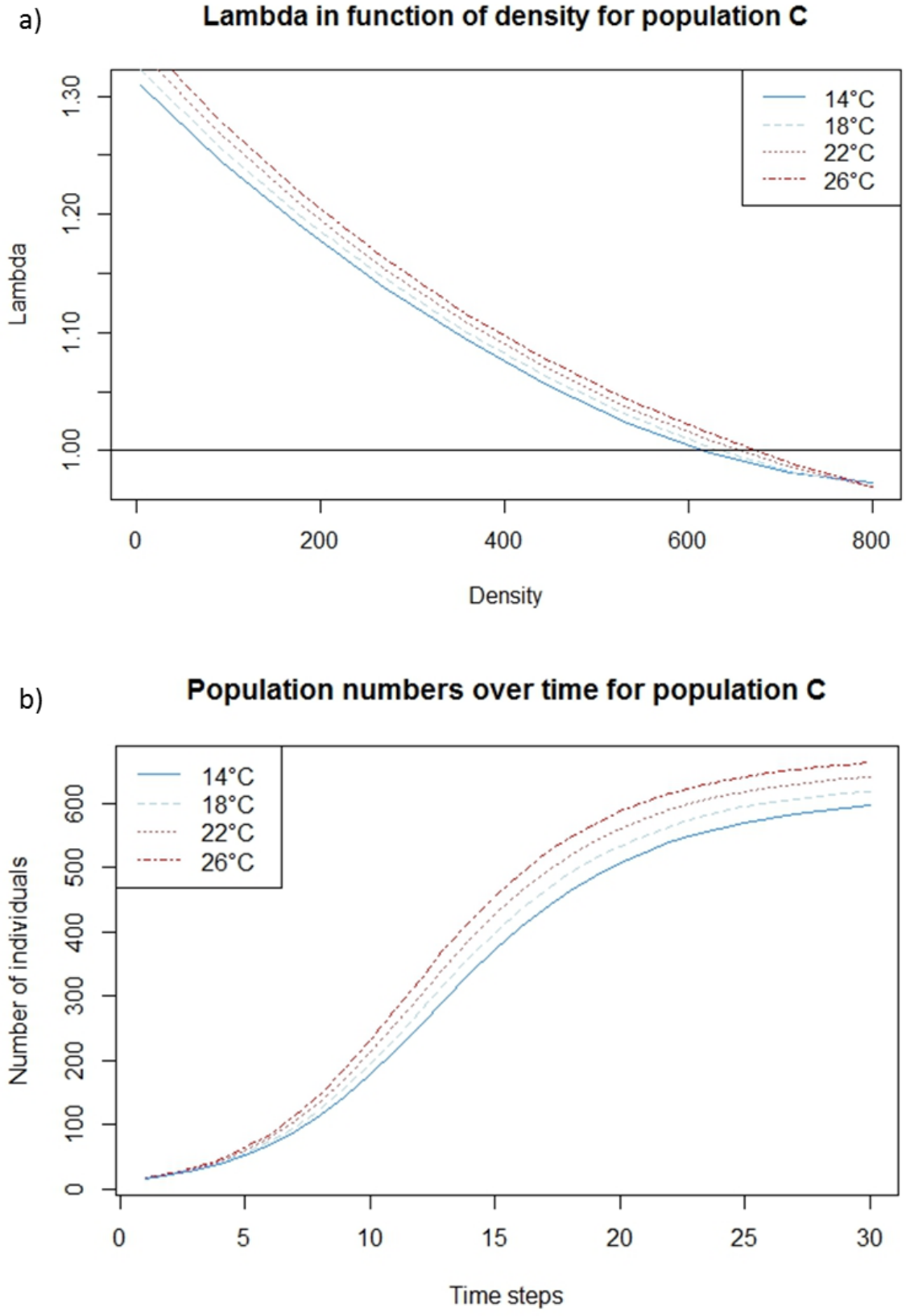

Figure 23: a) Graphical visualization of lambda of population C in function of density for different temperatures: 14°C (full blue line), 18°C (striped blue line), 22°C (dotted red line), 26°C (striped-dotted red line). b) Projection of the population sizes using the density-dependent lambda's given in a. for the different temperature regimes.

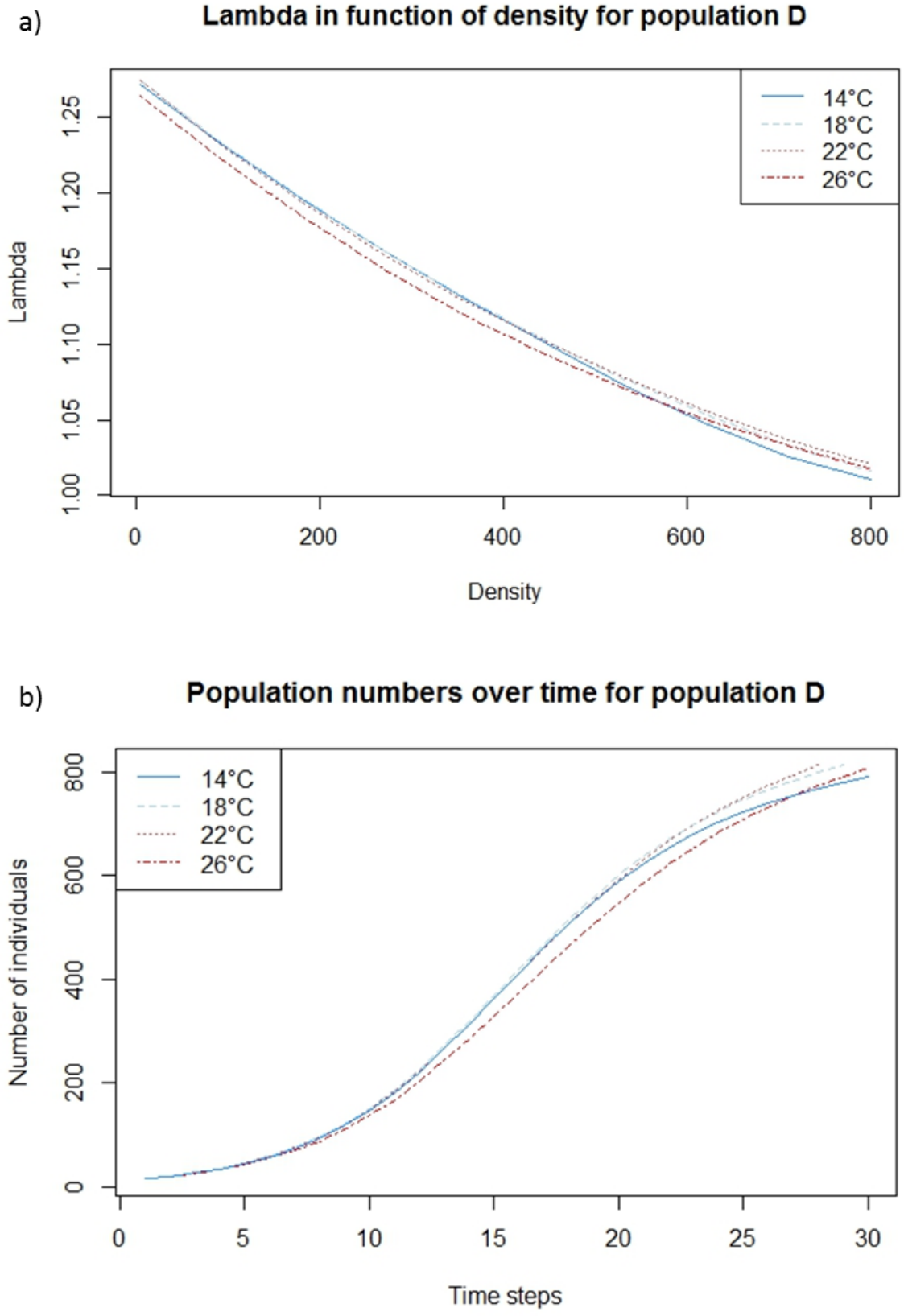

Figure 24: a) Graphical visualization of lambda of population D in function of density for different temperatures: 14°C (full blue line), 18°C (striped blue line), 22°C (dotted red line), 26°C (striped-dotted red line). b) Projection of the population sizes using the density-dependent lambda's given in a. for the different temperature regimes.

4.3.3. Effects of population kernels on lambda

Figures 25-29 show the results from the LTRE-like analyses in which the vital rates of all populations were compared to a baseline population across the four experimental temperatures and over a range of five density values (i.e. 4, 160, 278.4, 400 and 801). Overall we found that the Belgian populations perform better than the Norwegian populations, and this for all vital rates except offspring number. For this vital rate, at the second lowest density value, the Norwegian population B produces more offspring, while the Belgian population D produces the fewest offspring. The differences between population effects become smaller as density increases for all vital rates, except for survival

When replacing the average survival rate with a population-specific survival rate, all populations show a different pattern in their effect on lambda (Fig. 25). Overall, the Belgian populations C and D have positive effects on average lambda, except at high densities for population C. At this density value, replacing the average survival rate by the survival rates of 22°C and 26°C of population C results in a decrease in lambda. For the Belgian population D, the effects remain parallel across temperatures. For population C, there is a switch where the survival rates from the warmer regimes have a positive impact on lambda at low densities, but a negative impact at high densities. The two Norwegian populations show opposite patterns. Population A shows positive effects on lambda at lower densities, while population B shows positive effects on lambda at higher densities. For population A, the effects across temperatures remain parallel, with the warmest thermal regime having the less negative effect on lambda. For population B, however, the warmest regime has the most negative effect on lambda at lowest densities, but the most positive effect at the highest density value.

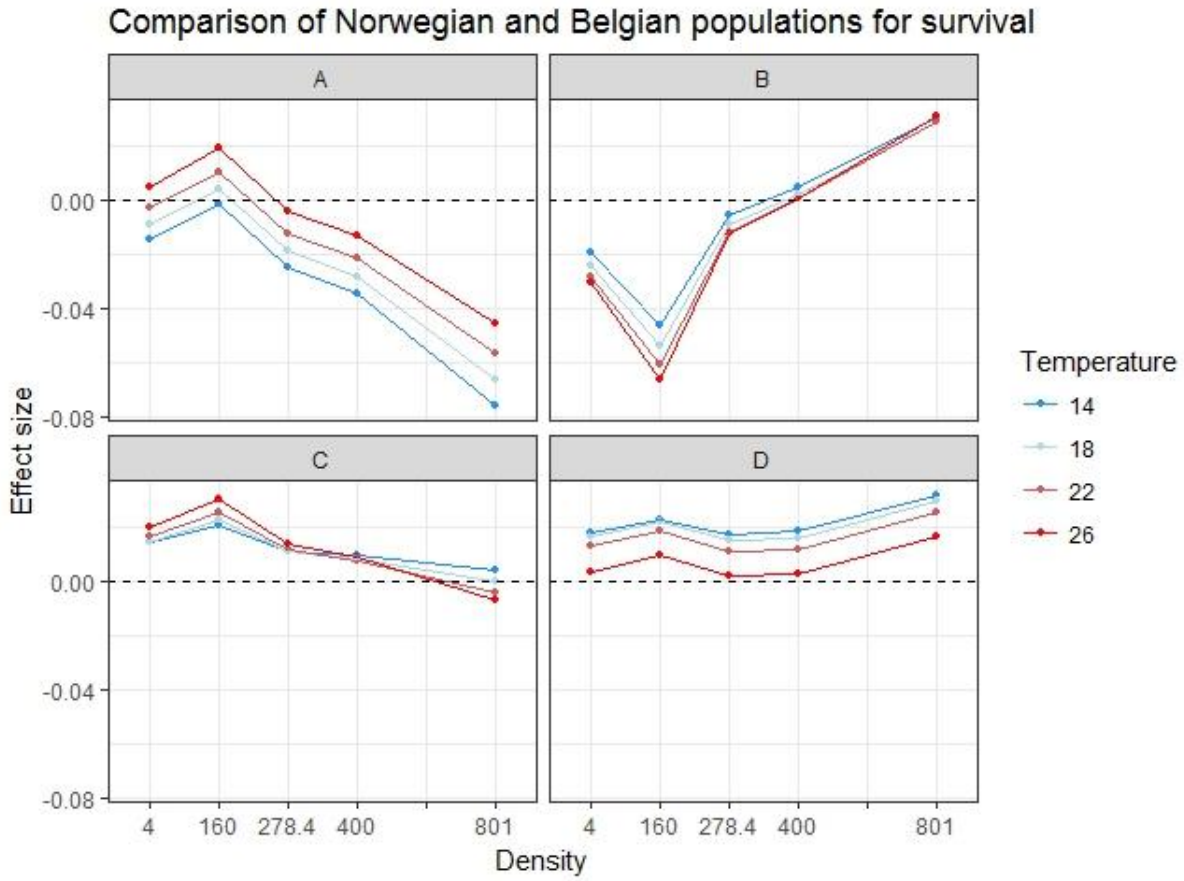

Figure 25: Visualization of LTRE-like analysis for survival of all populations to a total average population at the four temperature treatments: 14°C (blue), 18°C (light blue), 22°C (light red) and 26°C (red). The y-axis gives the effect size on lambda of the average population when changing the average survival kernel by the survival kernel of one of the four populations.

In all populations, replacing the average somatic growth rate with one of the population-specific somatic growth rate's results in stronger effects for the warmer thermal regimes (Fig. 26).The somatic growth rates of Norwegian populations A and B and the Belgian population C negatively impact the average population growth. However, this negative effect becomes less with increasing densities. For population A, B and C, somatic growth rate's from warmer thermal regimes result in stronger decreases in average lambda compare to growth rate's from the colder thermal regimes. Using the somatic growth of the Belgian population D has a positive effect on the average population growth, the opposite effect of the other populations. This effect is larger for higher temperatures.

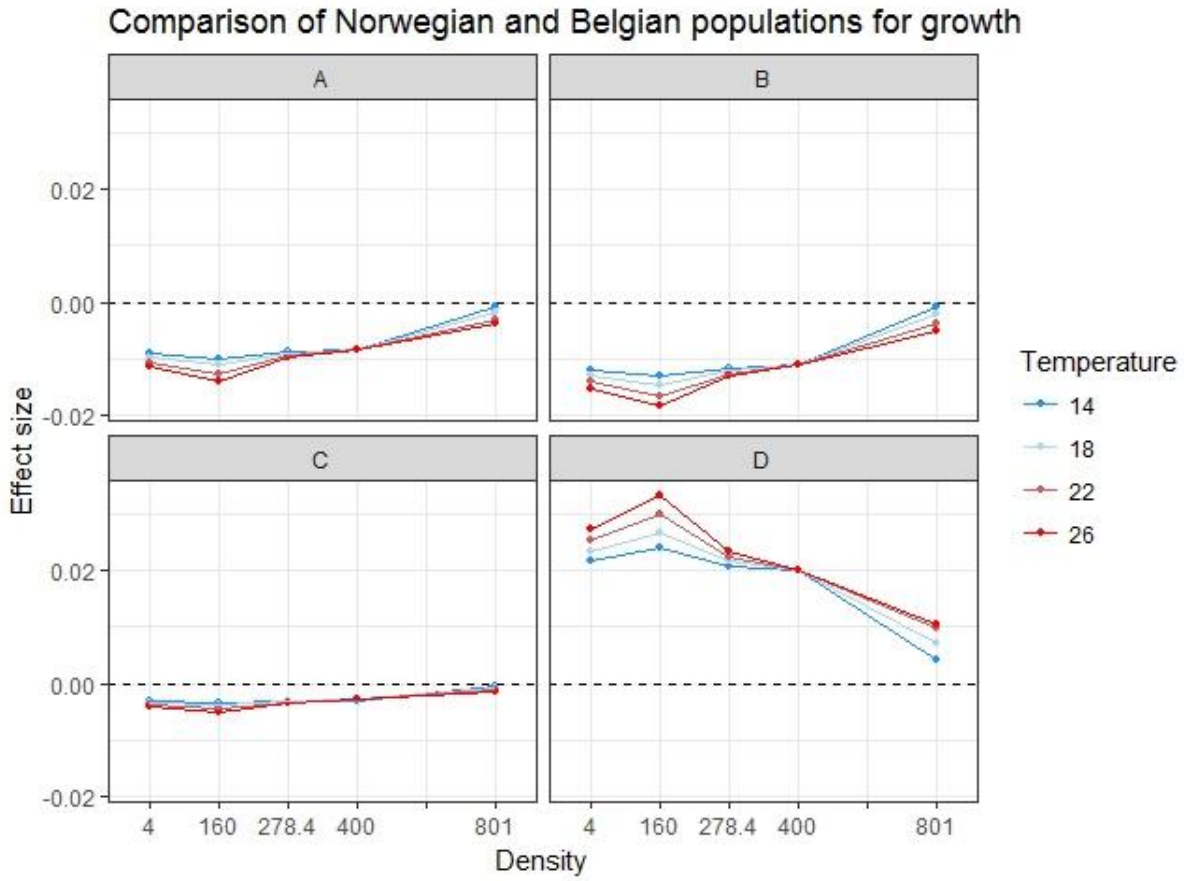

Figure 26: Visualization of LTRE-like analysis for somatic growth rate of all populations to a total average population at the four temperature treatments: 14°C (blue), 18°C (light blue), 22°C (light red) and 26°C (red). The y-axis gives the effect size on lambda of the average population when changing the average growth kernel by the growth kernel of one of the four populations.

Overall, replacing the average vital rate for offspring number with population-specific vital rates for offspring number shows that the warmest thermal regime treatment always had the largest effect on lambda, be it positive or negative (Fig. 27). For the Norwegian population A, replacing the average offspring number vital rate with the population-specific ones, resulted in a negative effect on average lambda, and this independent of temperature. This was different for the Norwegian population B, for which positive effects on lambda were found except for densities between 80-240. For the Belgian populations, population C has positive impact on lambda at low densities which become negative at higher densities, while population D has a negative impact on lambda at low densities, which become positive at higher densities.

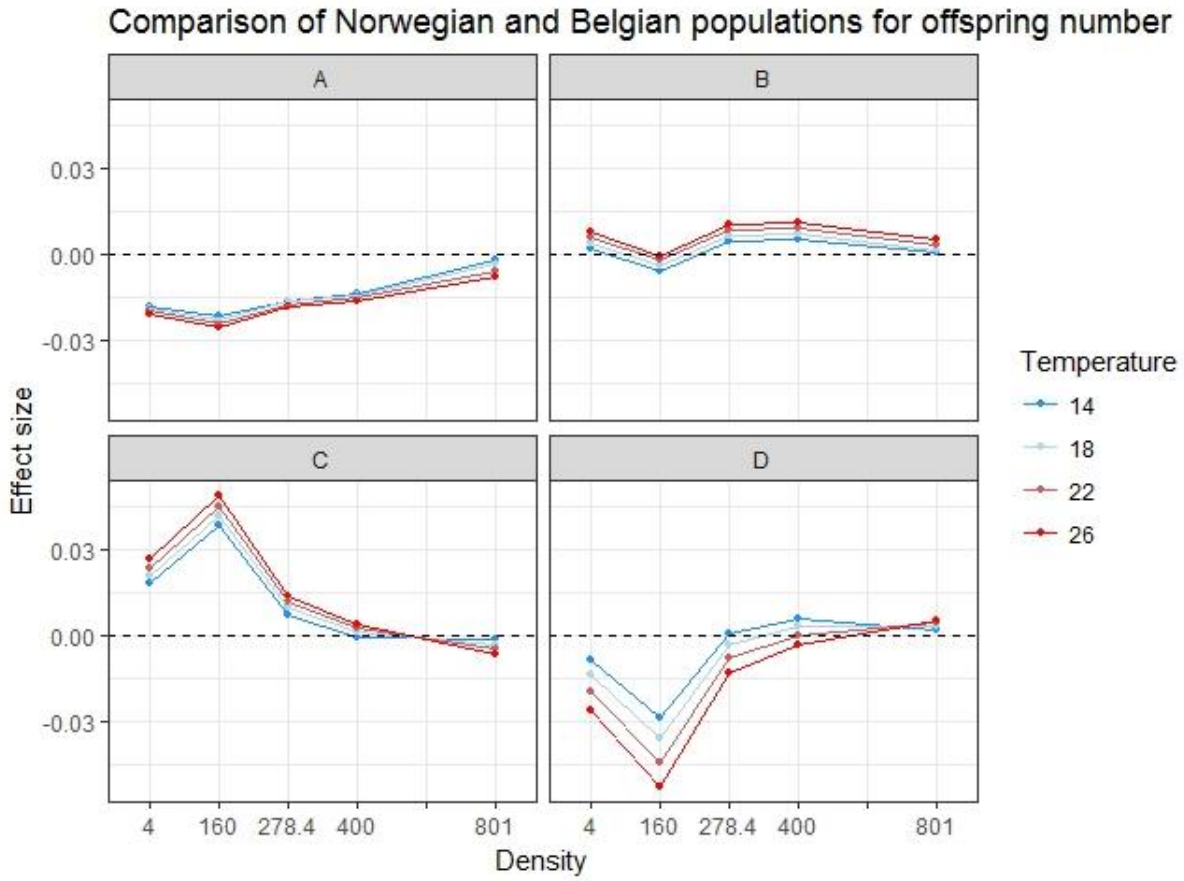

Figure 27: Visualization of LTRE-like analysis for offspring number of all populations to a total average population at the four temperature treatments: 14°C (blue), 18°C (light blue), 22°C (light red) and 26°C (red). The y-axis gives the effect size on lambda of the average population when changing the average offspring number kernel by the offspring number kernel of one of the four populations.

By replacing the average vital rate of offspring size with population-specific ones, we found in general that the changes in lambda are much smaller for offspring size than for any other vital rate (Fig. 28). Both Norwegian populations A and B showed negative impacts on lambda across the density range, independent of thermal regime. On the contrary, the two Belgian populations C and D showed positive effects on lambda across the density range. Overall, the warmest thermal regime had the largest effect on lambda, whether it was negative or positive. Furthermore, it seems that the pattern in population A and C and population B and D mirror each other.

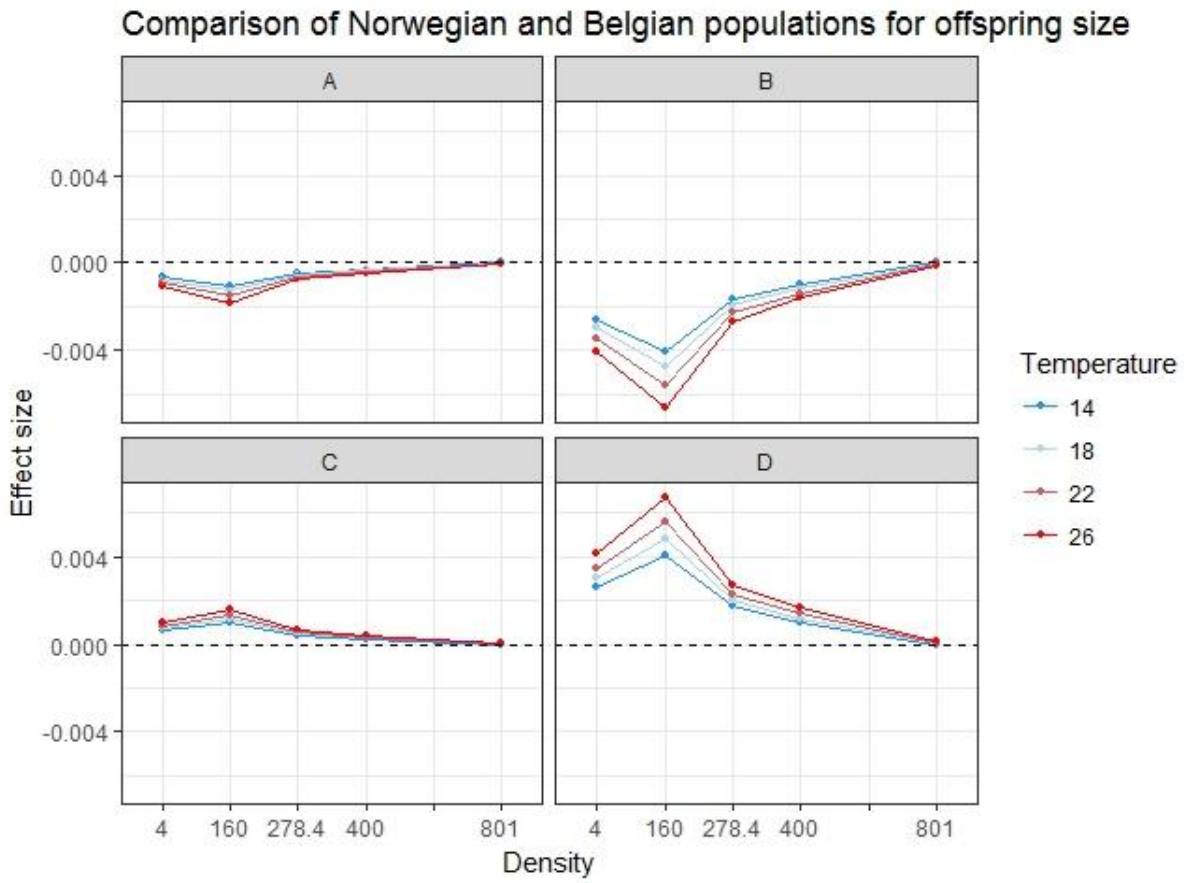

Figure 28: Visualization of LTRE-like analysis for offspring size rate of all populations to a total average population at the four temperature treatments: 14°C (blue), 18°C (light blue), 22°C (light red) and 26°C (red). The y-axis gives the effect size on lambda of the average population when changing the average offspring size kernel by the offspring size kernel of one of the four populations.

Generally, replacing average vital rates with any of the population-specific vital rates of the Norwegian population A has a negative impact on lambda. This is not the case for the other three populations. For the Norwegian population B, the vital rates for growth and offspring size always negatively impact lambda, while for survival and offspring number there is a positive impact but only at higher densities. The Belgian population C, generally, has a positive effect on lambda, except when using its vital rate of growth. However, this positive effect mostly disappears at higher densities. Last, the Belgian population D has mostly a positive impact on lambda. Only for the vital rate offspring number at low densities, there is a negative effect.

4.4. Within-population differences in thermal response

4.4.1. Clonal difference in vital rates

Figures 29-33 depict the vital rates of each clonal line and each temperature per population at an average density. Survival is mostly highest at the lower temperatures, except for the clones of population A. The pattern in offspring size in all populations is more organized by

temperature and not so much by clone. Larger offspring is produced at the highest temperature. The number of offspring in populations A and C is higher and occur at larger sizes that in populations B and D.

Population A consist of three clones: A2, A3 and A5 (Fig. 29). In general, the vital rates of all clones of population A are the highest under higher temperatures. The survival and number of offspring of clone A5 is the highest and that of clone A2 is the lowest. The pattern in somatic growth is less clear as there is a stronger overlap between clones at the different temperatures. For this vital rate clone A5 at the lowest temperature (14°C) has the lowest somatic growth rate, while clone A3 at the highest temperature (26°C) has the highest somatic growth rate. Offspring size is highest under the warmest thermal regime and keeps on increasing as size increases. All vital rates reach the highest values under higher thermal regimes and are at their lowest under colder temperatures.

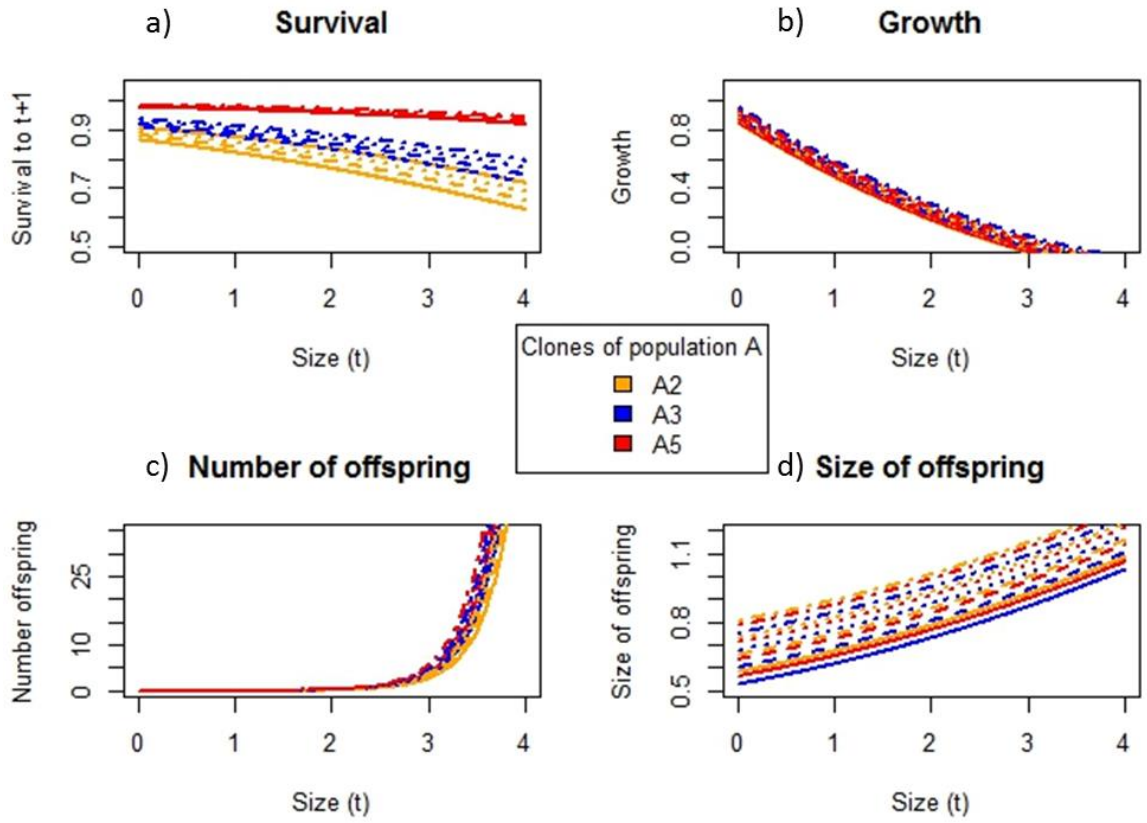

Figure 29: Visualization of vital rates a) survival, b) somatic growth, c) offspring number and d) offspring size. This is done for the clones of population A: A2 (yellow), A3 (blue) and A5 (red). The different thermal regimes are indicated by line type: 14°C (full line), 18°C (striped line), 22°C (dotted line), 26°C (striped-dotted line)

Survival of the clones in population B (Fig. 30) is highest in clone B1, slightly lower in B4 and lowest for clone B2. All clones show lowest survival in the warmest thermal regime. Clone B2 also has a lower somatic growth rate compared to the other two clones, with clone B4 the one

with highest somatic growth rate, and this independent of thermal regime. Clone B1 produces the most offspring under the warmest thermal regime, but this decreases fast under colder temperatures. The other two clones B2 and B4, have approximately equal clutch size independent of thermal regime. The size of offspring is largest in clone B2. Offspring size and number is highest under warmer thermal regimes, and this for all clones.

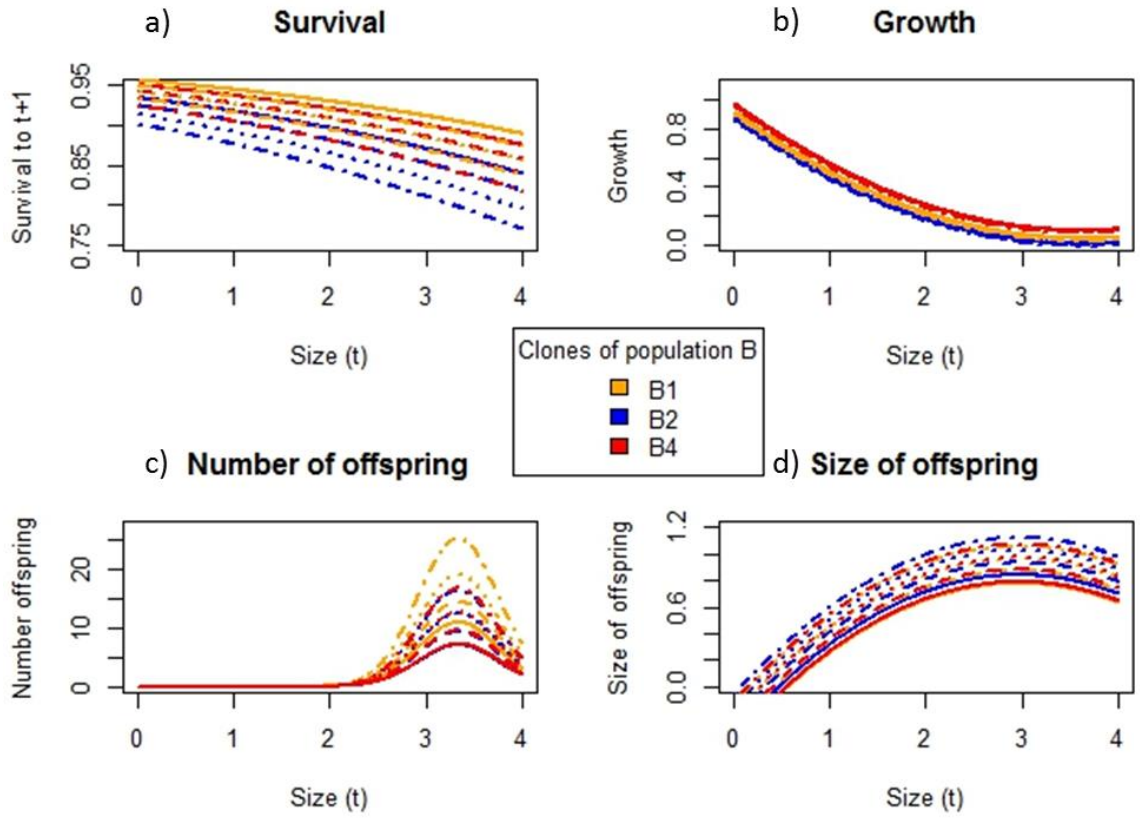

Figure 30: Visualization of vital rates a) survival, b) somatic growth, c) offspring number and d) offspring size. This is done for the clones of population B: B1 (yellow), B2 (blue) and B4 (red). The different thermal regimes are indicated by line type: 14°C (full line), 18°C (striped line), 22°C (dotted line), 26°C (striped-dotted line)

For population C there is less difference between clones for the four vital rates (Fig. 31). The survival of all three clones is very similar to one another. It is highest at 14°C in all clones, with the survival of clone C4 being slightly lower than the other two clonal lines. A similar pattern emerges for the somatic growth. For vital rates offspring number and size, there is a clear difference between thermal regimes, but less so between clones. Both vital rates are lowest at 14°C, and this for all clones.

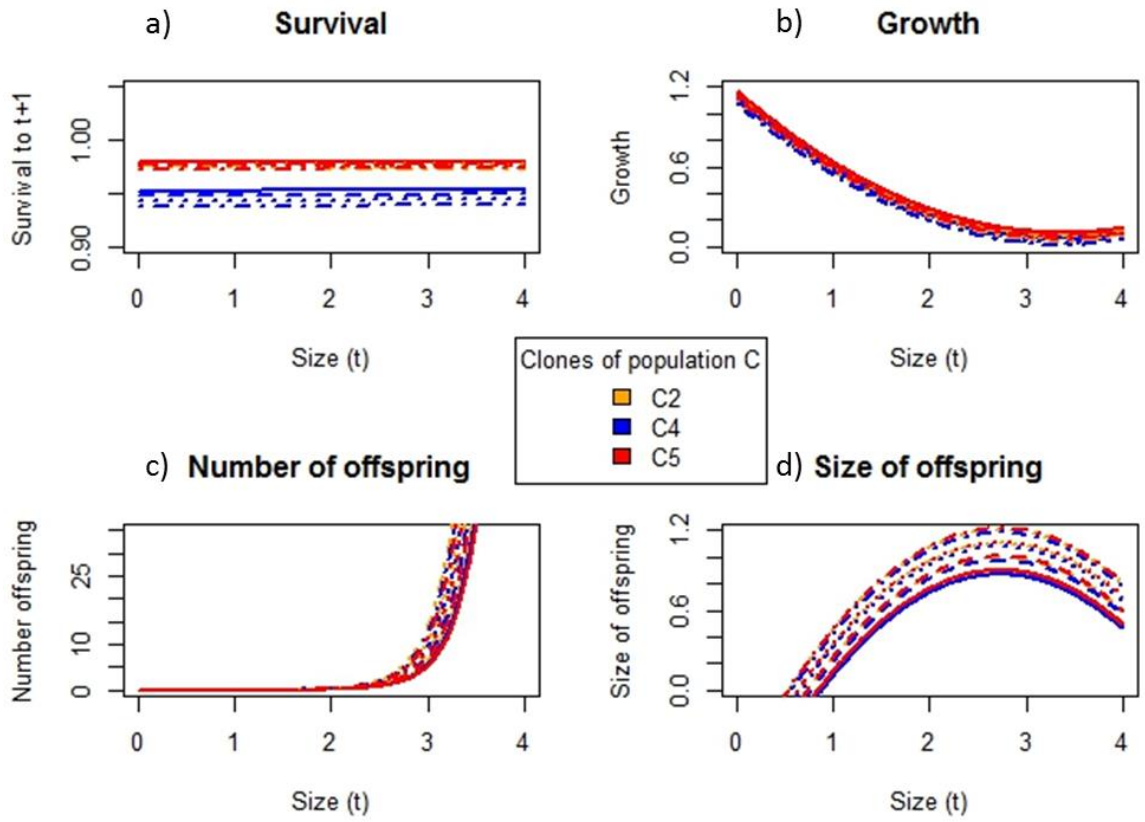

Figure 31: Visualization of vital rates a) survival, b) somatic growth, c) offspring number and d) offspring size. This is done for the clones of population C: C2 (yellow), C4 (blue) and C5 (red). The different thermal regimes are indicated by line type: 14°C (full line), 18°C (striped line), 22°C (dotted line), 26°C (striped-dotted line)

Contrary to previous results for survival, survival of clones in population D increase as the size increase. Lowest survival rates are found for the warmest thermal regime 26°C (Fig. 32). The survival of clone D2 is lowest at highest temperature, but becomes equal to the survival rate of clone D3 at colder temperatures, where clone D3 has the highest survival at each thermal regime. There is almost no variation in somatic growth among these clonal lines and among thermal regimes. Clone D2 produces the fewest offspring in all thermal regimes, whereas clone D3 produces similar but slightly more offspring than clone D1. The offspring size is again lowest for clone D2. However, for offspring size, clone D1 produces slightly larger offspring compared to clone D3. Offspring number is highest in all clones under the lower temperatures and it decreases as temperature increases. This is the opposite of the trend in offspring size, which is lowest under low temperatures and increases with temperature.

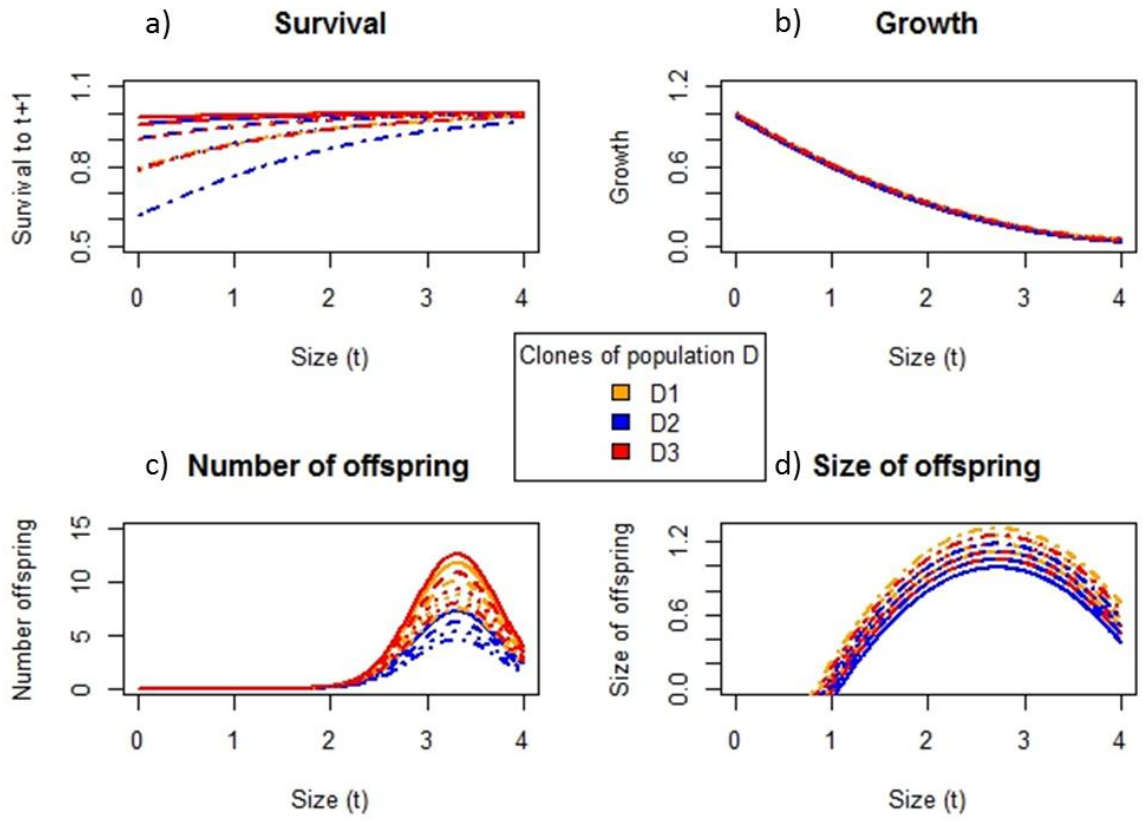

Figure 32: Visualization of vital rates a) survival, b) somatic growth, c) offspring number and d) offspring size. This is done for the clones of population D: D1 (yellow), D2 (blue) and D3 (red). The different thermal regimes are indicated by line type: 14°C (full line), 18°C (striped line), 22°C (dotted line), 26°C (striped-dotted line)

4.4.2. Effects of clonal kernels on lambda

Similar as previously we performed an LTRE-like analysis to determine clonal effects on the population average intrinsic population growth. For this analysis, we calculated lambdas for each clonal line and thermal regime over the same range of densities as used before.

We tested the effect of clone-specific vital rates on average within-population growth rate, as shown in figures 33-36. Overall, we observed that the effect of survival in relation to density varied across population and that the effect of survival in population D looks drastically different than the other populations. The differences in growth and reproduction among the clones of the same populations decrease as the density increases.

Only by replacing the offspring size kernel of clone A2 with the average offspring size kernel of population A, will positively affect the average intrinsic population growth. The other vital rate kernels of this clone have a negative effect on average lambda. Clone A3 has a positive influence on the average population growth via its growth and offspring number kernel, but a negative effect via its survival and offspring size kernel. The effect of both the survival and offspring number from clone A5 is positive, while the effect of the growth is negative, and the

effect of offspring size approximately neutral. (Fig. 33). The effects in survival are the highest in the lowest thermal regime and the intensity of the effects increase as density increases. This is in contrast with the effects of somatic growth, offspring number and size that are the largest under warmer thermal regimes.

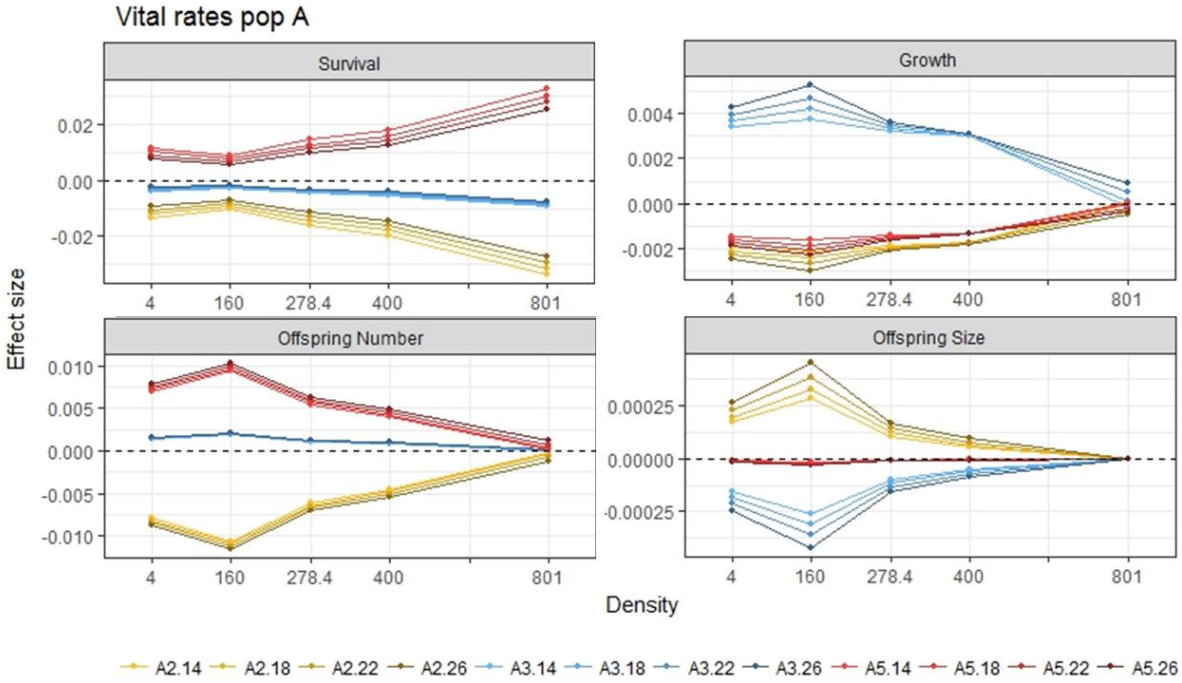

Figure 33: Visualization of LTRE-like analysis for the clones of population A. The clones are indicated in color: A2 (yellow), A3 (blue) and A5 (red). As the color darkens, the thermal regime increases. The y-axis gives the effect size on lambda of the average population when changing the average vital rate kernel by the vital rate kernel of one of the clones.

For population B, both the survival and the offspring number kernel of clone B1 have a positive effect on lambda, while its growth and offspring size kernels have a negative effect (Fig. 34). Replacing the average survival and offspring number kernel by the kernels of clone B2 affect the average population growth in a positive way. A negative effect is found when replacing the growth and offspring size kernel. The offspring size kernel of clone B4 is the only vital rate kernel that causes an increase in average population growth, the other vital rate kernels cause a decrease. Overall, all the effects on the average population growth rate, be they positive or negative, are strongest for the warmer thermal regime.

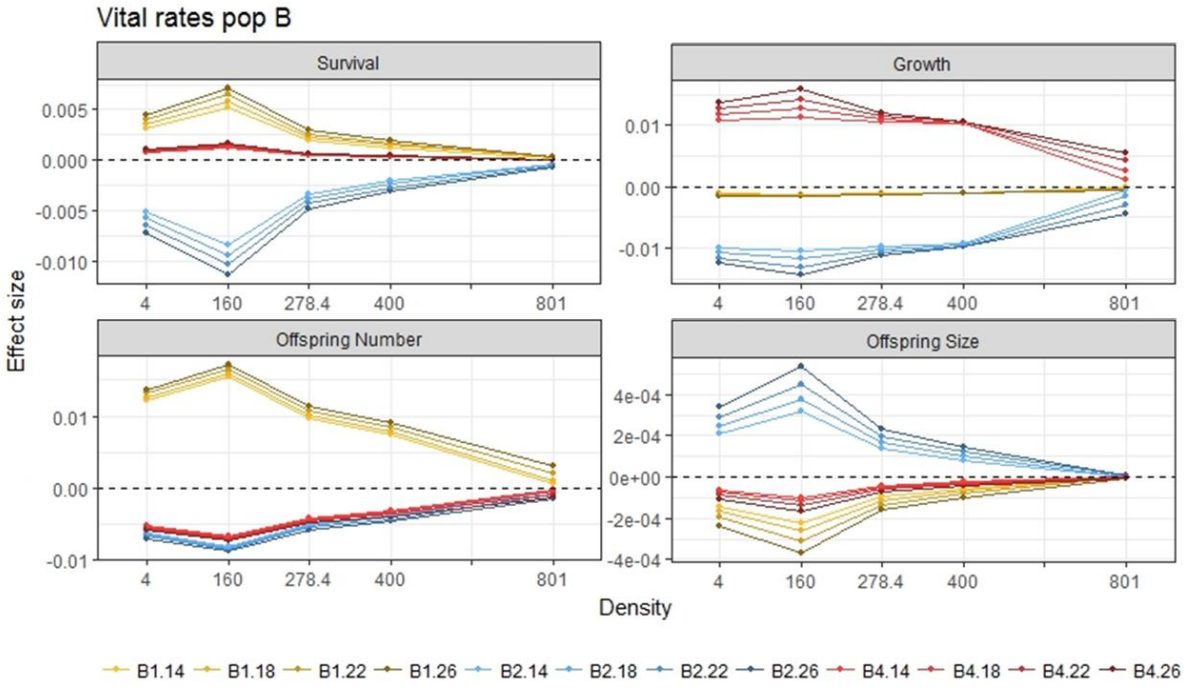

Figure 34: Visualization of LTRE-like analysis for the clones of population B. The clones are indicated in color: B1 (yellow), B2 (blue) and B4 (red). As the color darkens, the thermal regime increases. The y-axis gives the effect size on lambda of the average population when changing the average vital rate kernel by the vital rate kernel of one of the clones.

Replacing any average vital rate kernel with a kernel of clone C2 results in a higher population growth (Fig. 35). Clone C4 only has a positive effect when we use its kernel for offspring number. The other kernels cause a negative effect on the population growth. The effect of clone C5 is positive for the survival, somatic growth and offspring size kernels and negative for the offspring number kernel. All the vital rates are affecting the average population growth the most at higher temperatures.

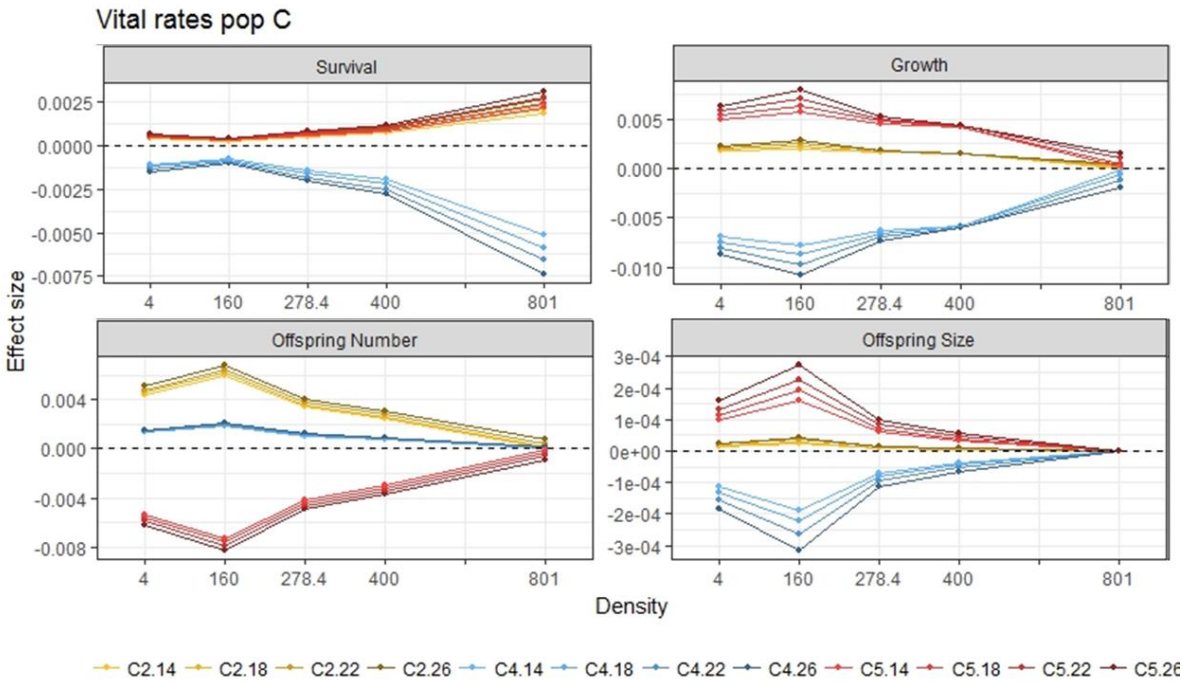

Figure 35: Visualization of LTRE-like analysis for the clones of population C. The clones are indicated in color: C2 (yellow), C4 (blue) and C5 (red). As the color darkens, the thermal regime increases. The y-axis gives the effect size on lambda of the average population when changing the average vital rate kernel by the vital rate kernel of one of the clones.

Replacing the average vital kernels with any of the vital kernels of clones D1 and D3 always has a positive effect on the population growth rate. The effect of clone D1 is highest for survival, growth and offspring size, while the effect of offspring number is largest for clone D3. Using the vital rate kernels of clone D2 results in a negative effect on the population growth rate. The effects of all vital rates of all clones are the highest under warmer thermal regimes.

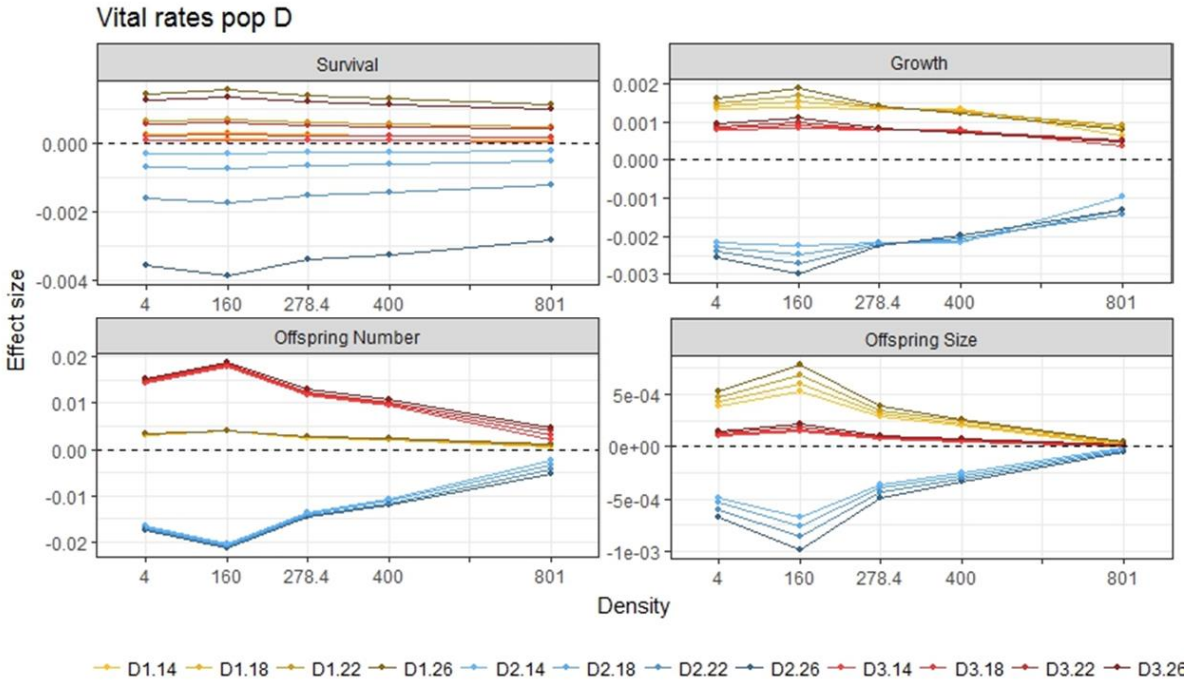

Figure 36: Visualization of LTRE-like analysis for the clones of population D. The clones are indicated in color: D1 (yellow), D2 (blue) and D3 (red). As the color darkens, the thermal regime increases. The y-axis gives the effect size on lambda of the average population when changing the average vital rate kernel by the vital rate kernel of one of the clones.

Finally, we calculated lambdas for each clonal line at each temperature treatment (Table 2). In a last step, we analyzed the variance in lambda within clones of the same population and across the populations to distinguish which is larger: intra- or inter-population variance. A linear model with nested design was made, while also accounting for temperature. The variance components were extracted and used to calculate the relative variance components. Most of the variation was unexplained, but 1% was explained. Clonal variation accounted 5.15e-04 of the total absolute variation, population explained 0.29e-04 and temperature 0.55e-04.

Table 2: Lambdas of each clonal line per population for all temperatures at an average population density.

	Population A				Population B			
	A	A2	A3	A5	B	B1	B2	B4
14	1.087	1.064	1.088	1.105	1.107	1.118	1.089	1.114
18	1.098	1.075	1.099	1.114	1.111	1.122	1.091	1.117
22	1.108	1.087	1.110	1.123	1.114	1.126	1.094	1.121
26	1.118	1.098	1.120	1.133	1.118	1.131	1.096	1.125
	Population C				Population D			
	C	C2	C4	C5	D	D1	D2	D3
14	1.120	1.125	1.113	1.121	1.152	1.156	1.136	1.164
18	1.127	1.133	1.120	1.128	1.152	1.157	1.136	1.165
22	1.135	1.141	1.127	1.136	1.150	1.155	1.132	1.163
26	1.143	1.149	1.135	1.144	1.140	1.146	1.120	1.155

5. Discussion

Anthropogenic-induced climate change is changing our world. One of its consequences is the rapidly increasing temperatures, expected to increase even further in the near future (IPCC, 2014). Increasing temperatures have important consequences for aquatic ecosystems, and can affect both biotic and abiotic factors (Scheffers et al., 2016). We therefore performed a temperature experiment using a key stone species of aquatic ecosystems, *Daphnia magna*. We used populations originating from a northern and a southern latitude. Mean temperature and temperature extremes show a gradient over latitude, which can be used to investigate thermal responses, like we did in our experiment (Geerts et al., 2014). Our main objective was to investigate the degree of genetic differentiation in the populations' thermal response. This was broken down into three research questions. First, we established the effects of temperature and density on population dynamics looking at four vital rate functions (survival, somatic growth, offspring number and size), and found that increasing density overall had negative effects on all vital rates, except offspring size. The effect of temperature varied across the vital rates. After that, we explored the difference in thermal response between the northern and southern populations. We found that the Belgian populations always perform better compared to the Norwegian ones, irrespective of the thermal regime they were reared in. Finally, we investigated intra-population variation in thermal response among the clonal lines and found different life-history strategies in the clones, indicating genetic variation.

5.1 Effects of temperature and density on vital rates

Using regression analysis we determined the effects of temperature and density on four vital rates: survival, somatic growth, offspring number and offspring size. Both temperature and density are known to have effects on each of these vital rates. For instance, temperature has been found affecting physiological processes in aquatic ectothermic animals, such as *Daphnia* (Paul et al., 2012). For the vital rates pooled across all four populations, we found an overall positive effect of temperature on offspring number and size. The number of offspring increases along warmer thermal regimes in populations B and C, however, was found to decrease for population D. Both a positive (Martínez-Jerónimo, 2012) and negative (Heugens et al., 2006; Chen et al., 2009) relation between offspring number and temperature has been found previously. Under good conditions, the produced clutch size will be large. Because increased temperatures in nature indicate the start of spring, which are considered to be good environmental conditions for daphniids (Paul et al., 2012), larger clutch sizes can be expected with higher temperatures. However, the decrease in offspring number can be due to a swift in energy allocation from reproduction and growth to survival under higher temperatures (Heugens et al., 2006). Smaller bodied individuals produce less offspring (Glazier, 1992).

Large clutch sizes often result in on-average smaller individual offspring (Mckee & Ebert, 1996). However, we also found a positive effect of temperature on offspring size in all populations. This means that higher temperatures result in more and larger offspring when under a constant density. The larger offspring size in *D. magna* can be explained by the fact that temperature might similarly influence the body size of offspring as it influences adult body size via the temperature-size rule of Atkinson (Atkinson 1994). For the two vital rates somatic growth and survival, we did not detect an overall pattern for temperature. Only for two populations, population A and D, we found a positive effect for somatic growth rate in population A, and a negative effect for survival in population D. Temperature is known to have a positive impact on growth, where individuals realize higher growth rate as temperature rises (e.g. Temperature-Size Rule by Atkinson (1994); Martínez-Jerónimo, 2012). The negative influence of temperature on survival in population D is not what we expected, but has been previously found in the literature. With increasing temperatures, the metabolic rate becomes higher and the need for nutrients consequently rises as well, and might thus decrease the survival of the individual (Wojtal-Frankiewicz, 2012). To conclude, population-specific vital rates respond in different manners to temperature, which is a more nuanced image of population dynamics than our hypothesis that all vital rates of all populations would react similarly.

Density has also been found to impact *Daphnia* vital rates. For example, increased density has been found to reduce adult body size of *Daphnia* (Boersma et al, 1999) and to decrease *Daphnia magna*'s clutch size, due to earlier reproduction (Guisande, 1993). Increased density leads to increased effort into offspring production because crowding is associated with lower resource availability under which *D. magna* produces larger sized offspring as a stress response (Glazier, 1992). Moreover, the survival also decreases under stressful conditions, such as crowding (Jansen et al., 2011). These findings are in line with our results. We found an overall significant negative effect of density on clutch size, indicating that all four populations decreased the number of offspring with increasing densities. In addition, we found a significant positive effect of density on offspring size, indicating while less offspring was produced, they were larger. The somatic growth rate of all populations is negatively impacted by density. Only the survival of the northern populations A and B is significantly impacted and this in a negative and positive way, respectively. This indicates that the effect of density on survival appears to depend on the population. It has already been stated that the response to crowding are likely to be clone-specific (Burns, 2000), thus explaining the variation across populations. These effects combined translate into fewer, but larger offspring produced while the somatic growth rate decreases, which is reflected in our results where density negatively impacted growth, survival and number of offspring, but positively offspring size.

5.2. Differences among populations

5.2.1. Differences in density

Populations were found to differ in their realized densities, but this only at colder temperatures. At 14°C, the Norwegian population A had significantly lower density values compared to the two Belgian populations C and D, and the Norwegian population B had significantly lower density values compared to the Belgian population D. Population A has the lowest densities at 14°C, 18°C and 22°C. At higher temperatures, no density differences between populations were found. This could be because the density of the Belgian population D is negatively influenced by temperature, while no such effect was found for the other populations. As this population has the highest density at low temperatures, this might reduce the density differences between populations at higher temperatures.

5.2.2. Thermal differences

Previous studies have found that *D. magna* populations of different latitudes show adaptation to temperature (Geerts et al., 2014). For example, Geerts et al. (2014) found that southern *D. magna* populations were adapted to withstand higher temperature compared to northern populations. The temperature difference at different latitudes has also shown its effect for populations dynamics, as populations from southern latitudes are expected to mature faster (Chopelet et al., 2008). Populations originating from different latitudes can have varying thermal ranges under which they can survive, depending on their local temperature (Geerts et al., 2014) or their acclimatization temperature (Yampolsky et al., 2014a). In this thesis, we found that the relation between the intrinsic population growth rate and density is negative, but the extent of the decrease in lambda over density differs across populations and thermal regimes. The threshold at which the intrinsic population growth starts to decrease (i.e. lambda < 1) varies among populations and thermal regimes. This threshold is reached first by population A, then population C. Population B reaches this threshold only for the coldest thermal regime and population D never reaches it and thus keeps on growing in population size, although at a lower rate.

All populations, except the Belgian population D, have highest population growth at the warmest thermal regime (26°C). For population D, it varies according to the population density, where at intermediate density levels, the population has largest growth in the coldest thermal regime (14°C). This result is confirmed by the LTRE-like analysis later on, where we found that all vital rates of all populations, with the exception of survival in population D, reach the highest values under warmer temperature. A reason that population D does not have its highest intrinsic population growth at the warmest thermal regime could be that the individuals metabolic rate demands far more energy under higher temperatures (Geerts et al., 2014). This means that the energy is mostly put into survival and less in somatic growth and reproduction, thus lowering the intrinsic population growth rate (Heugens et al., 2006). We also observed that

the density of population D decreases as temperature increases, which fits into this explanation. We observe that all populations prefer warmer temperatures under the same experimental conditions, implying that the difference is rather due to phenotypic plasticity and not thermal adaptation. It could also be that the populations are thermally adapted, but to similar conditions, so there is no difference in thermal response.

Different populations have different life-history strategies. For example, the Belgian population D, overall, performs best for three out of the four vital rates (somatic growth, survival and offspring size), but worst for offspring number. On the contrary, the Norwegian population A is overall at the lower side in all its vital rates. However, these population differences are highly dependent on density. For instance, while for offspring size at low densities there are clear differences between all populations, these differences disappear at high densities. The reduction in these differences could be because some clones respond stronger to the increase in density than others, yet all respond in the same direction. For other vital rates, such as offspring number, our results show that population B and D suffer less from crowding compared to population C, which performs best under low densities, but worst under high densities. Various trade-offs between the vital rates can be distinguished. The trade-off between offspring size and number is well known and is probably also at play in *D. magna*, which can explain the observed increase in offspring size while the number declines (Glazier, 1992). This trade-off has also been established in other species, such as for birds (Lack, 1947), lizards (e.g. Sinervo & Licht, 1991) and fish (e.g. Elgar, 1990), all oviparous animals. We found this particular negative correlation in our *Daphnia* populations, as studies did before our study (Glazier, 1992; Mckee & Ebert, 1996). Another trade-off observed in both latitudes, is between growth and some offspring property (either number or size), while the effect of survival varies across populations. According to the energy allocation strategy, the available amount of material and energy is divided among physiological processes (Lika & Kooijman, 2003). Therefore, we could expect a trade-off between survival, somatic growth and reproduction, depending on the environmental conditions and food availability (Martínez-Jerónimo, 2012).

The change in intrinsic population growth due to changes offspring size is, in all populations and under all temperatures, smaller by a factor of ten in comparison to the other vital rates. This implies that either offspring size is not as important to the intrinsic population growth as the other vital rates or that it is much more constant in the populations than any other vital rate and less influenced by external factors. We did find in our results that offspring size is under significant influence of maternal size. The effect of temperature and density on offspring size could be indirect via the maternal size, thus buffering possible effects. The importance of the mothers' fitness and genotype for determining the offspring size has been stated before (Glazier, 1992; Mckee & Ebert, 1996).

Overall, Belgian populations do better than their Norwegian counterparts. It has been found that *D. magna* produced more and smaller offspring under poor conditions and larger but less offspring in good environments (Mckee & Ebert, 1996). This was also observed in our results. Both Norwegian populations produced smaller offspring, yet more offspring compared to the Belgian population D. It is, however, still less than the number of offspring produced by population C. It could be that the Norwegian populations are less stress-resistant than the Belgian populations and therefore, they experienced more stress induced by the experimental environment. We observed that certain clones, mostly from the northern populations, produced male offspring and dormant eggs during the culturing phase of the experiment, indicating that they were experiencing stress (Caceres, 1998). A stressful environment is known to influence the population dynamics of *D. magna* populations (Jansen et al., 2011). Stress experienced in the past can also influence life history along with testing thermal stress to which the individual is exposed (Jansen et al., 2011). This effect can be transferred across generations leading to adaptive responses during the testing phase of the experiment. Additionally, it has been suggested that responses to density can be clone-specific (Burns, 2000). So it could be possible that the Norwegian populations are more sensitive to crowding and have a stronger response to increased densities than the Belgian populations.

Another possible explanation for the fact that Belgian populations perform better than the Norwegian ones, could be due to the loss of genetic diversity as the distance to the ancient hotspot increases. Across Eurasia, genetic differentiation is mainly driven by geographic distance (Fields et al., 2015). There is limited evidence for the hypothesis that this hotspot might be located in the Middle East and Africa (Fields et al., 2015). Belgium is far away from this hotspot but Norway even further, which could explain why the Belgian clones perform better than the Norwegian ones. Moreover, the clones of populations A, B and D experienced additional stress of being transferred to Belgium from Norway, where they were hatched. The clones of population C had already been used before in the lab and could already be accustomed to the specific lab conditions under which we performed the experiment. The clonal lines of population C were hatched earlier than the clones of the other populations and this difference in 'age' could be carried on in differences in our results. The hatched eggs could also be produced at different time point in the season but we do know that the thermal response can differ within seasonal clones (Carvalho, 1987; Paul et al., 2012). Finally, existing in an experimental environment instead of a natural one could itself impose stress and selection on the tested organism (De Meester et al., 2011). This again could interfere with the natural response yielding in an artificial response of the test subject.

To conclude, our results are not in accordance with our expectation that the northern populations would reach higher intrinsic population growth under colder thermal regimes. Therefore, we could not provide confirmation of thermal adaptation. A possible explanation as why we did not find a thermal difference in our populations retrieved from two different

latitudes could be that the latitudes did not differ enough in their climatic conditions. The latitude of the ponds where the northern and southern populations were retrieved from is used as proxy for their climatic conditions. It is assumed that due to the different latitude, the regional climates differ, yet this assumption is not completely true (Yampolsky et al., 2014a). The climate in Flanders is classified as Cfb under the Köppen Geiger climate classification (Peel et al., 2007). Cfb is characterized by a warm temperate oceanic climate that is fully humid with a warm summer (Chen & Chen, 2013; Kottek et al., 2006). However, Trondheim has been classified into multiple categories over time. Peel et al. (2007) classified it as Dfc, while noting that the coast of southern and northwest Norway would be expected to be classified as Cfb. At that time, temperature data was missing so it was not classified as such. Dfc is described as a subarctic snow climate that is fully humid with a cool summer and cold winter (Chen & Chen, 2013; Kottek et al., 2006).

Both Kottek et al. (2006) and Rubel et al. (2017) provided kmz files that can be layered into Google Earth. These digital maps make classifying the climate of Flanders and Trondheim more easy. It is assumed that the data for the 2017 maps is more complete than the data available in 2006. Flanders has always been classified as Cfb (a and b on Fig. 38), but with increasingly available data, the classification of Trondheim changed from Dfc to Cfb (c and d on Fig 38). This thus implies that the amount of precipitation and the air temperature does not differ significantly between the two sampling latitudes and that the populations are actually adapted to similar conditions. This could lead to similar responses in experimental conditions and might explain why we did not observe significant difference between the populations originating from both latitudes. However, the Köppen Geiger climate classification is based on vegetation and only incorporates precipitation and air temperature. While those factors can be similar for the two sampling latitudes, other factors, such as hours of sunlight, altitude and local climate are not taken into account and could still differ between the two experimental latitudes.

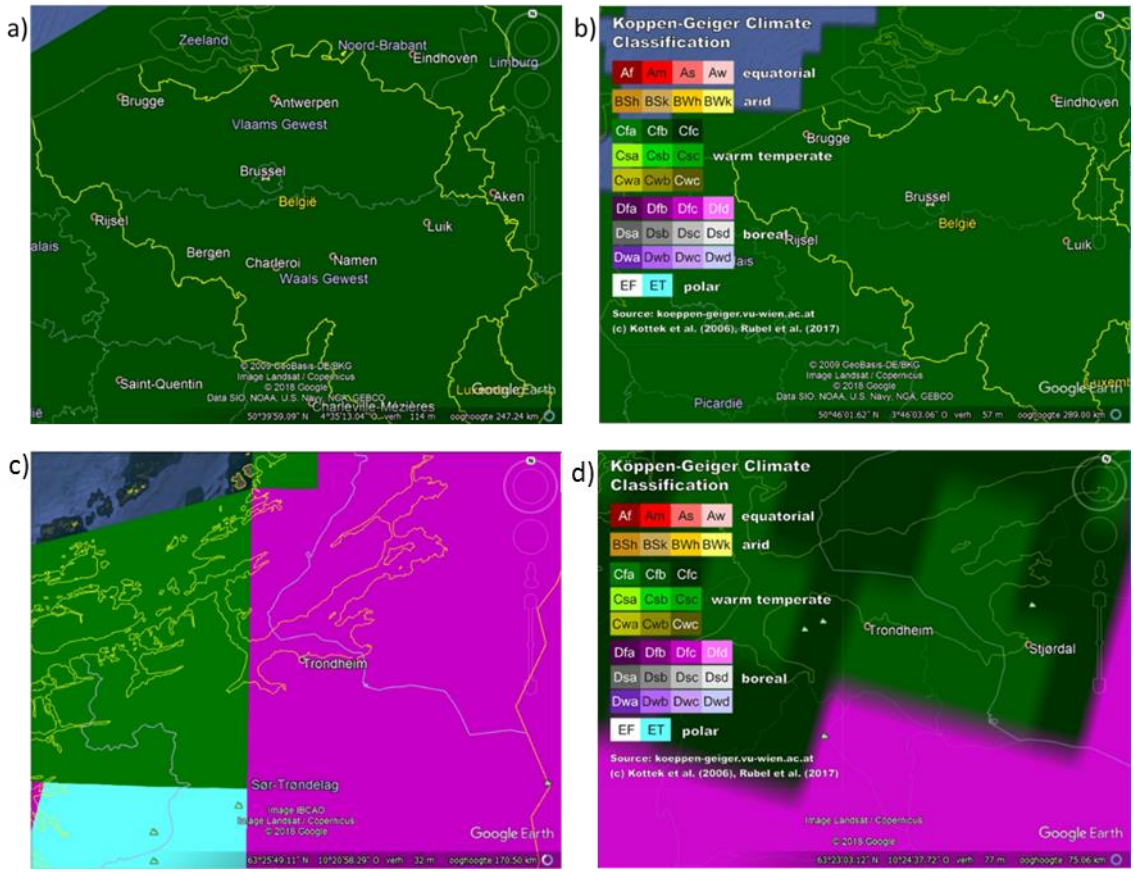

Figure 38: Köppen Geiger classification of both sampling latitudes. a) Classification of Flanders in 2006. b) Classification of Flanders in 2017. Flanders is classified as Cfb consistently classified as Cfb. c) Classification of Trondheim as Dfc in 2006. b) Classification of Trondheim as Cfb/Cfc in 2017. The maps of 2006 were retrieved from Kottek et al. (2006), the maps of 2017 from Rubel et al. (2017)

5.3. Clonal differences

Genetic variation in populations is an important mechanism to cope with climate change (Hoffmann & Sgro, 2011). Natural populations of *D. magna* have been shown to harbor substantial genetic variation for thermal tolerance (Mitchell & Lampert, 2000; Geerts et al., 2014; Fields et al., 2015). In addition, responses to density effects can be clone-specific, varying in their relation with vital rates across populations and clones (Burns, 2000). We therefore used three different clones from each population in our experiment to detect how much genetic variation in the vital rates there is in response to the different thermal regimes and density changes (Geerts et al., 2014). Growth and reproduction decrease as density increases, but the relation of survival with density changes depending on the population to which the clonal line belongs.

More specific, based on our LTRE-like analysis, we could detect multiple life-history strategies among the clones, indicating that populations possess genetic variation for life-history strategies. There are some clones who always perform worse (e.g. clone D2) or better (e.g. clone D1) than the average clone within their population. These strategies are unlikely to occur under natural conditions. One would expect that the 'loser' clone would go extinct and only the

'Darwinian Demon' would remain, heavily reducing genetic diversity (Law, 1979). However, it is important to note that these results are obtained from monocultures and thus we lack information about the competition qualities of each clone. In natural settings, populations consist of multiple clonal lines occurring at the same time, and this can influence the results. Overall, the strongest differences between clones occur at lower densities, while the clonal differences reduce with higher densities, indicating that at high densities, multiple clones might be able to coexist.

In a way, we can say that there was bias in clonal selection. Initially, five clonal lines per populations were reared of which three were selected to use in the experiment. The clones we selected were the ones that were capable of living in an experimental environment. This to avoid clones that were unable to cope with experimental conditions and might have gone extinct during the experiment (Yampolsky, et al., 2014a).

Finally, we analyzed the variation in lambda among and between populations to examine where the largest portion of variance derives from. We found that the variation between clones of the same population was larger than the variation due to temperature. The variation that was due solely to the population itself was the smallest. This result indicate that the differences between population are mainly caused by variation among the clones of the same population, so the genetic variation within a population. Within-population variation in clones of *D. magna* is often found in research (Mitchell & Lampert, 2000; Fields et al., 2015).

5.4. Integral projection models as an analytical tool

In this thesis, integral projection modelling (IPM) was used as an analytical tool to investigate the effects of temperature and density to population dynamics of four populations originating from two different latitudes. IPMs have been increasingly used during the last decades, and have more extensive possibilities than traditional population models, because they do not require to divide the population in discrete stages (Easterling et al., 2000; Merow et al., 2014) and have been used to tackle all sorts of population analysis such as density dependence, complex life cycles, immigration and emigration … (Coulson, 2012; Rees et al., 2014). Because IPMs use regression models, they have the advantage that environmental factors (e.g. hours of sunlight, precipitation,…) but also other factors affecting demographic rates (e.g. population density, clone, body mass, infection,…) and even random factors can be included in the model. The influence of exploratory variables on the population dynamics can be taken into account when explaining the underlying mechanisms of the observed trends. The relation between the factors and the vital rate can be modelled as linear or non-linear, and as additive or non-additive (Coulson, 2012). In our model, we tried to include a quadratic effect of temperature, because this would yield a more accurate relation of temperature and the vital rates. We were, however,

unsuccessful in modelling this quadratic effect due to the way our data was collected, thus excluding the possible presence of a thermal optimum.

A practical advantage of including all these factors in one regression model for each vital rate, is that shifts between populations and thermal regimes can be examined by simply filling out different variables in the regression function. One does not have to divide the dataset into separate subgroups to calculate for different temperatures and populations. By including all factors into one regression model for each vital rate, we retain the statistical power of the complete dataset.

Deriving eigenvalues from the population matrix, we could estimate population growth (lambda) for each of the four population at the different thermal regimes, and detect whether populations differed in their intrinsic growth rate. Using the Life Table Response Experiment (LTRE)-like analysis, we were able to identify differences in vital rates between populations, but also between clones within populations, and how these differences alter population growth rate. We were also capable of exploring the relation between the intrinsic population rate, calculated based on the IPMs, and temperature or density.

To conclude, IPMs allowed insights in the population dynamics of the different populations under varying thermal regimes. Moreover, they not only allow to represent the population dynamics based on the observed data, but also to make predictions under different temperature or density scenarios. Making predictions is an important feature of IPMs and can deal with sparse data by interpolating the missing data (Merow et al., 2014). A downside is that the quality of the model greatly depends on the quality of the obtained data and the regression models, because the model makes an abstraction of the data (Coulson, 2012). Consequently, the selection of the best fitted regression model is of high priority. Choosing the wrong model could lead to erroneous predictions. Validating predictions can be done by using part of the data to build to model and then verify if the predicted values obtained with the model correspond with the unused, observed data. A method to increase the goodness of the model is bootstrapping the data. Bootstrapping calculates standard errors for the lambdas that increase certainty in the results (Rees et al., 2014).

6. Conclusion and future perspectives

The main objective of this thesis was to investigate the degree of genetic differentiation in thermal response of natural populations. It is important to strive for more in-depth knowledge about thermal responses in face of the looming threat of climate change. We used a keystone species as test subject, because changes in their behavior, morphology, physiology and so on, will likely impact other species in the community. In this thesis, we attempted to unravel population dynamics of populations from different latitudes. Although we did not find confirmation of thermal adaptation, our method showed the capability of doing so.

The first research question examined the effect of factors, in particular temperature and density, on the vital rates. We found that the effect of temperature varied across vital rates and populations. It was positive for somatic growth and offspring size, negative in survival and varied for offspring number. The effect of increasing density was overall negative, except for offspring size.

The second research question explores differences between the populations, both in density and thermal differences. We found that differences in density manifested themselves only at low temperatures. The populations showed varying degrees of temperature- and density dependence, which we were able to detect. The results provided no support for thermal adaptation, contrary to our expectations. All populations, except population D, performed better under higher temperatures. Throughout our results, we observed that population D frequently exhibits different patterns than the other populations. Looking at the effect of vital rates of a specific population, temperature and density revealed that the populations employ different life-history strategies. Overall, we found that the Belgian populations do better than the Norwegian populations. We were able to detect differences between all populations based on their modelled vital rates.

Our third research question concerns the differences among clones of the same population in their thermal tolerance. We also investigated the variation division of the lambdas of each clone and at each temperature. Clones of the same population differed from each other in their life-history strategy. Some strategies were unlikely to occur naturally and the same trade-offs as in the population differences were found. Variation in life-history strategies indicate the presence of genetic variation in the population, which is important for the evolutionary potential of the population. From the variation analysis, we detected variation in intrinsic population growth can be explained more by variation within the population than the variation across population or by temperature.

Lastly, we evaluated the use of Integral Projection Models in this type of research. We felt that IPMs are much more extensive than matrix population models. They are highly adaptable to many types of populations and can be modelled as desired. By manually changing average population kernels by population-specific kernels, we could identify which kernel from which

population and under which thermal regime and density caused the largest change in the average intrinsic population growth rate.

Although pools, ponds and lakes could be considered as model systems, results from this experiment cannot be considered as substitutes for natural conditions (De Meester et al., 2005). The experiment we conducted only exposed *D. magna* to a limited and controlled number of stressors (i.e. temperature and crowding). While the experiment is not a true replication of the natural situation, it did provide a better understanding on how natural D. magna populations of different latitudes vary in their response to temperature and crowding and how these stressors further alter population dynamics. In natural systems, however, many more stressors are present (Jansen et al., 2011), and these might also interact with each other, creating a complex feedback system, which is very difficult (maybe even impossible) to replicate in an experiment. While our results do not reflect a true natural situations, they show that populations harbor genetic and phenotypic (via phenotypic plasticity to temperature and crowding) variation in their vital rates. In this thesis, due to time limits, we could only analyzed populations consisting of one clonal line. Future analysis on the mixed populations will provide additional information on how clones originating from the same population interact and alter population dynamics, but also which clone is the superior competitor. Additional research is needed for a more complete picture of population dynamics in natural settings, at least for this species.

Future perspectives

Our experiment exposed *D.magna* populations to their immediate final temperature starting from the acclimatization temperature of 20°C. Sudden heath shock is very artificial and in natural conditions such a warming would occur gradually (De Meester et al., 2011). This discrepancy between the experimental and natural process of warming could result in a different response to the imposed selection in the lab than the response that would occur naturally. We therefore suggest to gradually increase the acclimatization temperature to the experimental temperature to simulate gradual warming

In this thesis, we only analyzed the data retrieved from the monocultures. A step to a more natural-like system is the analysis of the mixed cultures, where competition between clones of the same or of different populations is also at play. But to understand those interactions better, the populations themselves need to be understood first, which is what we did in this thesis.

Due to unforeseen circumstances during the experiment, a generalist parasite was found to affect all populations. This additional stress likely influences the population dynamics of clonal lines that are already under thermal stress. The susceptibility of *Daphnia* to a parasite is genetically determined with minimal influence of plasticity (Miner et al., 2012). Organisms facing multiple stressors, in this case thermal stress and parasitic infection, have an increased

risk of mortality. We tried to minimize the effect of the infection by using the data from before the infection was noticed. But it could still be that clonal lines, already under heavy thermal stress, could have higher mortality due to the combination with infection. The interaction between parasite and *D. magna* is interesting to study, as well as the effects of a parasitic infection on the population dynamics. But again, to better understand those interactions, the population dynamics of the uninfected populations must be examined first, which is what we did here.

We showed that IPMs are a very potent tool for unraveling population dynamics, but we believe that many more objectives can be supported by the use of IPMs. Additional research using IPMs for this purpose, to expand the current use in order to hopefully unravel even more complex pattern should be pursued. IPMs are sufficiently simple yet sophisticated to be establish as commonly used tool for this type of research.

7. References

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Addendum

Risk analysis

We mainly worked with watery media, which don't pose a risk to the handlers. We did use 70% ethanol to rewrite the labels and 100% ethanol to store the samples. Ethanol, C2H5OH (CAS 64-17-5), is a clear liquid with a characteristic odor which easily evaporates. It is a highly flammable liquid and vapor and therefore classified as a dangerous substance. Ethanol is not classified as carcinogenic nor mutagenic. It is not dangerous to the environment. The following H- and P-sentences are associated with ethanol: H225, H319, P210, P280, P305+P351 + P338, P337 +P313 and P403+235, P233, P240, P241, P242, P243, P28, P303+P361+P353, P370+P378, P403+P235 and P501.

Working with ethanol should be done under ventilated conditions, e.g. fume hood, local exhaust,… and in the absence of ignition sources, e.g. open flames, heat, hot surfaces, sparks, static discharge,…. No smoking is allowed in the neighborhood and measures to avoid the build-up of static charges should be taken, e.g. containers should be earthed, during liquid transfer.

Routes of exposure can be inhalation, ingestion or skin/eye contact.

Ethanol can cause serious eye irritation and can be irritant to the skin. Working safely with ethanol therefore requires protective anti-static cloths such as a lab coat, safety goggles and nitrile gloves. When in contact with skin, rinsing off should be sufficient to avoid damage. Ethanol should be stored in a cool and well ventilated area. The containers should be tightly closed. Ethanol is incompatible with alkali metals, oxidizing agents and peroxides and can form violent reactions. Examples are nitric acid, silver nitrate, mercuric nitrate, or magnesium perchlorate. Addition of alcohols to highly concentrate hydrogen peroxide forms powerful explosives. Explodes on contact with calcium hypochlorite.

Supplementary tables for model selection

Table S1: Model selection for the growth vital rate regressions based on AIC.

Model	ΔAIC
size (t+1) ~ size (t) + size² (t) + temp + dens + popu + temp:dens + (1|popu:clone)	**0 (-567.7144)**
size (t+1) ~ size (t) + size² (t) + temp + dens + popu + (1|popu:clone)	5.5792
size (t+1) ~ size (t) + size² (t) + temp + dens + popu + temp:dens + temp :popu (1|popu:clone)	16.2513
size (t+1) ~ size (t) + temp + dens + popu + temp:dens + (1|popu:clone)	16.3267

size (t+1) ~ size (t) + size² (t) + temp + dens + popu + temp:dens + dens :popu + (1|popu:clone)	20.8725
size (t+1) ~ size (t) + temp + dens + popu + (1|popu:clone)	21.5884
size (t+1) ~ size (t) + size² (t) + temp + dens + popu + temp:popu + (1|popu:clone)	24.9449
size (t+1) ~ size (t) + size² (t) + temp + dens + popu + dens:popu + (1|popu:clone)	25.9549
size (t+1) ~ size (t) + temp + dens + popu + temp:dens + temp:popu + (1|popu:clone)	33.8035
size (t+1) ~ size (t) + temp + dens + popu + temp:dens + dens:popu + (1|popu:clone)	36.2902
size (t+1) ~ size (t) + size² (t) + temp + dens + popu + temp:dens+ dens:popu + temp:popu + (1|popu:clone)	36.5272
size (t+1) ~ size (t) + temp + dens + popu + dens:popu +(1|popu:clone)	41.2906
size (t+1) ~ size (t) + temp + dens + popu + temp:popu + (1|popu:clone)	42.0958
size (t+1) ~ size (t) + size² (t) + temp + dens + popu + dens:popu + temp:popu + (1|popu:clone)	44.7746
size (t+1) ~ size (t) + temp + dens + popu + temp:dens+ dens:popu + temp:popu + (1|popu:clone)	53.1332
size (t+1) ~ size (t) + temp + dens + popu + dens:popu + temp:popu + (1|popu:clone)	61.2628

Table S2: Model selection for the survival vital rate regressions based on AIC.

Model	ΔAIC
surv ~ size (t) + temp + dens + popu + dens:popu + temp:popu + (1|popu:clone)	**0 (692.3381)**
surv ~ size (t) + size² (t) + temp + dens + popu + dens:popu + temp:popu + (1|popu:clone)	0.1687
surv ~ size (t) + temp + dens + popu + temp:dens + dens:popu + temp:popu + (1|popu:clone)	1.6563
surv ~ size (t) + size² (t) + temp + dens + popu + temp:dens + dens:popu + temp:popu + (1|popu:clone)	1.832
surv ~ size (t) + temp + dens + popu + dens:popu + (1|popu:clone)	2.5221
surv ~ size (t) + size² (t) + temp + dens + popu + dens:popu + (1|popu:clone)	2.6775

surv ~ size (t) + temp + dens + popu + temp:dens + dens:popu + (1|popu:clone)	4.2448
surv ~ size (t) + size² (t) + temp + dens + popu + temp:dens + dens:popu + (1|popu:clone)	4.3915
surv ~ size (t) + size² (t) + temp + dens + popu + temp:popu + (1|popu:clone)	11.2387
surv ~ size (t) + temp + dens + popu + temp:popu + (1|popu:clone)	12.1816
surv ~ size (t) + size² (t) + temp + dens + popu + temp:dens+ temp:popu + (1|popu:clone)	12.8908
surv ~ size (t) + size² (t) + temp + dens + popu + (1|popu:clone)	13.4276
surv ~ size (t) + temp + dens + popu + temp:dens + temp:popu + (1|popu:clone)	13.8159
surv ~ size (t) + temp + dens + popu + (1|popu:clone)	14.3239
surv ~ size (t) + size² (t) + temp + dens + popu + temp:dens + (1|popu:clone)	15.3229
surv ~ size (t) + temp + dens + popu + temp:dens + (1|popu:clone)	16.2146

Table S3: Model selection for the number of offspring vital rate regressions based on AIC. All models without size² showed significantly higher AIC values, so they are not included into this table.

Model	ΔAIC
offspring ~ size (t) + size² (t) + temp + dens + popu + temp:dens + dens:popu + temp:popu + (1|popu:clone)	**0 (4380.981)**
offspring ~ size (t) + size² (t) + temp + dens + popu + temp:dens + dens:popu + (1|popu:clone)	2.591
offspring ~ size (t) + size² (t) + temp + dens + popu + dens:popu + temp:popu + (1|popu:clone)	17.337
offspring ~ size (t) + size² (t) + temp + dens + popu + dens:popu + (1|popu:clone)	21.632
offspring ~ size (t) + size² (t) + temp + dens + popu + temp:dens + temp :popu + (1|popu:clone)	53.029
offspring ~ size (t) + size² (t) + temp + dens + popu + temp:dens + (1|popu:clone)	58.393
offspring ~ size (t) + size² (t) + temp + dens + popu + temp:popu + (1|popu:clone)	66.984
offspring ~ size (t) + size² (t) + temp + dens + popu + (1|popu:clone)	71.626

Table S4: Model selection for the offspring size vital rate based on AIC.

Model	ΔAIC
sizeoff ~ size(t) + size²(t) + temp + dens + popu + (1|popu:clone)	**0 (-105.070)**
sizeoff ~ size(t) + temp + dens + popu + (1|popu:clone)	0.374
sizeoff ~ size(t) + size²(t) + temp + dens + popu + temp:dens + (1|popu:clone)	6.312
sizeoff ~ size(t) + temp + dens + popu + temp:dens + (1|popu:clone)	7.316
sizeoff ~ size(t) + size²(t) + temp + dens + popu + temp:popu + (1|popu:clone)	17.288
sizeoff ~ size(t) + temp + dens + popu + temp:popu + (1|popu:clone)	18.816
sizeoff ~ size(t) + size²(t) + temp + dens + popu + dens:popu + (1|popu:clone)	19.918
sizeoff ~ size(t) + temp + dens + popu + dens:popu + (1|popu:clone)	20.296
sizeoff ~ size(t) + size²(t) + temp + dens + popu + temp:dens + temp:popu + (1|popu:clone)	23.047
sizeoff ~ size(t) + temp + dens + popu + temp:dens + temp:popu + (1|popu:clone)	25.486
sizeoff ~ size(t) + size²(t) + temp + dens + popu + temp:dens + dens:popu + (1|popu:clone)	26.416
sizeoff ~ size(t) + temp + dens + popu + temp:dens + dens:popu + (1|popu:clone)	27.435
sizeoff ~ size(t) + size²(t) + temp + dens + popu + dens:popu + temp:popu + (1|popu:clone)	37.864
sizeoff ~ size(t) + temp + dens + popu + dens:popu + temp:popu + (1|popu:clone)	39.421
sizeoff ~ size(t) + size²(t) + temp + dens + popu + temp :dens + dens:popu + temp:popu + (1|popu:clone)	43.655
sizeoff ~ size(t) + temp + dens + popu + temp :dens + dens:popu + temp:popu + (1|popu:clone)	46.198

Full summary of regression models

Growth

```
Linear mixed model fit by REML
t-tests use  Satterthwaite approximations to degrees of freedom ['lmerMod']
```

```
Formula: GR ~ size + size2 + popu + temp + dens + temp:dens + (1 | popu:clo
ne)
   Data: df

REML criterion at convergence: -589.7

Scaled residuals:
    Min      1Q  Median      3Q     Max
-2.7447 -0.6455 -0.1059  0.5855  4.0784

Random effects:
 Groups     Name        Variance Std.Dev.
 popu:clone (Intercept) 0.000457 0.02138
 Residual               0.033836 0.18395
Number of obs: 1198, groups:  popu:clone, 12

Fixed effects:
             Estimate Std. Error         df t value Pr(>|t|)
(Intercept)  9.571e-01  5.390e-02  5.439e+02  17.756  < 2e-16 ***
size        -4.865e-01  5.096e-02  1.185e+03  -9.547  < 2e-16 ***
size2        6.109e-02  1.208e-02  1.185e+03   5.057 4.93e-07 ***
popuB       -7.574e-03  2.344e-02  8.100e+00  -0.323  0.75479
popuC        1.553e-02  2.321e-02  7.800e+00   0.669  0.52284
popuD        8.354e-02  2.322e-02  7.800e+00   3.597  0.00731 **
temp         1.253e-04  5.419e-03  1.183e+03   0.023  0.98156
dens        -7.001e-02  5.572e-03  1.189e+03 -12.564  < 2e-16 ***
temp:dens    2.169e-02  5.372e-03  1.188e+03   4.037 5.76e-05 ***
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Correlation of Fixed Effects:
          (Intr) size   size2  popuB  popuC  popuD  temp   dens
size      -0.933
size2      0.885 -0.984
popuB     -0.226  0.006 -0.006
popuC     -0.211 -0.016  0.019  0.512
popuD     -0.216 -0.001 -0.004  0.511  0.518
temp       0.027 -0.054  0.066  0.027  0.021  0.018
dens       0.066 -0.059  0.073 -0.062 -0.097 -0.082  0.046
temp:dens -0.002  0.002  0.001 -0.017 -0.015  0.019  0.073  0.231
```

Survival

```
Generalized linear mixed model fit by maximum likelihood (Laplace Approxima
tion) ['glmerMod']
 Family: binomial  ( logit )
Formula: surv ~ size + popu + temp + dens + dens:popu + temp:popu + (1 |
popu:clone)
   Data: df
Control: glmerControl(optimizer = "bobyqa", optCtrl = list(maxfun = 2e+05))

     AIC      BIC   logLik deviance df.resid
   692.3    764.8   -332.2    664.3     1292

Scaled residuals:
    Min      1Q  Median      3Q     Max
-9.1418  0.1572  0.2300  0.3264  0.7120

Random effects:
 Groups     Name        Variance Std.Dev.
```

```
 popu:clone (Intercept) 0.09393  0.3065
Number of obs: 1306, groups:  popu:clone, 12

Fixed effects:
            Estimate Std. Error z value Pr(>|z|)
(Intercept)  2.25982    0.46425   4.868 1.13e-06 ***
size        -0.13211    0.18304  -0.722 0.470426
popuB        0.16938    0.36651   0.462 0.643985
popuC        1.42321    0.45608   3.121 0.001805 **
popuD        1.78782    0.53228   3.359 0.000783 ***
temp         0.16261    0.18023   0.902 0.366929
dens        -0.35316    0.19577  -1.804 0.071247 .
popuB:dens   1.03226    0.27427   3.764 0.000167 ***
popuC:dens  -0.08596    0.38308  -0.224 0.822460
popuD:dens   0.42845    0.36438   1.176 0.239654
popuB:temp  -0.32940    0.24469  -1.346 0.178228
popuC:temp  -0.29753    0.34826  -0.854 0.392916
popuD:temp  -1.15270    0.43862  -2.628 0.008588 **
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

Offspring number

```
Generalized linear mixed model fit by maximum likelihood (Laplace Approxima
tion) ['glmerMod']
 Family: poisson  ( log )
Formula: offspring ~ size + size2 + popu + temp + dens + temp:dens + dens:p
opu +      temp:popu + (1 | popu:clone)
   Data: df
Control: glmerControl(optimizer = "bobyqa", optCtrl = list(maxfun = 2e+05))

     AIC      BIC   logLik deviance df.resid
  4381.0   4462.4  -2174.5   4349.0     1180

Scaled residuals:
    Min      1Q  Median      3Q     Max
-4.7045 -0.7460 -0.2692 -0.0316 30.7614

Random effects:
 Groups     Name        Variance Std.Dev.
 popu:clone (Intercept) 0.02711  0.1646
Number of obs: 1196, groups:  popu:clone, 12

Fixed effects:
             Estimate Std. Error z value Pr(>|z|)
(Intercept) -18.37927    1.13772 -16.154  < 2e-16 ***
size         11.11720    0.84076  13.223  < 2e-16 ***
size2        -1.49323    0.15418  -9.685  < 2e-16 ***
popuB         0.53836    0.15992   3.366 0.000761 ***
popuC         0.60620    0.16050   3.777 0.000159 ***
popuD         0.26072    0.16005   1.629 0.103310
temp          0.10816    0.04903   2.206 0.027372 *
dens         -1.02483    0.06608 -15.510  < 2e-16 ***
temp:dens    -0.11626    0.02633  -4.416 1.00e-05 ***
popuB:dens    0.15547    0.07909   1.966 0.049327 *
popuC:dens   -0.15660    0.07848  -1.995 0.045996 *
popuD:dens    0.30079    0.07715   3.899 9.67e-05 ***
popuB:temp    0.03639    0.05731   0.635 0.525467
popuC:temp    0.03748    0.05785   0.648 0.517064
popuD:temp   -0.10486    0.05759  -1.821 0.068646 .
```

```
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

Offspring size

```
Linear mixed model fit by REML
t-tests use  Satterthwaite approximations to degrees of freedom ['lmerMod']
Formula: sizeoff ~ size + size2 + popu + temp + dens + (1 | popu:clone)
   Data: df

REML criterion at convergence: -125.1

Scaled residuals:
    Min      1Q  Median      3Q     Max
-2.0594 -0.6706 -0.1902  0.4154  3.9335

Random effects:
 Groups     Name        Variance  Std.Dev.
 popu:clone (Intercept) 0.0002775 0.01666
 Residual               0.0324413 0.18011
Number of obs: 279, groups:  popu:clone, 12

Fixed effects:
             Estimate Std. Error        df t value Pr(>|t|)
(Intercept)  -0.36797    0.47819 270.92000  -0.770  0.44227
size          0.96953    0.36753 271.00000   2.638  0.00882 **
size2        -0.17079    0.06951 271.00000  -2.457  0.01463 *
popuB        -0.05319    0.03356   7.90000  -1.585  0.15213
popuC         0.03451    0.03305   7.56000   1.044  0.32865
popuD         0.08455    0.03378   8.11000   2.503  0.03640 *
temp          0.08931    0.01171 270.22000   7.630 4.05e-13 ***
dens          0.03952    0.01221 269.61000   3.236  0.00136 **
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Correlation of Fixed Effects:
      (Intr) size   size2  popuB  popuC  popuD  temp
size  -0.994
size2  0.983 -0.996
popuB  0.008 -0.047  0.051
popuC -0.051  0.005  0.005  0.489
popuD -0.053  0.032 -0.043  0.463  0.464
temp  -0.065  0.031 -0.007  0.046  0.047 -0.008
dens  -0.268  0.307 -0.326 -0.065 -0.106  0.016 -0.150
```

Full summary of ANOVA, TukeyHSD and linear model

ANOVA & TukeyHSD

```
                    Df   Sum Sq Mean Sq F value   Pr(>F)
pop_combi            3   868099  289366  11.336 2.43e-07 ***
regime               3   506633  168878   6.616 0.000195 ***
pop_combi:regime     9   690343   76705   3.005 0.001487 **
Residuals         1300 33184430   25526
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
76 observations deleted due to missingness

Tukey multiple comparisons of means
    95% family-wise confidence level
```

```
Fit: aov(formula = n ~ pop_combi * regime, data = df)

$pop_combi
         diff         lwr      upr     p adj
B-A 24.458113  -7.8895231 56.80575 0.2098451
C-A 64.778930  32.6190977 96.93876 0.0000015
D-A 56.229526  23.9295855 88.52947 0.0000484
C-B 40.320818   8.5155021 72.12613 0.0062548
D-B 31.771413  -0.1755649 63.71839 0.0518982
D-C -8.549405 -40.3062101 23.20740 0.8999792

$regime
             diff       lwr        upr     p adj
18-14  46.7309129  14.11800  79.343822 0.0013543
22-14  22.7763438 -10.06656  55.619245 0.2815113
26-14  -0.7273225 -33.16467  31.710023 0.9999311
22-18 -23.9545691 -55.71364   7.804501 0.2117616
26-18 -47.4582354 -78.79773 -16.118745 0.0005957
26-22 -23.5036663 -55.08242   8.075092 0.2223361

$`pop_combi:regime`
                   diff         lwr         upr     p adj
B:14-A:14   17.6707317  -73.809751 109.1512142 0.9999991
C:14-A:14   95.1666667    2.663667 187.6696662 0.0363277
D:14-A:14  109.8243243   16.201308 203.4473405 0.0060085
C:14-B:14   77.4959350   -9.251111 164.2429812 0.1436578
D:14-B:14   92.1535926    4.213191 180.0939943 0.0291633
D:14-C:14   14.6576577  -74.345941 103.6612559 0.9999999
B:18-A:18   27.5000000  -57.129989 112.1299894 0.9992686
C:18-A:18   50.0188261  -34.117690 134.1553420 0.8017721
D:18-A:18   93.4606866    9.324171 177.5972025 0.0135796
C:18-B:18   22.5188261  -61.617690 106.6553420 0.9999323
D:18-B:18   65.9606866  -18.175829 150.0972025 0.3371710
D:18-C:18   43.4418605  -40.198270 127.0819914 0.9222960
B:22-A:22   61.2480769  -26.025983 148.5221371 0.5409361
C:22-A:22   83.6276224   -1.665564 168.9208086 0.0615955
D:22-A:22    7.7087912  -78.533343  93.9509253 1.0000000
C:22-B:22   22.3795455  -62.346560 107.1056508 0.9999427
D:22-B:22  -53.5392857 -139.220620  32.1420483 0.7353368
D:22-C:22  -75.9188312 -159.581589   7.7439266 0.1264854
B:26-A:26    0.9873016  -82.220234  84.1948370 1.0000000
C:26-A:26   37.8599483  -44.845627 120.5655233 0.9731358
D:26-A:26   24.5792929  -57.644280 106.8028654 0.9997309
C:26-B:26   36.8726467  -47.263869 121.0091626 0.9820659
D:26-B:26   23.5919913  -60.070766 107.2547491 0.9998691
D:26-C:26  -13.2806554  -96.444201  69.8828897 0.9999999
```

Linear model

```
Call:
lm(formula = n ~ pop_combi * regime, data = df)

Residuals:
    Min      1Q  Median      3Q     Max
-345.65 -108.65    9.41  109.30  532.93

Coefficients:
                  Estimate Std. Error t value Pr(>|t|)
(Intercept)          204.500     19.971  10.240  < 2e-16 ***
pop_combiB            17.671     26.649   0.663 0.507384
pop_combiC            95.167     26.947   3.532 0.000427 ***
pop_combiD           109.824     27.273   4.027 5.98e-05 ***
regime18              59.690     26.509   2.252 0.024508 *
regime22              40.577     26.947   1.506 0.132353
regime26              38.989     26.124   1.492 0.135827
```

```
pop_combiB:regime18    9.829     36.303   0.271 0.786623
pop_combiC:regime18  -45.148     36.426  -1.239 0.215401
pop_combiD:regime18  -16.364     36.668  -0.446 0.655476
pop_combiB:regime22   43.577     36.831   1.183 0.236953
pop_combiC:regime22  -11.539     36.653  -0.315 0.752951
pop_combiD:regime22 -102.116     37.080  -2.754 0.005971 **
pop_combiB:regime26  -16.683     36.023  -0.463 0.643348
pop_combiC:regime26  -57.307     36.146  -1.585 0.113117
pop_combiD:regime26  -85.245     36.297  -2.349 0.018998 *
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Residual standard error: 159.8 on 1300 degrees of freedom
  (76 observations deleted due to missingness)
Multiple R-squared:  0.05858, Adjusted R-squared:  0.04772
F-statistic: 5.393 on 15 and 1300 DF,  p-value: 1.122e-10
```

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