

The effect of positively autocorrelated thermal variance
on the reproduction and individual growth of
the nematode *Caenorhabditis elegans*

by

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A thesis

presented to the University of Waterloo

in fulfillment of the

thesis requirement for the degree of

Master of Science

in

Biology

Waterloo, Ontario, Canada, 2015

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Author's Declaration

I hereby declare that I am the sole author of this thesis. This is a true copy of the thesis, including any required final revisions, as accepted by my examiners.

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Abstract

The effect of uncorrelated and positively autocorrelated temperature variance on the offspring production and individual development of the nematode *Caenorhabditis elegans* was examined at two different temperature means: a mean closer to the optimal temperature (20°C) and a lower, more suboptimal mean (16°C). Variance in temperature was introduced with a computer-controlled incubators, which changed the target temperature every 40 minutes. Offspring production was measured as the number of offspring produced by five adults during a 72-hour period, and individual growth was measured via estimated body length when the individuals were 75-81 hours old, and through time to maturation, observed every 12 hours.

Uncorrelated variance had no effect on the offspring production, time to maturation, or body length for either mean temperature. However, when the temperature series was positively autocorrelated, where the condition at a certain time is dependent on previous conditions, it resulted in a decrease in offspring number, longer time to maturation, and shorter body length at mean 20°C. The negative effects of variance observed at mean 20°C were absent at mean 16°C, which is consistent with literature that suggests that mean temperature can influence the effect of variance on biological performance. The significant negative effect was only observed in positively autocorrelated treatments.

Acknowledgement

I would like to thank Dr. Kim Cuddington for taking me on as an undergraduate lab technician, and later as a graduate student. Without her guidance and patience I would have never been able to get through my graduate work. I also thank the members of Cuddington lab - Laura Bell, Z. Tasker Hull and Stephi Sobec-Swant - for their help in lab work and writing, as my experiment would never have been the same without their input and help. I thank my committee members Dr. Kirsten Müller and Stephen Murphy for their valuable advice. I also thank Dr. Bill Taylor and Dr. Jonathan Witt for their input and help on statistics and my research material. I thank Dr. Trevor Charles and members of his lab for lending me necessary equipment and materials when I first started out. I thank Dr. Cheryl Duxbury and Gracia Murase for their insight and knowledge in microbiology and *E. coli* culturing. This project was funded by an NSERC discovery grant to Kim Cuddington and the University of Waterloo.

I also thank my loved ones, my family and friends who helped me get through times of great personal difficulty. I thank Vinay Daté, a faithful friend who proofread my work countless times and helped me with his knowledge in microbial work. Without him I would never been able to finish this work. I also thank Jaeyoung Lee, Willam Owen Parry, Michael Hrivnak, Bojana Ristic, and Scott Lisicky for their help in proofreading my thesis and their kind words of encouragement.

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Chapter 1 – Literature review

1.1. Introduction

Environmental variability can translate into changes in organismal performance, which may cause detrimental effects (e.g. increased risk of extinction) on the population and individual levels (Lewontin and Cohen, 1969; Lande and Orzack, 1988; Tuljapurkar, 1989). However, this prediction may vary depending on the mechanisms by which the change in the abiotic variable (e.g. temperature) causes the change in organismal performance (e.g. reproductive rate) (Izem and Kingsolver, 2005; Kjærsgaard *et al.*, 2013).

Temperature has a strong effect on organismal performance due to its ability to affect biochemical reaction rates and molecular stability (Somero, 2002). This change in performance with respect to changing temperature is best summed up in a curve, commonly referred to as the thermal performance curve (Huey and Kingsolver, 1989). In terrestrial ectotherms which have no internal means of regulating body temperature and small body mass, such as the fruit fly *Drosophila melanogaster* (Dillon *et al.*, 2009; Bozinovic *et al.*, 2013) or the nematode *Caenorhabditis elegans* (Anderson *et al.*, 2007), the thermal performance curve displays a non-linear, asymmetric shape (Izem and Kingsolver, 2005; Deutsch *et al.*, 2008; Dillon *et al.*, 2009). This asymmetry interacts with the mean and magnitude of thermal fluctuations, potentially changing the magnitude or direction of the effect that thermal variance has on an organism, depending on where the mean is located within the curve (Ruel and Ayres, 1999; Bozinovic *et al.*, 2011; Folguera *et al.*, 2011; Kjærsgaard *et al.*, 2013).

As ongoing global climate change is expected to increase the magnitude and frequency of thermal variance in addition to increases in mean temperature (Burroughs 2007), a detailed understanding of their thermal performance curves is expected to become more important in predicting the dynamics of small populations (Folguera *et al.*, 2009; Paaijmans *et al.*, 2013; Estay, Lima & Boznovic, 2013).

In addition to variance, abiotic factors such as temperature often display positive temporal autocorrelation (Pelletier, 1998; Pelletier, 2002; Cyr and Cyr, 2003; Vasseur and Yodzis, 2004), where an element in a time step is dependent on previous elements. Although stochastic population models incorporating positive autocorrelation predict possible changes in population dynamics (Lawton 1988; Johst and Wissel 1996; Ripa and Lundberg, 1996), positive autocorrelation is rarely considered in models and experiments addressing the effect of thermal variance on ectotherms.

I review the literature on population models that incorporate the effects of unstructured environmental variance in density-independent models. In addition, I describe positive temporal autocorrelation in abiotic factors and models which incorporate such variation. Then I describe the common shapes of the thermal performance curves found in small terrestrial ectotherms and how they may influence the effect of thermal variance on organisms, with specific details regarding the thermal performance curve and other temperature related traits in the model organism *Caenorhabditis elegans*. Finally, I will discuss previous experimental efforts to test predictions regarding the effect of thermal variance on populations or individuals, and the lack of studies incorporating autocorrelated variation.

1.2. Stochasticity and its effect on populations

Population models predict that fluctuations in vital rates result in reduced population size, faster time to extinction, increased chance of extinction and reduced probability of establishment compared to that predicted by the model under constant conditions. I will explain the general modeling framework that has been used, and examine some of the more influential of these works. In general, single species models, including both unstructured and stage/age structured models, predict that fluctuations in vital rates, survival, and reproductive parameters associated with life history parameters, result in decreased average population size and increased probability of extinction.

1.2.1. The effect of uncorrelated environmental variance on populations

The dynamics of populations have both deterministic and stochastic aspects. Deterministic aspects of population growth comprise those factors that do not vary randomly with time, such as the maximum number of offspring per individual. Stochastic aspects comprise random variation due to small population size (demographic stochasticity), and variation in vital rates caused by fluctuations in the environment (environmental stochasticity).

When a population model only accounts for the deterministic aspect of population growth, the end result will always be the same as long as the initial conditions are identical - for example, in a simple geometric model $N_{t+1} = N_t\lambda$ where λ and N_t represent the per capita population growth rate and the population size at time t , respectively, the population will grow without limit and the change in population size, $N_t - N_{t+1}$, will increase as the population size becomes larger.

Lewontin and Cohen (1969) modified this geometric growth model to include the effect of a varying per capita growth rate, resulting in the model $N_{t+1} = N_t \lambda_t$. In this model, λ_t represents the per capita population growth rate at time t , where its value at every time step is drawn from a normal distribution with given mean and variance. Lewontin and Cohen found that for a given mean λ , a population with a varying per capita growth rate will always be smaller than a population with a constant rate. In fact, a population can go extinct (reach a size of zero in a given duration), even if unbounded population growth was predicted under constant conditions.

This finding can be explained by recognizing that when there is variation, the realized population growth rate is the natural log of the growth rate, which can be represented as the geometric mean of the growth rates at each time step. The geometric mean, as it is the arithmetic mean of the log transformed values of the elements in the series, is always smaller than the arithmetic mean unless all the elements in the set are the same. Due to this discrepancy, if environmental fluctuations alter per capita population growth rate, the expected population growth rate and population size will always be smaller in variable environments than in constant ones. Thus, sufficiently large environmental variance can cause extinction despite the fact that the arithmetic mean of the per capita growth is positive and would, in more constant environments, result in indefinite population growth.

The method used in Lewontin and Cohen's work (1969) can be extended to more complicated models, such as stage structured matrix models, in which the population growth rate is described by a matrix derived from the life history of the species instead of a single number (λ in the above examples), where each element of the matrix is drawn from a normal distribution with a given mean and variance for each timestep. Much like their unstructured counterpart (e.g., Lewontin and Cohen, 1969), these models predict detrimental impact of variance on population

growth and persistence, with larger variance causing larger detrimental effects (Lande and Orzack, 1988; Tuljarpurka, 1990).

In summary, stochastic fluctuation in vital rates in density independent single species models, both structured and unstructured, results in reduced population growth rate, reduced population size, and increased probability of extinction. However, in nature, environmental variables such as temperature are often positively correlated, with possible effects on individual and population growth. In the next section, I will briefly describe why temporal autocorrelation can be an important factor in predicting the effect of environmental stochasticity, and review models regarding its impact.

1.2.2. Positive autocorrelation in environmental signals and its predicted effect

Positive autocorrelation is a characteristic that can be found in signals with variance, referring to when elements in a time step are dependent on each other. For example, in a system with strong positive autocorrelation, a higher than average temperature observed one day would likely be followed by a similarly high temperature the next day. In contrast, in a system with no autocorrelation, the chance of such an occurrence is lower. If both systems have the same mean and variance, the chances of good and bad conditions in both the positively autocorrelated and uncorrelated time series are the same, but the distribution of such conditions is different. Namely, long periods of similar conditions, such as continued runs of high temperature, are more likely to happen in positively autocorrelated environments.

Early stochastic models such as Lewontin and Cohen (1969) assumed that the elements of the environmental time series are independent of each other (i.e. not autocorrelated). However, there is strong evidence that many environmental parameters such as temperature are

autocorrelated (Pelletier 1998; Pelletier 2002; Cyr and Cyr 2003; Vasseur and Yodzis 2004).

Autocorrelation in temperature is especially detectable in coastal regions, where there are large bodies of water which store heat (Steele, 1985; Vasseur and Yodzis, 2004).

Lawton (1988) argued that continued runs of bad conditions (e.g. low food density) are considerably harder to survive compared to a short period of bad conditions, and that the consequences of a prolonged period of bad conditions outweigh the potential benefit of a continued run of good conditions (e.g. high food density). Therefore, the negative consequences of environmental variance would be magnified in a positively autocorrelated environment. Similarly, in a simple density independent model with randomly fluctuating population growth rate but no net population increase, the presence of positive autocorrelation increases the chance of extinction (Foley, 1994).

While prolonged runs of bad conditions in a positively autocorrelated environment can exert a negative effect on population growth, the same positive autocorrelation may reduce the chance of catastrophic events, which can also be beneficial (Schwager *et al.*, 2006). Using a density dependant model, Schwager and the authors demonstrated that the magnitude of the variation, which determines the likelihood of extreme catastrophic events, is one of the factors which determines whether positive temporal autocorrelation has a positive or negative effect on a population's risk of extinction. The authors predict that positive autocorrelation will decrease the extinction risk if the magnitude of environmental fluctuation is high, while the opposite is the true when the magnitude of fluctuation is small.

However, more recent models (Van de Pol *et al.*, 2011; Engen *et al.*, 2013) which incorporate field data into their predictions show that positive autocorrelation has a smaller effect on population extinction than previously expected, sometimes even a negligible one (Engen *et*

al., 2013). When model predictions and field data for the population growth of the Eurasian oystercatcher *Haematopus ostralegus* were compared to variation in winter temperature, field data suggested that the extinction risk was relatively insensitive to autocorrelation despite the model predicting the opposite (Van de Pol *et al.*, 2011). The authors attributed this result to the relative insensitivity of population growth rate to change in temperature, suggesting that understanding the mechanism connecting the change in the abiotic factor and vital rates is important in predicting the effect of positively autocorrelated environmental variance. In the next section, I will look at how change in temperature affects vital rates, and how this property influences the predictions regarding the effect of uncorrelated environmental variance.

1.3. The effect of variance in temperature on population and individual growth

As reviewed above, fluctuation in vital rates due to environmental variance may affect population growth negatively if the magnitude of variance is sufficiently large (Lewontin and Cohen, 1969; Lande and Orzack, 1988; Tuljarpurka, 1990). However, this prediction may not hold depending on various factors, one of which is the relationship between environmental variance and the biological performance of an organism. I will review the changes in ectotherm performance with respect to temperature, and explain how the shape of the generic thermal performance curve interacts with environmental stochasticity and its effect on population and individual growth.

1.3.1. The thermal performance curve and its implications in a fluctuating environment

Change in body temperature corresponds to change in biological performance of the organism, such as reproductive rate (Harvey and Viney, 2007; Diloh et. al., 2009), feeding rate (Kingsolver and Gomulkiewicz, 2003), or body growth rate (Byerly, Cassada & Russel, 1976; Kingsolver and Gomulkiewicz, 2003), due to its influence on biochemical reaction rates and molecular stability (Somero, 2002). As this relationship is continuous and non-linear, a common way of representing the effect of change in body temperature on an organism is the thermal performance curve, a curve describing the change in performance of the organism with respect to change in temperature (Huey and Kingsolver, 1989; Izem and Kingsolver, 2005).

The shape of the thermal performance curve in ectotherms varies depending on the type of organism and the environment they are in. For example, some aquatic organisms, such as the Brown trout *Salmo trutta*, display more symmetrical thermal performance curves (Elliot, Hurley & Fryer, 1995) than terrestrial ectotherms like the fruit fly *Drosophila melanogaster* (Dillon et al., 2009). The width of the thermal performance curve, which indicates the thermal range of the organism, is narrower in ectotherms found in low latitudes (i.e. tropical regions) compared to high latitudes (i.e. temperate regions) (Deutsch et al., 2008).

In small terrestrial ectotherms (i.e. smaller reptiles, insects and other invertebrates), where changes in environmental temperature translate more readily into changes in body temperature due to their limited mechanism for body temperature regulation, change in thermal performance in response to temperature increase follows a common pattern (Kingsolver and Gomulkiewicz, 2003). Performance increases as temperature increases, until a point where biological performance is maximized. Once temperature increases over this threshold, commonly referred to as the optimal temperature, performance rapidly declines, often with serious

consequences such as sterilization or death (Anderson et al., 2007; Deutsch et al., 2008). A generic thermal performance curve in small terrestrial ectotherms reflects this pattern, with a convex section representing an accelerating increase in biological performance in lower temperatures, followed by a section of close to linear increase in intermediate temperatures, then ending with a section of sharp decline at high temperatures (Figure 1-1) (Izem and Kingsolver, 2005; Ikemoto, 2005). Organisms that show this kind of thermal performance curve include caterpillars (Paaijmans et al., 2013), the Atlantic salmon *Salmo salar* (Jonsson et al., 2001), the fruit fly *Drosophila melanogaster* (Dillon et al., 2009) and the nematode *Caenorhabditis elegans* (Byerly, Cassada & Russel, 1976; Venette and Ferris, 1997; Anderson et al., 2007)

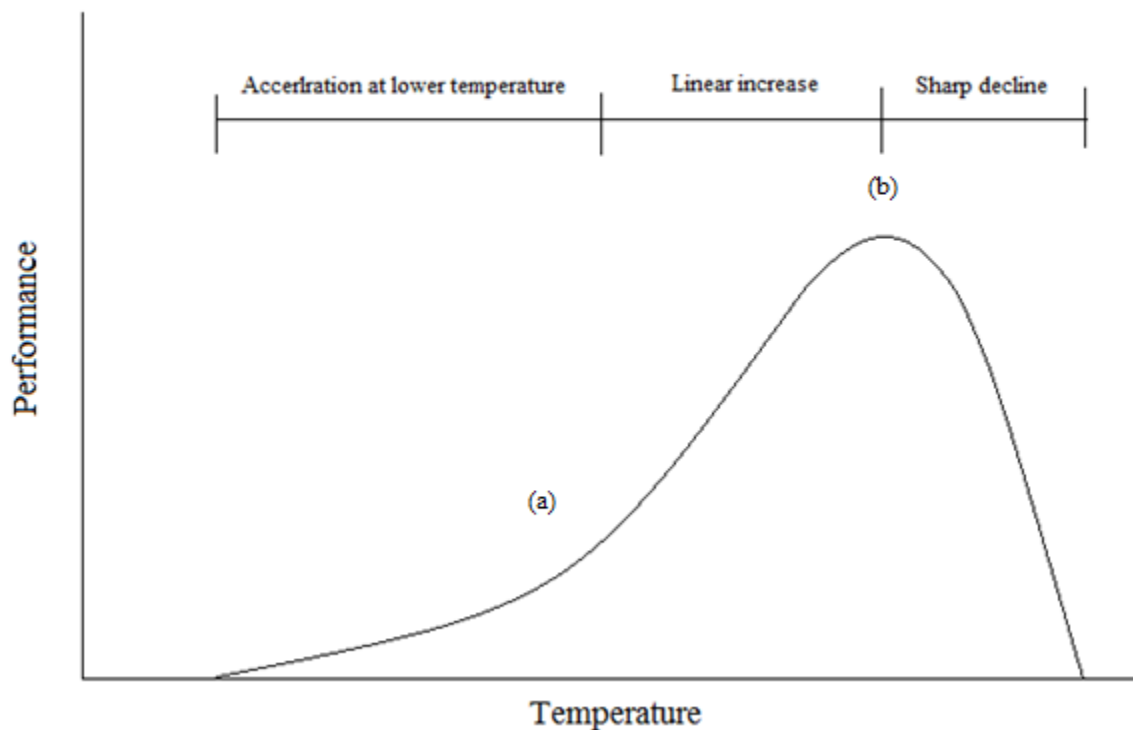


Figure 1-1. A hypothetical thermal performance curve with three distinct sections, showing the changes in biological performance (e.g. reproductive rate) as temperature increases.

The non-linear, asymmetric shape of thermal performance curves suggests that both mean and variance are important in assessing the effect of temperature on organismal performance, as sufficiently large thermal fluctuation may elevate or depress the overall performance compared to the performance expected from its linear counterpart, depending on where the fluctuation is located on the thermal performance curve (Ruel and Ayres, 1999). For example, in the hypothetical thermal performance curve (Figure 1-1), thermal variance when the mean is located at the lower thermal range of the organism (a), would have a very different impact on biological performance compared to when the mean is located near the peak region of the performance curve (b), as potential highs and lows caused by thermal variance of the same magnitude will have very different effects depending on where the mean is located on the curve.

As variance near the peak region of thermal performance curve is more likely to result in a sharp drop off in performance due to high temperature, the temperature at which a species' long-term fitness is maximized is not same as the temperature at which a temperature dependant trait (e.g., population growth rate) is maximized (Martin and Huey, 2008; Deutsch et al., 2008). This gap between the ideal temperature for long-term fitness and maximum biological performance grows larger as the thermal performance curve becomes more asymmetric (Martin and Huey 2008). Laboratory studies and field data support this prediction in small terrestrial ectotherms, such as lizards and insects (Martin and Huey 2008; Diloh et. al., 2009; Paaijmans et al., 2013).

Furthermore, an instance of 'good' variance (i.e. higher temperature which may result in higher body function) may have an unexpected effect on overall long-term performance. In an organism where change in body temperature instantaneously translates into change in biological performance (e.g. growth and locomotory rate in caterpillars; Kingsolver and Gomulkiewicz,

2003), a short period of high performance due to increased temperature can contribute to the overall performance of the organism to a degree that is disproportionate to the duration of the ‘good’ condition (Kingsolver and Gomulkiewicz, 2003). The reverse is also true if the brief exposure to the extreme temperature condition can result in irreversible damage to an organism, such as death or sterilization (Anderson et al., 2007; Deutsch et al., 2008).

Considering the influence that the shape of the thermal performance curve has on both the individual and population level, both the mean and variance of a thermal environment should be considered in order to make accurate predictions about biological performance (Paaijmans *et al.*, 2013; Vasseur et al., 2014). A comparison between two models which did and did not incorporate thermal variance showed that the model incorporating thermal variance produced more varied responses, including greater risk for decreased performance, while the model which incorporated only mean temperature predicted an overall increase in performance (Vasseur et al., 2014). In the next section, I will review experiments which tested some of the predictions regarding the effect of thermal variance on organismal performance.

1.3.2. Experimental studies regarding the effect of thermal variance

Recent experimental studies directly manipulate temperature to affect the vital rates of the experimental organisms to test the effect of thermal variance on the organism, with some studies focusing specifically on the effect of shifts in mean temperature. In this section, I will review experimental studies which test the effect of temperature on individual and population performance.

Bozinovic et al. (2011) studied the fruit fly *Drosophila melanogaster* under conditions of thermal variation with two different means, and observed the changes in maximal population

growth rate, survival rate and tolerance to an extreme condition. There were four temperature treatments used in the experiments: two constant conditions at low (17°C, which was the lower boundary of thermal tolerance for *D. melanogaster*) and high temperatures (23°C, which was the high boundary of thermal tolerance for *D. melanogaster*), and varying conditions ($\pm 5^\circ\text{C}$) at both high and low means. The authors observed that the population growth rate increased under varying conditions as compared to constant conditions when the mean temperature was low, but that the trend reversed when the temperature was high. This result is consistent with predictions based on a typical thermal performance curve, where variance has a positive effect when the mean temperature is lower than the thermal optimum. Survival rate also closely followed this trend.

Folguera et al. (2011) studied the terrestrial woodlouse *Porcellio laevis* under three temperature treatments (Constant at 24°C, 24 $\pm$ 4°C, 24 $\pm$ 8°C), measuring changes in maximum size, survival, individual growth, metabolic rates and heat shock protein expression. The treatments were chosen in consideration to the thermal range of the study organism, similar to the study described above. The authors found positive effects on metabolic rate and growth rate in the treatment with medium variance, compared to the constant treatment. However, all three variables were negatively affected when the magnitude of temperature fluctuation became larger. The survival rate was highest in constant conditions, and decreased as the magnitude of the variance increased.

Kjaersgaard et al. (2013) exposed the yellow dung fly *Scatophaga stercoraria* to two different temperature conditions, which shared a mean temperature (18°C, which was the median of the known temperature tolerance range of *S. stercoraria*) but differed in the magnitude of variance ($\pm 3^\circ\text{C}$ and $\pm 5^\circ\text{C}$). Longevity, survival rate, body size, and wing shape were measured,

and were compared against three constant temperature treatments, 12°C, 18°C and 24°C. Kjaersgaard and co-authors observed that large fluctuations had detrimental effects on *S. stercoraria*, resulting in slower development, individual growth and lower survival rate. This result is consistent with predictions made from the shape of the thermal performance curve of *S. stercoraria*, as the large fluctuation treatment included the sharp drop-off region in the thermal performance curve. The treatment with smaller fluctuation had less detrimental results, compared to the constant treatment with same mean.

Overall, experiments testing the effect of varying environments display the interaction between mean and variance predicted by the shape of the thermal performance curve of the organism. When the temperature mean is close to the optimal temperature, variance has a detrimental effect on both populations and individuals. The detrimental effect increases as the magnitude of the variance increases, as the asymmetrical shape of the thermal performance curve predicts that the detrimental effect of high temperature will outweigh the positive effect, if any, of lower temperature. When the temperature mean is lower than the thermal optimum, variance has a positive effect on both individual and population performance, as the positive effect of variance outweighs the negative effect of variance. The beneficial effect of variance, even if it exists at a given mean, diminishes if magnitude of the variance becomes sufficiently larger.

In the next section, I will review experimental studies which focused on the thermal performance curve and other temperature related physiological and behavioral responses of the model organism *C. elegans*.

1.3.3. Thermal performance of *Caenorhabditis elegans*

Caenorhabditis elegans is a model organism in genetics and cell biology, and its biological performance variables such as generation time (Byerly, Cassada & Russel, 1976), body growth (Byerly, Cassada & Russel, 1976), and reproductive rate (Venette and Ferris, 1997; Anderson et al., 2007; Bagasse et. al., 2015) are strongly influenced by change in temperature. Due to its small body mass, body temperature in a wildtype adult *C. elegans* is very heavily influenced by the surrounding temperature (Anderson et al., 2007). While some strains of *C. elegans* show more definitive temperature preferences, wildtype *C. elegans* shows no clear thermal preference other than avoiding temperatures higher than 25°C (Anderson et al., 2007) and preferring the mean temperature at which it was reared (Hedgecock and Russel, 1975).

In regular lab conditions, a wildtype *C. elegans* hermaphrodite produces approximately 400 sperm during its last larval stage (L4), then irreversibly switches to oocyte production once matured. Therefore an adult *C. elegans* has a finite supply of sperm cells, which are prone to damage caused by heat stress at temperatures above 25°C (Bagasse et. al., 2015). Sperm cells start to be permanently damaged above 27°C which may lead to a sterile individual (Petrella, 2014, Bagasse et. al., 2015), although lethality due to high temperature in *C. elegans* does not happen until 31 - 33°C (Venette and Ferris, 1997). The natural preferred temperature of *C. elegans* in the wild is 17°C (Martin and Huey, 2008), although the rate of population growth increases with temperature until it peaks at 24-26°C (Byerly, Cassada & Russel, 1976; Venette and Ferris, 1997; Martin and Huey, 2008). *C. elegans* development stops altogether at 7°C (Felix and Braendle, 2010).

The generation time of *C. elegans* decreases as temperature increases - the time for an individual to hatch from an egg, mature, and lay an egg at 16°C is 90 hours, whereas it takes 65

and 47 hours at 20°C and 25°C, respectively (Byerly, Cassada & Russel, 1976). Body length, regardless of temperature, corresponds to the molting stage the individual is in, although maximum size at adulthood is smaller at lower temperatures (16°C).

Egg production rate is another measure of *C. elegans* performance found in the literature. Egg laying rate is highest at 20-21°C (Byerly, Cassada & Russel, 1976; Bagasse *et. al.*, 2015), although the rate remains relatively high at 25°C (Byerly, Cassada & Russel, 1976). However, the total number of eggs laid at 25°C (170 eggs per individual) is much lower compared to the number laid at 16°C (275 eggs) or 20°C (280 eggs) (Byerly, Cassada & Russel, 1976). This is due to the heat stress sperm cells receive at temperatures over 25°C (Bagasse *et. al.*, 2015). Both egg laying rate and total egg production sharply decline at 28°C and above (McMullen *et al.*, 2012). The total number of eggs produced over a set duration, however, does have the shape of a typical thermal performance curve. Egg production at young age (60-100 hours) peaks at 24-26°C (McMullen *et al.*, 2012), and stops at a lower boundary of 12-14°C and a high boundary of 29-30°C (Byerly, Cassada & Russel, 1976).

Per capita population growth rate also displays a similar pattern (Venette and Ferris, 1997, shown in the figure 1-2). Growth rate increases linearly with temperature from 10°C to 25°C, until it reaches the maximum rate of increase at 25 - 26°C. The individual no longer reproduces at 30°C (Venette and Ferris, 1997).

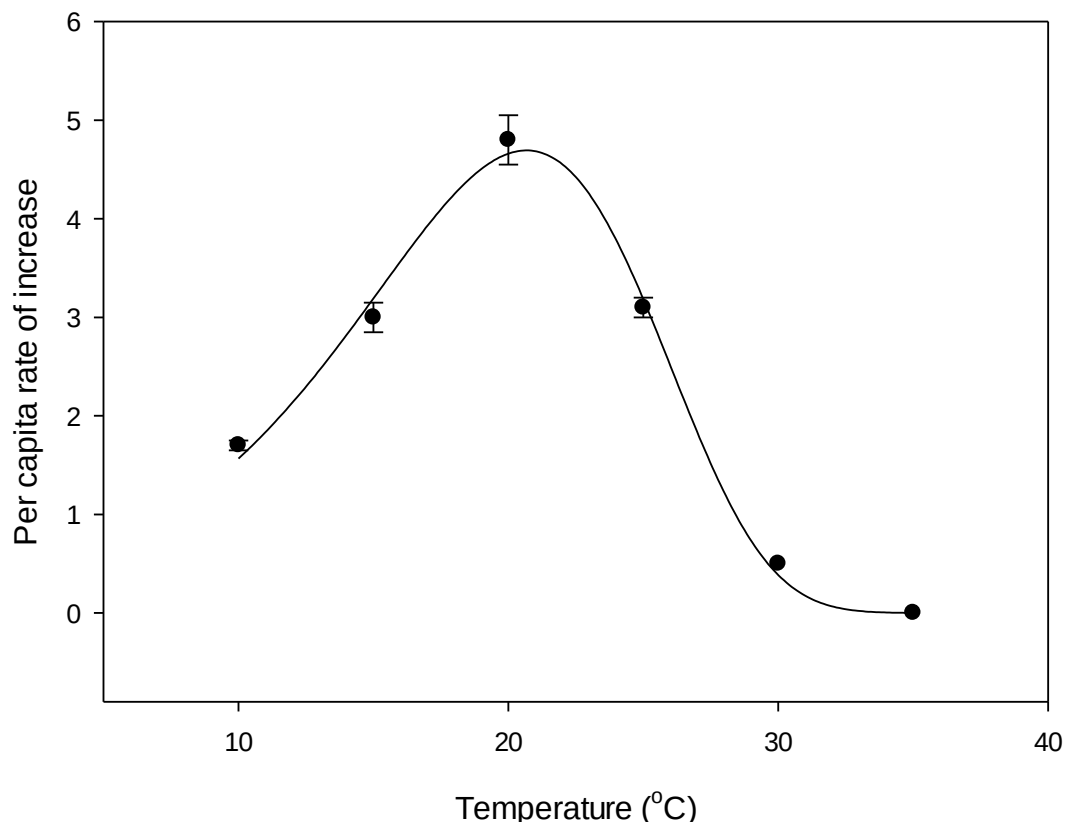


Figure 1-2. Thermal performance curve of *C. elegans* for rate of increase with respect to increase in temperature, obtained from 6 replicates per each constant temperature. Bars indicate 95% confidence interval (unseen bars are obscured by the data point), and data points indicate the mean per capita rate of increase. The curve was fitted to the weibull distribution in Sigmaplot 12.5. Redrawn from: Venette, R. C., & Ferris, H. (1997). Thermal constraints to population growth of bacterial-feeding nematodes. *Soil Biology and Biochemistry*, 29(1), 63-74.

In summary, some elements of the biological performance of *C. elegans* (e.g. generation time, population increase) show a thermal response similar to the generic thermal performance curve described in the previous section. Although responses such as egg laying rate do not follow this generic shape, *C. elegans* can be considered as an ectotherm with a typical thermal performance curve with respect to individual growth and offspring production.

1.4. Summary

In density independent models, increased environmental variance results in decreased population size, increased extinction risk, and decreased chance of establishment (Lewontin and Cohen, 1969; Lande and Orzack, 1988; Tuljapurkar, 1990). However, this predicted negative effect may change depending on the type of environmental variable affecting the organism (Drake, 2005).

Temperature, due to its characteristic non-linear, asymmetric relationship with biological performance in small terrestrial ectotherms (Paaijmans *et al.*, 2013; Vasseur *et al.*, 2014), can change the direction and magnitude of the effect of variance, depending on the mean temperature (Deutsch *et al.*, 2008). Both mathematical models and experimental studies agree that varying temperature can have a positive effect on populations or individuals when the mean temperature is suboptimal (e.g. at the edge of temperature tolerance range of the organism), while detrimental effects can happen in more optimal conditions (e.g. signal mean at the middle of temperature tolerance range).

Variance in environmental signals is not restricted to simple changes in magnitude - it may also display positive temporal autocorrelation, which increases the likelihood of prolonged runs of a similar environmental condition. Model studies predict that positive autocorrelation can either increase or decrease the effect of variance (Foley, 1994; Schwager *et al.*, 2006; Van de pol *et al.*, 2011) based on various factors such as the magnitude of the variance, type of environmental variable, and other life history traits (e.g. density dependence; Petchey *et al.* 1997) of the population. Despite the prevalence of autocorrelation in certain environments (Steele, 1985; Vasseur and Yodzis, 2004) no studies incorporate it with the thermal performance curve.

As variance and autocorrelation in environmental temperature is expected to increase due to global climate change (Folland et al., 2002; Katz et al., 2005; Burroughs 2007), understanding the effect of stochastic temperature change is important in making accurate predictions regarding population growth and extinction (Paaijmans *et al.*, 2013; Vasseur et al., 2014). Although there are a number of experimental studies testing the effect of thermal variance on population or individual growth, none of them addressed the effect of autocorrelated thermal variance.

A quick literature search on Scopus.com (retrieved August 1st, 2015), returned 299 papers published in the last 10 years (386 with no date restrictions) that includes the term ‘thermal performance curve’ in the abstract. However, none of these articles mention autocorrelation or red noise (a commonly used term referring to positive autocorrelation). A similar search on Web of Science (<https://apps.webofknowledge.com>; retrieved August 1st, 2015) returned one result that mentions autocorrelation out of a total of 194 papers that include the term ‘thermal performance curve’, and this paper (Laakso et al., 2006) was not directly related to the interaction between the thermal performance curve and positive autocorrelation.

Although this result is not conclusive, autocorrelation does not appear to be a commonly discussed topic in the context of the effect of thermal variance on ectotherms or the thermal performance curve. This gap in literature is concerning, as the presence of positive temporal autocorrelation in thermal variance may influence model predictions based on the shape of the thermal performance curve.

Chapter 2 – The effect of thermal variance on *C. elegans* body length and offspring production

2.1. Introduction

In small terrestrial ectotherms (e.g., caterpillars, nematodes) change in environmental temperature is directly linked to change in biological performance (e.g., body growth) due to the influence temperature has on the rate of biochemical reactions and on molecular stability (Somero, 2002). In these organisms, the change in performance with respect to increasing temperature often takes the shape of an asymmetric curve, which peaks at the optimum temperature, then shows a rapid decline in performance above this optimum temperature (Kingsolver and Gomulkiewicz, 2003; Izem and Kingsolver, 2005). Due to this asymmetry, fluctuation in temperature will have different effects on the organism depending on the magnitude of the fluctuation, and where the mean temperature is located with respect to the optimal temperature (Izem and Kingsolver, 2005; Martin and Huey, 2008; Deutsch et al., 2008). This also means that in a fluctuating environment, organisms will have better long term viability at means lower than the optimum temperature (Martin and Huey, 2008; Deutsch et al., 2008).

The prediction that a fluctuation in temperature can have different effects on a population depending on the mean temperature becomes more important in the context of the ongoing problem of global warming. As previously mentioned, an organism's preferred temperature is often several degrees lower than its thermal optimum to minimize the detrimental effect of thermal variance (Martin and Huey, 2008). However, if the habitat's mean temperature rises, it would bring the mean temperature closer to the peak of the thermal performance curve, which could result in potential negative effects that would be difficult to predict based on

changes in mean temperature alone (Paaijmans et al., 2013). A few studies predict that climate change will increase the magnitude of thermal variance in addition to increase in mean (Folland et al., 2002; Katz et al., 2005; Burroughs 2007), therefore the possible detrimental effect of climate change may be more severe than previously expected.

In addition to a simple change in magnitude (i.e. variance), a stochastic temperature signal may be temporally autocorrelated, defined as the dependence of an element in a time series on the other elements in the same series. Positive autocorrelation results in increased probability of runs of similar conditions. Air and water temperatures are known to be positively autocorrelated (Mandelbrot and Wallis 1969; Cyr and Cyr 2003; Vasseur and Yodzis 2004). This positive autocorrelation is greater near large bodies of water (Steele, 1985; Vasseur and Yodzis, 2004).

Population models predict that variance in environmental signals will result in increased probability of extinction compared to constant conditions (Lewontin and Cohen, 1969; Lande and Orzack, 1988; Tuljapurka, 1989). Introducing positive autocorrelation can dampen or amplify this effect on population growth, depending on the magnitude of variance (Schwager *et al.*, 2006), the type of environmental variable (Van de pol *et al.*, 2011), and the organism's demographic characteristics (Petchey *et al.* 1997; Schwager *et al.*, 2006). However, despite the abundance of literature on the thermal performance curve and its effect on population, literature which addresses the effect of positively autocorrelated thermal variance is very rare, especially experimental studies.

In this chapter, I will use a model system composed of the nematode *Caenorhabditis elegans* and its food source *Escherichia coli* to test the effect of uncorrelated and autocorrelated thermal variance on reproduction and individual growth at two different means. The nematode *C.*

C. elegans is a model organism commonly used in cell biology and genetics. *C. elegans* is an ectotherm with small body mass (Anderson et al., 2007) and therefore lacks a sufficient buffer against outside temperature change. As a result, biological performance in *C. elegans*, (e.g. body growth), is very sensitive to change in environmental temperature (Venette and Ferris, 1997; Anderson et al., 2007; Bagasse et. al., 2015), making it an ideal organism to test the effect of thermal variance.

Population growth in *C. elegans* peaks at 24-26°C (Byerly, Cassada & Russel, 1976; Venette and Harris, 1997), followed by a sharp decline in reproductive rate at higher temperatures. *C. elegans* sperm cells start to show signs of heat stress above 25°C and start to become sterile at 27°C (Bagasse et. al., 2015), rendering both male and hermaphrodites sterile even though lethality due to high temperature does not occur under 30°C (Bagasse et. al., 2015). Low temperature does not have permanent negative effects such as sterilization until the temperature drops below 0°C, although development halts at 7°C (Felix and Braendle, 2010). The generation time of *C. elegans*, the time for an individual to hatch from an egg then mature and produce its first egg, decreases as temperature increases until it reaches the minimum at 25°C (Byerly, Cassada & Russel, 1976).

I will compare each of three different measures of organismal performance of *Caenorhabditis elegans* – offspring production, body length after 72 hours, and time until maturation – under six different thermal conditions representing the constant condition, uncorrelated variance and strongly autocorrelated variance at two different mean temperatures. I will test the following two hypotheses:

- 1) Introducing thermal variance will decrease biological performance of *C. elegans* in comparison to the constant condition, and this decrease in performance will diminish as

the mean temperature of the thermal variance decreases further away from the optimum temperature. Previous theoretical works have demonstrated that fluctuation in vital rates can result in a decrease in overall performance (Lewontin and Cohen, 1969; Ruel and Ayres, 1999) and this reduction in performance can change magnitude or direction depending on where the mean is located in the thermal performance curve (Martin and Huey, 2008; Deutsch et al., 2008) Based on this hypothesis, I predict that at mean 20°C, *C. elegans* in treatments with variance will show smaller offspring numbers, longer time until maturation, and shorter body length as a result of the negative effect of fluctuating temperature compared to those observed in constant conditions. At 16°C, the reduction in offspring numbers and body size and increase in development time will be smaller, or the direction may even be reversed.

- 2) The presence of positive temporal autocorrelation, given the same mean temperature and variance, will enhance the asymmetry of the effects of the two extreme conditions (i.e. high and low extremes of temperature) on the biological performance of *C. elegans*. Positive autocorrelation results in increased chance of prolonged runs of extreme conditions (Lawton, 1988; Schwager *et al.*, 2006), and high temperature extremes (>27°C) have very severe consequences with respect to *C. elegans* biological performance, such as sterilization or death. In comparison, the consequences of low temperature extremes (<7°C) in *C. elegans* are comparatively weak, therefore the decrease in biological performance due to positive autocorrelation will also decrease as the mean becomes increasingly lower than the optimal temperature, and may enhance the positive effect of variance, depending on the conditions. Based on this hypothesis, I

predict that autocorrelated treatments will display lower offspring numbers, longer development time, and smaller body size in comparison to uncorrelated treatments at mean 20°C, and this reduction in biological performance, albeit smaller, will still be present at 16°C, due to the asymmetry in the negative consequences of extreme conditions.

2.2. Methods

I conducted experiments investigating the effect of different temperature conditions on the biological performance of *C. elegans*, using *E. coli* OP50-1 as the food source. Both experiments used the same six temperature treatments; 1) constant temperature at mean 16°C, 2) constant temperature at mean 20°C, 3) mean temperature of 16°C with uncorrelated variance of 5°C, 4) mean temperature of 16°C with positively autocorrelated variance of 5°C, 5) mean temperature of 20°C with uncorrelated variance of 5°C, 6) mean temperature of 20°C with positively autocorrelated variance of 5°C. Under these temperature treatments, three types of data - number of offspring produced by 5 adults, body length at 72 hours, and time until maturation - were measured. In this section, I will explain the methods I used.

2.2.1. Strains and culture methods used

The nematode *Caenorhabditis elegans* has a relatively fast generation time and its growth and reproductive rates are very sensitive to changes in temperature (Byerly, Cassada & Russel, 1976; Lewis and Fleming, 1995; Venette and Ferris, 1997), which makes it an ideal organism for studying the effect of varying temperature on reproduction. The *C. elegans* strain BC15866 (McKay et al., 2004), which contains mutants with the *dpy-5* gene, which is responsible for the short-type, dumpy phenotype (Thacker, Sheps & Rose, 2006) was used. Each individual in the strains has a fluorescent marker, sEx15866. Maintenance and handling of *C. elegans* was done according to the procedures described by Lewis and Fleming (1995) and Wood (1998). The worms were kept in a 5 cm petri plate filled with nematode growth medium (Stiernagle, 2006),

inoculated with a standardized culture of *E. coli* OP50-1. This plate setup was used both in keeping *C. elegans* populations for general lab use and experimental studies.

The fecundity of *C. elegans* diminishes as it ages (Byerly, Cassada & Russel, 1976), therefore it was important to make sure that individuals of fixed, known age were used across every treatment. Age synchronization of *C. elegans* was achieved by moving five to ten fertile adults into a plate pre-inoculated with *E. coli*. The adults were introduced and incubated at a constant temperature of 20°C, and were then removed from the plate after 6 hours, which ensured that all eggs on the plate were laid within 6 hours of each other.

E. coli strain OP50 is the most commonly used food source for *C. elegans*, as its inability to synthesize uracil limits its growth on standard nematode growth medium. This keeps overgrowth of *E. coli* from hindering movement and reproduction of *C. elegans* and makes transport and observation of *C. elegans* easier (Lewis and Fleming, 1995; Wood 1998). To prevent contamination of the medium, streptomycin is present in the standard nematode growth medium. Therefore, the streptomycin resistant mutant of OP50, called OP50-1, was used in this experiment.

The *E. coli* culture was produced with a standard liquid broth for *E. coli* that was incubated at 37°C for 18 hours post-inoculation, and kept at 4°C afterwards. The amount of *E. coli* cells in the broth was standardized by obtaining *E. coli* cells from a streak plate and incubating the cells in fresh 500ml liquid media prepared at 37°C on a heated shaker for 18 hours, as described by Lewis and Fleming (1995). Then a cuvette full of sterile culture was taken as a blank and the optical density (OD) of the *E. coli* broth at 600 nm was measured. Liquid culture for inoculating the petri plates was diluted to achieve the measured OD₆₀₀ of 0.740A, corresponding to a mean cell density of 3.15×10^5 Colony Forming Units (CFU) /ml, counted by

the serial dilution method. The *E. coli* culture was remade every 2 weeks to prevent back mutation or contamination.

2.2.2. Generation and implementation of temperature series

The temperature series used for the experiment were generated in MATLAB. The temperature series used were generated using the Autoregressive (1) [AR(1)] process in MATLAB (for an annotated copy of the MATLAB code see appendix 1). Sequences were generated to change temperature every 40 minutes for 72 hours, which resulted in series with 108 temperature points in total.

Each temperature series was entered into a Memmert ICP-500 incubator, using the manufacturer supplied software Celsius (version 10.1.4). The same software also recorded the actual temperature inside the incubator every minute. As heating and cooling of the incubator was gradual and the speed of heating and cooling were not symmetrical, a small difference between input and output sequences was expected. To ensure that these differences did not significantly differ between non-constant treatments, the output temperature sequence was sampled to match the length of the input temperature sequence, and the difference between the input and output mean, standard deviation, and autocorrelation (calculated with Durbin-Watson statistics in MATLAB) was compared using two-way ANOVA, after the number of replicates per treatment was equalized using the SAS procedure Surveyselect.

In temperature series with high autocorrelation, some series had extreme cases of prolonged runs of high or low conditions in the first 1/3 of the series, which made comparison within the same treatment difficult. To solve this problem, the first 1/3 of each series was analyzed using the SAS procedure AUTOREG, and any series with a Durbin-Watson statistic of

± 6.0 or greater was discarded before use. Each series had 108 data points which were 40 minutes apart, and the same temperature series was used again if the experiment was required to be longer than 72 hours.

2.2.3. Testing the effect of thermal variance on *C. elegans* reproduction.

I designed an experiment to measure the effect of different thermal conditions on *C. elegans* offspring production, in which I measured the number of offspring produced from 5 age-synchronized adults during a 72-hour experimental period.

Each replicate consisted of five 5cm petri plates inoculated with 75 μ l of standardized *E. coli* liquid broth (as described in section 2.2.1.), each with five 98-104 hours old young adults. The experimental plates were placed in a temperature controlled incubator for 72 hours, then the plates were taken out of incubator and the number of offspring on the plate were counted at 25x magnification, using a grid placed under the petri dish as a visual aid. The number of offspring in a replicate (the sum of the individuals counted on 3 randomly selected plates) after 72 hours was analyzed with a Generalized Linear Model in SAS (version 9.2). Each treatment was replicated between 6 to 11 times, as some replicates experienced unexpected disruption in temperature control due to network problems. In addition, microbial contamination, due to back mutation in *E. coli*, contamination in *C. elegans*, or other microbes (e.g. fungus, mold), resulted in significant difficulty in counting and a lower number of individuals counted. Therefore the contaminated plates were discarded to ensure accurate counting on all plates. Some treatments were less susceptible to network problems (e.g., constant treatments) and therefore there were very large differences in the number of replicates (11 replicates for the constant treatment at mean 20°C, and 6 for autocorrelated treatments) between treatments. As a result, the number of replicates per

treatment was equalized using random selection instead of using other statistical methods that are able to work around small inequalities in the number of replicates.

The number of replicates per treatment and number of plates per replicate were equalized with the SAS procedure Surveyselect, resulting in 6 replicates per treatment. The Kolmogorov-Smirnov test for normality reported that the offspring number data was not normal ($p = 0.010$), thus the data was log transformed to satisfy the assumption of normality for ANOVA ($p = 0.104$ after transformation). Two-way ANOVA was done to analyse the effect of mean temperature and type of variance (constant, uncorrelated and uncorrelated) and any possible interaction between the two factors.

2.2.4. Testing the effect of thermal variance on *C. elegans* development

To assess the effect of thermal variance on *C. elegans* individual growth and development, body length and time until maturation were measured using the same treatments as previously described. Five age-synchronized eggs were transferred to a 5-cm petri plate filled with nematode growth medium and inoculated with *E. coli*. Each replicate consisted of five plates, each with 5 eggs on them.

The individual growth of *C. elegans* was estimated using photos taken at 20x magnification so that time consuming steps such as measuring body length could be done later. This approach also minimized the effect of the temperature change caused by taking the plates out of the incubator. Every 12 hours from the start of the experiment, each plate was taken out of the incubator and photos of each individual on the plate were taken at 20x magnification. From these photos the larval stage (L1, L2, L3, L4, young adult and mature adult with eggs) of each individual was determined. This step was repeated every 12 hours, until every female *C. elegans*

on the plate was a mature adult with eggs and the eggs were found on the plate. For each plate, only the time when the first mature adult was observed was recorded, as a 12 hour period was long enough for the rest of the *C. elegans* on the plate to mature.

The body lengths of individuals after a 72 hour period were estimated from the photo using the manufacturer provided software AxioVision (version 4.8.2.0). When *C. elegans* individuals were photographed curled up, up to 4 straight lines were used to estimate body length (figure 2-1).

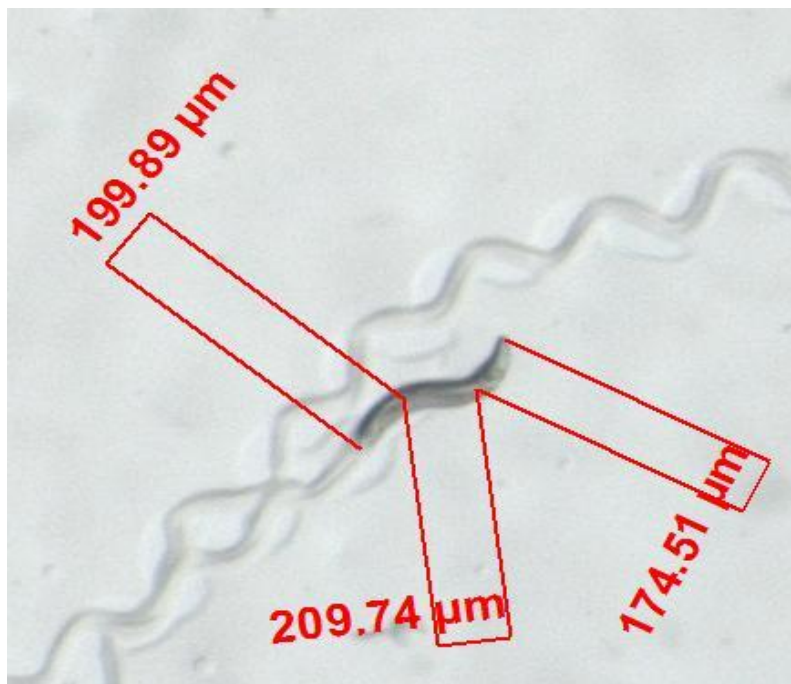


Figure 2-1. An example of *C. elegans* body length estimation (photo taken at 20x magnification).

For each replicate, the mean time until first mature adult was found was calculated, then analyzed using the Kruskal-Wallis test in SAS using the procedure Npar1way. A non-parametric test was used as the maturation time data was ordinal because it was measured every 12 hours and therefore violated the assumptions of an ANOVA. There were at least 4 replicates per treatment, with a total of 24 samples.

The number of *C. elegans* measured for individual length was sampled using the SAS procedure Surveyselect to account for missing individuals or organisms that were unable to be photographed (e.g. the organism was right next to the petri plate wall), then the mean length of *C. elegans* at 72 hours was calculated for each replicate. Two-way ANOVA in SAS was used to analyze the effect of mean temperature and variance. Logarithmic transformation was used to meet the assumption of normality for two-way ANOVA ($p = 0.150$ after transformation, using Kolmogorov-Smirnov test).

2.3. Results

I collected input and output temperature from each incubator, the number of offspring found on experimental plates after 72 hours, time until maturation, and body length of *C. elegans* under different temperature treatments. The experiments counting the number of offspring produced and those measuring body length and time until maturation were done separately, at two different periods. Therefore the input and output temperature series in each experiment will be described separately.

2.3.1. Comparison of input and output temperature signal (for the offspring experiment)

I determined if the temperature signals produced by the incubator were similar to the intended signals. In non-constant treatments, observations indicated that cooling of the incubator was slower than heating, especially when the transition between cooling and heating was abrupt, causing the input and output temperature to be different. Due to this asymmetry in cooling and heating, I expected that the output temperature may be skewed in treatments with frequent switching between heating and cooling, with possible interaction effects.

The calculated input and output mean, variance, and autocorrelation indicated that in uncorrelated treatments, output mean temperature was higher than input at both means. Output standard deviation decreased compared to input standard deviation, and autocorrelation in output was higher than in input. (Table 1).

Table 1. The calculated mean (of total 6 replicates per treatment) of input and output mean, standard deviation (StDv) and autocorrelation (AC) of temperature series used in offspring production experiment.

Mean Temperature (°C)	Treatment	Variable	Input	Output
16	Uncorrelated	Mean	16±0	16.06±0.03
		StDv	5±0	4.60±0.05
		AC	0±0.01	0.13±0.01

20	Correlated	Mean	16±0	15.90±0.05
		StDv	5±0	5.04±0.10
		AC	0.9±0.01	0.89±0.02
	Uncorrelated	Mean	20±0	20.06±0.03
		StDv	5±0	4.65±0.05
		AC	0±0	0.09±0.02
	Correlated	Mean	20±0	19.91±0.00
		StDv	5±0	4.82±0.01
		AC	0.89±0.01	0.90±0.25

Seven replicates per treatment were randomly selected using the SAS procedure Surveyselect to account for uneven replicate numbers per treatment, with a total sample size of 42. To determine if the difference between input and output temperature was influenced by input mean, standard deviation, or autocorrelation, two-way ANOVA ($\alpha = 0.05$) was used.

The mean temperature signal had no significant effect on the difference between input and output mean, ($F_{3, 20} = 9.48$, $p = 0.763$), while autocorrelation increased the output temperature mean compared to the treatments without autocorrelation, ($F_{3, 20} = 9.48$, $p = <0.001$). There was no interaction effect between the two factors, ($F_{3, 20} = 9.48$, $p = 0.782$).

Mean temperature also did not have a significant effect on the difference between input and output standard deviation, ($F_{3, 20} = 19.8$, $p = 0.291$), but autocorrelation decreased the difference in standard deviation, ($F_{3, 20} = 19.8$, $p = <0.0001$). There was no significant interaction between mean and autocorrelation, ($F_{3, 20} = 19.8$, $p = 0.837$).

The difference in input and output autocorrelation was not significantly affected by the mean temperature of the series, ($F_{3, 20} = 13.7$, $p = 0.074$), but intended autocorrelation significantly decreased the difference between the input and output autocorrelation, ($F_{3, 20} = 13.7$, $p = <0.001$) compared to constant treatments. There was no significant interaction effect between the mean temperature and autocorrelation, ($F_{3, 20} = 13.7$, $p = 0.700$).

2.3.2. Comparison of input and output temperature signal (for the body length and time until maturation experiments)

As with the previous chapter, I compared the difference between input and output mean, standard deviation, and autocorrelation in temperature signals sent to and received from the incubators to ensure that there was no unexpected bias in temperature signals due to asymmetry in heating and cooling (Table 2). The mean temperature of a series had no significant effect on the difference between its input and output mean, ($F_{3,12} = 4.82$, $p = 0.397$), but the autocorrelated signals had a higher output temperature than uncorrelated signals, ($F_{3,12} = 4.82$, $p = 0.0094$). No significant interaction effect was found between the mean temperature and autocorrelation, ($F_{3,12} = 4.82$, $p = 0.0642$).

Table 2. The calculated mean and standard deviation, presented after $\pm$ symbol, of input and output mean, standard deviation (StDv) and autocorrelation (AC) of temperature series used in the body length and time until maturation experiments. There were total of 4 replicates per treatment

Mean Temperature	Treatment	Variable	Input	Output
16	Uncorrelated	Mean	16 $\pm$ 0	16.11 $\pm$ 0.03
		StDv	5 $\pm$ 0	4.54 $\pm$ 0.05
		AC	0 $\pm$ 0	0.14 $\pm$ 0.01
	Correlated	Mean	16 $\pm$ 0	15.93 $\pm$ 0.01
		StDv	5 $\pm$ 0	4.99 $\pm$ 0.01
		AC	0.9 $\pm$ 0.01	0.91 $\pm$ 0.01
20	Uncorrelated	Mean	20 $\pm$ 0	20.03 $\pm$ 0.03
		StDv	5 $\pm$ 0	4.70 $\pm$ 0.05
		AC	0 $\pm$ 0	0.09 $\pm$ 0.02
	Correlated	Mean	20 $\pm$ 0	19.93 $\pm$ 0.00
		StDv	5 $\pm$ 0	5.00 $\pm$ 0.01
		AC	0.89 $\pm$ 0.01	0.90 $\pm$ 0.00

Change in mean temperature also did not have a significant effect on the difference between input and output standard deviation, ($F_{3, 12} = 6.97$, $p = 0.569$), but increased autocorrelation decreased the difference between input and output standard deviation, ($F_{3, 12} =$

6.97, $p = 0.0015$). There was no significant interaction between mean and autocorrelation, ($F_{3, 12} = 6.97$, $p = 0.0747$).

The difference between input and output autocorrelation was not significantly affected by the mean temperature of the series ($F_{3, 12} = 8.98$, $p = 0.691$), but increased autocorrelation decreased the difference, ($F_{3, 12} = 8.98$, $p = 0.0005$). There was a significant interaction effect between the mean temperature and autocorrelation, ($F_{3, 12} = 8.98$, $p = 0.0487$), where an increase in mean temperature decreased the difference between input and output autocorrelation in the series with no initial autocorrelation, while the opposite trend was shown when the intended autocorrelation was 0.9.

2.3.3. Effect of variance and autocorrelation on *C. elegans* reproduction

The number of *C. elegans* offspring produced after 72 hours was compared between three different temperature signals (constant, uncorrelated, autocorrected) for each of two different mean temperatures (figure 2-2).

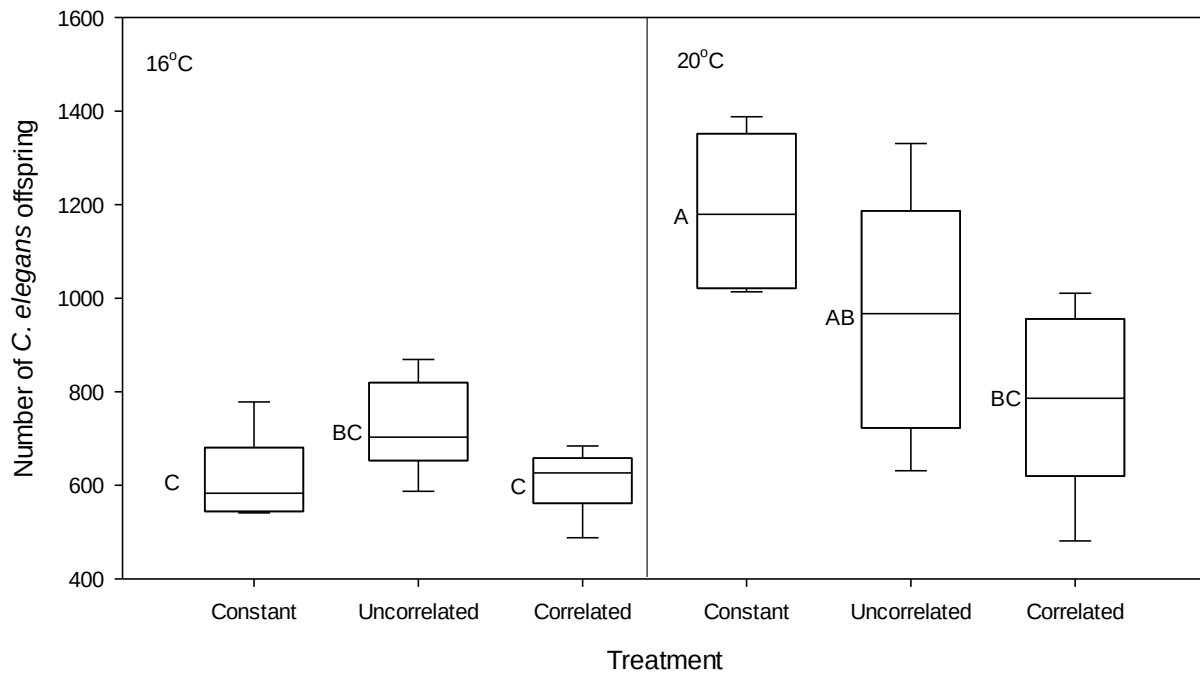


Figure 2-2. Number of *C. elegans* offspring counted after 72 hours at three different variance treatments (constant, uncorrelated and autocorrelated) at two different temperature means (16°C and 20°C). Letters next to each box indicate grouping done by Tukey's post-hoc test. Means that share a letter are not significantly different from each other. The middle line in the boxplot indicates the median, and the edges of the box indicate the first and third quartiles, and the two edges of the bar indicate the minimum and maximum.

The effect of mean temperature and variance on the number of offspring produced was determined using two-way ANOVA. The number of *C. elegans* offspring produced within the 72 hour experimental period was higher at mean 20°C compared to 16°C, ($F_{5,30} = 9.82$, $P < .0001$). The number of offspring at mean 20°C was lower in treatments with variance compared to the constant treatment, ($F_{5,30} = 9.82$, $P < 0.0244$). There was a significant interaction between variance and temperature, ($F_{5,30} = 9.82$, $P < 0.0199$). One-way ANOVA analysis of the number

of offspring produced indicated that changes in variance did not have any effect on the number of offspring at mean 16°C, ($F_{2, 15} = 3.30$, $P < 0.0648$), but resulted in a significant reduction in the number of offspring at mean 20°C, ($F_{2, 15} = 4.64$, $P < 0.0269$) (Figure 2-3).

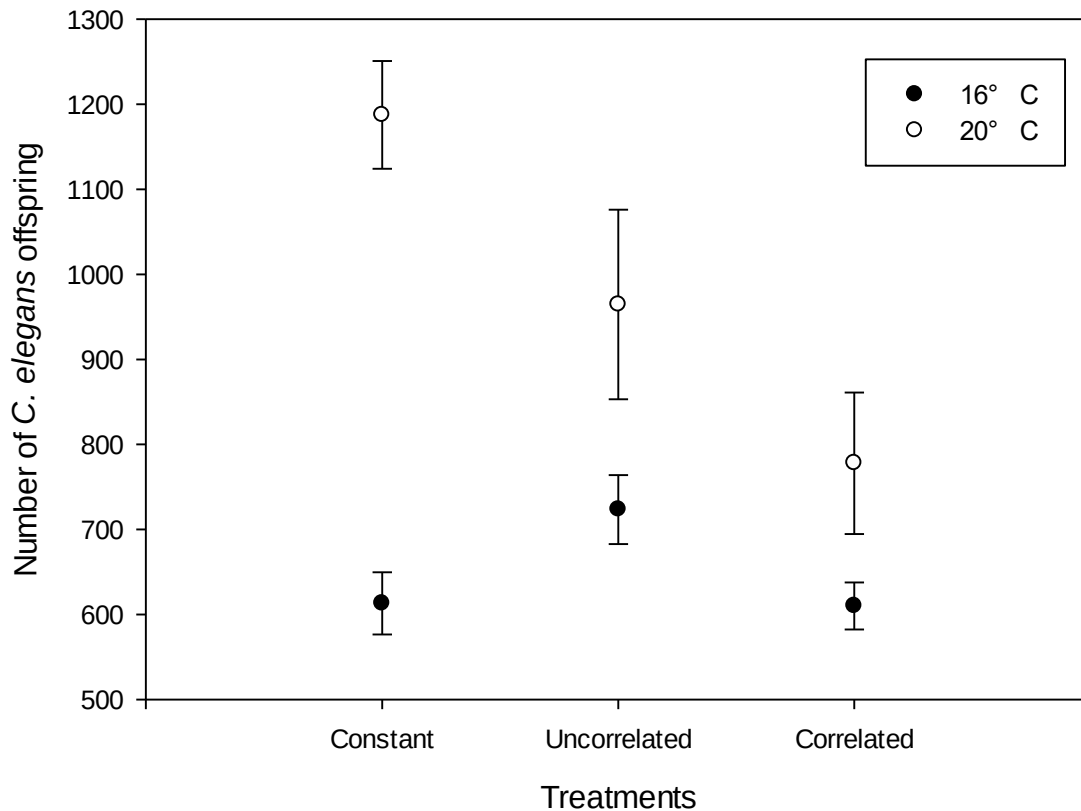


Figure 2-3. Interaction plot comparing the effect of different variance treatments on offspring production at two different temperature means (16°C and 20°C). The dots indicate the mean and error bars indicate standard error.

2.3.4. The effect of temperature on time to maturation in *C. elegans*

The effect of variance on the mean time until maturation for each replicate was analyzed using the Kruskal-Wallis one-way non-parametric test. When the mean temperature was 16°C, change in variance had no effect on the time until maturation, ($DF = 2$, $p = < 0.44$). However, autocorrelated treatments had significantly longer time to maturation at mean 20°C (Figure 2-4).

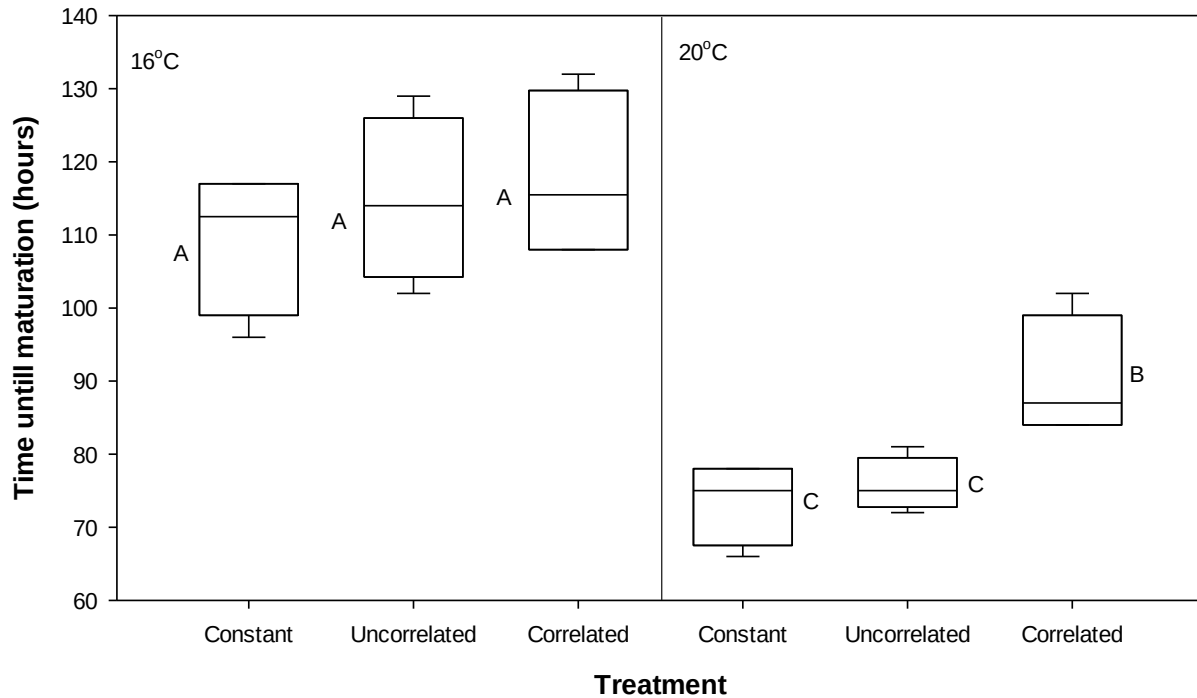


Figure 2-4. Mean time until a mature *C. elegans* was found at three different thermal conditions (constant, uncorrelated and autocorrelated) at two different temperature means (16°C and 20°C). Letters next to each box indicate grouping done by pairwise comparison. Means that share a letter are not significantly different from each other. The middle line in the boxplot indicates the median, and the edges of the box indicate the first and third quartiles, and the two edges of the bar indicate the minimum and maximum.

2.3.5. The effect of temperature on *C. elegans* body length

I measured the length of *C. elegans* individuals after 72 hours to determine the effect of mean temperature and variance in temperature signal on the individual growth of *C. elegans*. Two-way ANOVA was used to determine the effect of each treatment on *C. elegans* body length, estimated in micrometers. (Figure 3-6). Mean temperature had a significant effect on the body length of *C. elegans*, as the treatments at mean 20°C had longer body length than the treatments at mean 16°C, ($F_{5, 18} = 86.32$, $p < 0.0001$). Variance also had a significant effect on the body length, ($F_{5, 18} = 9.14$, $p = 0.0018$), but there was no significant interaction effect between the two factors ($F_{5, 18} = 1.19$, $p = 0.3281$) (figure 2-5).

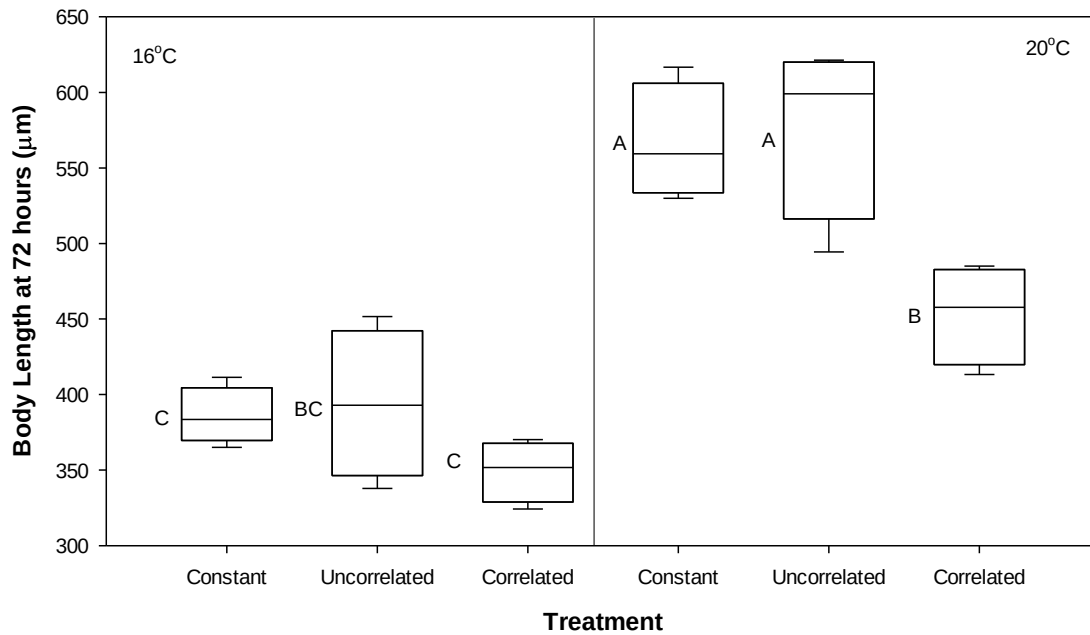


Figure 2-5. Body length measured at 72 hours after the experiment for *C. elegans* under three different variance treatments (constant, uncorrelated and autocorrelated) at two different temperature means (16°C and 20°C). Letters indicate grouping done by Tukey's post-hoc test. Means that share a letter are not significantly different from each other. The middle line in the boxplot indicates the median, and the edges of the box indicate the first and third quartiles, and the two edges of the bar indicate the minimum and maximum.

One-way ANOVA, conducted separately for each temperature mean, indicates that body length does not differ between treatments with variance at mean 16°C, ($F_{2,9} = 1.78$, $p = 0.2225$), while the autocorrelated treatment had significantly shorter body length than the constant and uncorrelated treatments at mean 20°C ($F_{2,9} = 8.90$, $p = 0.0074$).

2.4. Discussion

The effect of uncorrelated variance in temperature on ectotherm performance has been thoroughly examined (Siddiqui and Barlow 1972; Bozinovic *et al.* 2011). Experimental studies have shown that variance in environmental temperature will have a negative effect on the population growth and fecundity of an organism when the mean temperature is located close to the thermal optimum of the organism (Siddiqui and Barlow 1972; Bozinovic *et al.*, 2011). However, the effect of thermal variance can be positive (Bozinovic *et al.*, 2011) when the mean temperature is near the lower limit of the organism's thermal range. This difference in the effect of variance is mainly attributed to asymmetry in the thermal performance curve of ectotherms (Izem and Kingsolver, 2005) and the difference between the consequences of breaking the lower limit and the upper limit of the thermal range in these organisms (Deutsch *et al.*, 2008). The asymmetry in the consequences of breaking the lower and upper thermal limit may have a larger impact when environmental temperature is positively autocorrelated, where longer periods of extreme conditions are more common (Lawton, 1988), resulting in a larger effect on organismal performance compared to the uncorrelated condition.

2.4.1. The effect of variance and autocorrelation on offspring production

At mean 20°C, uncorrelated variance did not significantly affect the number of *C. elegans* offspring produced after 72 hours. This lack of significant reduction in offspring numbers is unexpected, as theoretical models such as Lewontin and Cohen (1969) predicted that stochastic change in the rate of population increase will result in reduced population size. Reduction in population growth rate due to thermal fluctuation when the mean temperature is close to the optimal temperature was also observed experimentally (Bozinovic *et al.*, 2011). Although this

apparent lack of negative effect on offspring production may simply be due to the magnitude of variance not being large enough to significantly influence *C. elegans* offspring production, a more likely cause is asymmetry in *C. elegans*' thermal performance curve with respect to egg production.

The reproductive ability of *C. elegans* diminishes at high temperatures ($< 27^{\circ}\text{C}$) due to thermal stress on germ cells (Bagasse et. al., 2015), especially on sperm cells. An adult *C. elegans* hermaphrodite does not produce new sperm cells, therefore thermal damage to the sperm cells will reduce the total number of eggs that it is able to fertilize. However, although the lifetime total number of eggs produced by *C. elegans* is lowered at high temperatures ($< 25^{\circ}\text{C}$) the egg laying rate is not significantly slowed (9.1 eggs/hr at 20°C , 8.1 eggs/hr at 25°C) (Byerly, Cassada & Russel, 1976). This result suggests that number of eggs laid would not appear to significantly decrease as a consequence of high temperature until the later stages of life, where sperm cell mortality will reduce the total number of eggs that the individual is able to produce. Therefore reduction in *C. elegans* reproductive rate due to sperm cell damage may not be visible during the 72-hour experimental period.

Furthermore, the egg laying rate of *C. elegans* is much lower at 16°C (5.4 eggs/hr) in comparison to higher temperatures (Byerly, Cassada & Russel, 1976). Therefore the negative effect of low temperature (fewer eggs laid in a given period) is more visible than the negative effect of high temperature (lower lifetime egg production) in a short experimental period. This asymmetry, which enhances the comparative benefits of high temperature, may explain why there was no significant reduction in offspring numbers as expected. If the experimental period were longer - where the full consequences of higher temperatures would be more visible and the

rate of population increase would be the main variable to consider, rather than egg production - a reduction in offspring number due to thermal variance may be observed.

At the same mean (20°C), strong positive temporal autocorrelation resulted in significant reduction in number of offspring produced compared to the constant treatment. The most likely cause of this reduction in offspring size is asymmetry in the biological consequences of the high extreme and low extreme in the treatment. The temperature range of the mean 20°C treatments was about $\pm 12^\circ\text{C}$, with a high extreme of 32°C and a low of 8°C. 32°C is very close to the lethal temperature of wildtype *C. elegans* (33°C), and egg production ceases at about 30°C (McMullen et al., 2012).

Low temperature has much less lasting consequences in the given temperature range, as the worst possible consequence of extreme low temperatures is very slow development speed, which halts at 7°C (Venette and Ferries, 1997; Bagasse et. al., 2015). This asymmetry is further amplified in longer periods of thermal extremes, as a long period of high temperature is more detrimental to sperm cell viability than multiple short exposures to the same high temperature, even if the total period remains the same (Bagasse et. al., 2015). The estimated thermal performance of *C. elegans* population growth suggests that there is an asymmetry in performance between 32°C and 8°C (Venette and Ferries, 1997), where the expected population increase at mean 32°C is less than half of the expected increase at 8°C. Strong positive autocorrelation increases the likelihood of continued runs of extreme conditions, and therefore the asymmetry in performance at extreme temperatures is more exaggerated, resulting in significant reduction in offspring production.

Another possible factor that could be contributing to smaller offspring number in autocorrelated treatment at mean 20°C is the discrepancy between input and output mean

temperature in autocorrelated treatments. The incubators used for the experiments, due to their mechanical limitations, displayed a delay in implementing the input temperature in regions where a drastic change in temperature occurred in a short time period (Figure 2-6). This delay in execution and failure to reach local maxima or minima in time (shown in section (a) of Figure 2-6) resulted in a decrease in output mean temperature in positively autocorrelated treatments, which was sometimes as high as 0.1°C. Although expected decrease in egg production due to 0.1°C decrease in mean temperature is very small, it may be a contributing factor.

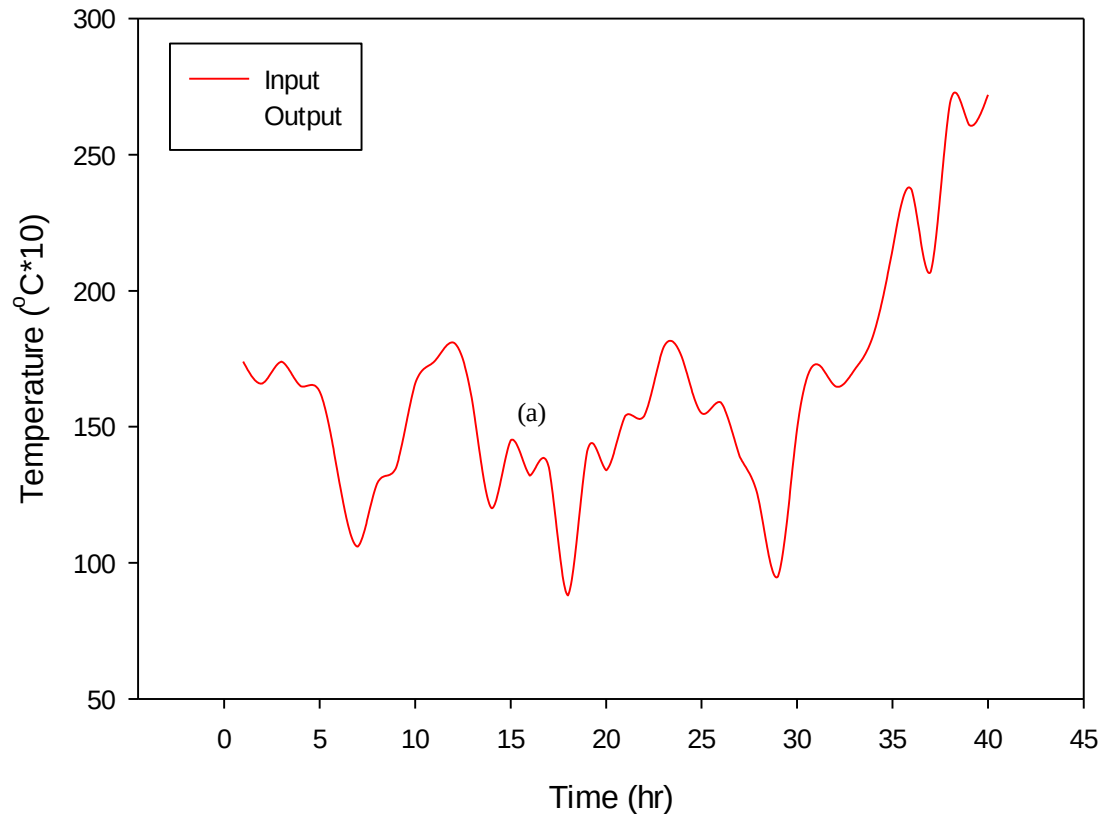


Figure 2-6. An example of input-output temperature discrepancy in the Memmert ICP-500 incubator used in the experiments. The sequence was sampled from a positively autocorrelated series (intended degree of autocorrelation of 0.9) at mean 20°C with standard deviation of 5°C (Sampled series input mean: 16.38°C, input standard deviation 4.20°C, input autocorrelation: 0.737, output mean: 16.29°C, output standard deviation 4.19°C, output autocorrelation: 0.762).

Another consequence of this slower-than-expected transition in temperature is exaggeration of autocorrelation in uncorrelated treatments, where a sudden change in temperature is expected to happen more frequently, regardless of the mean temperature. As a result, the realised temperature sequence for uncorrelated input was more autocorrelated than intended. Similarly, as the output temperature failed to precisely reach the local minima or maxima in uncorrelated treatments, output standard deviation was also smaller than the input.

At mean 16°C, there was no significant difference between the numbers of offspring produced under variable or constant conditions. This is surprising considering that egg laying rate benefits significantly from increased temperature with very few consequences that would be visible within the experimental period, especially at mean 16°C. However, egg production is known to stop at temperatures under 7°C, which may have balanced out the potential benefits of increased temperature. As with the uncorrelated treatment at 20°C, it is also possible that a standard deviation of 5°C was not large enough to result in a significant difference in *C. elegans* offspring production on the scale of that observed under the uncorrelated treatment at 20°C.

The introduction of positively autocorrelated variance at mean 16°C also did not have any significant effect on number of offspring produced, compared to both constant conditions and conditions with uncorrelated variance. Most of the acute detrimental effects of high temperature in *C. elegans*, such as sterilization or heat death, start to happen above 28°C (McMullen et al., 2012). In a treatment with an expected temperature range of 4-28°C, the asymmetry which caused the decrease in offspring number at mean 20°C is absent, resulting in the absence of any visible negative impact.

An interaction effect was observed between mean temperature and autocorrelated variance. Autocorrelated variance had a negative effect on offspring production when the mean

temperature was close to optimum (20°C), but it had no effect on offspring production when the mean was suboptimal (16°C). This change in the effect of autocorrelated variance due to a shift in mean temperature is consistent with studies (e.g. Izem and Kingsolver, 2005; Ikemoto, 2005; Vasseur *et al.*, 2014) which predict that the effect of variance will change in both magnitude and direction depending on the mean temperature. The expected negative effect of high temperature is more detrimental at mean 20°C, as the range where high temperature will exert long term negative effect on *C. elegans* reproduction is smaller at mean 16°C (26 - 28°C) compared to its counterpart at mean 20°C (26 - 32°C). As a result, the chance that a period of high temperature will have negative impact on *C. elegans* reproduction is higher at mean 20°C, whereas at mean 16°C it is less likely.

Apart from the effects of differences in mean temperature and variance, depletion of the food source may affect fecundity. The treatment which is expected to have the highest resource consumption, constant condition at 20°C, did not display signs of resource limitation such as dauer formation (Wood, 1998; Anderson et al., 2007) and the experimental plates displayed limited lawn growth throughout the experimental period. The threshold concentration of *E. coli* suitable for *C. elegans* population growth is 5.1×10^3 (Venette and Ferris, 1998), which is much lower than the concentration measured on the experimental plate, 3.15×10^5 . Therefore the lack of food source is unlikely to have been the factor in reduction in *C. elegans* offspring production found in autocorrelated treatment at mean 20°C.

2.4.2. The effect of variance and autocorrelation on time to maturation in *C. elegans*

I found that, at a mean temperature of 20°C, changes in variance had a significant effect on the time until maturation, as there was a significant increase in time until maturation in

autocorrelated treatments. Larval growth speed in *C. elegans* with respect to temperature has a typical asymmetric shape commonly found in other small ectotherms, where the growth speed increases with temperature until it peaks at 25.5°C, followed by sudden decline in growth above 27°C, as high temperature results in heat stress on the *C. elegans* cell division process (Begssae *et al.*, 2015).

Unlike egg production, peak performance of larval growth speed is very close to the end of the curve (within 2°C) and therefore the asymmetry in the curve is more prominent. However, there was no significant difference in time until maturation between the constant treatment at 20°C and uncorrelated variance at the same mean. The possible reason for this result is that mean temperature of 20°C is sufficiently far enough from the peak temperature for *C. elegans* larval growth that the reduction in biological performance due to thermal variance may be low. Martin and Huey (2008) have shown that organisms may thrive at what would be considered as ‘suboptimal’ means, where the temperature is too low for an organism to function at maximum performance, because it provides a sufficient buffer to a potentially detrimental temperature fluctuations. This explanation would be consistent with the significant increase in time until maturation in *C. elegans* found in autocorrelated treatment, as the asymmetry in the thermal performance curve of *C. elegans* larval growth would be exaggerated under strong positive autocorrelation.

At mean 16°C, thermal variance, either with or without autocorrelation, had no significant effect on time until maturation. At mean 16°C, the potential benefits of increased temperature are similar to the disadvantages of decreased temperature, as the thermal performance curve of *C. elegans* larval growth within the thermal range found in 16°C treatment (from 4- 6°C to 26- 28°C) is mostly linear (from 7°C to 26°C) and asymmetry is only found in

the tail end of the relevant part of the curve. This symmetry would also explain why introduction of positively autocorrelated variance at mean 16°C also did not have any significant effect on time until maturation, compared to both constant conditions and conditions with uncorrelated variance.

2.4.3. The effect of variance and autocorrelation on body length

Although the maximum body length of *C. elegans* is longest at mean 20°C, and shorter at 16°C and 25°C, body length is proportional to the developmental stage of the individual (Byerly, Cassada & Russel, 1976) until older age (>100 hours). Therefore, both body length and time until maturation are expected to respond similarly to thermal variance, as both are proportional to each other for the early part of the *C. elegans* life cycle.

At a mean temperature of 20°C, there was no difference in the body length of *C. elegans* exposed to uncorrelated thermal variance and those experiencing constant conditions. Similar to time until maturation, the mean of 20°C is far from the temperature at which larval growth peaks, which would reduce the negative effect of thermal variance on *C. elegans* body length (Martin and Huey, 2008). Worms which were exposed to autocorrelated variance at mean 20°C had significantly shorter body length compared to individuals which grew under constant condition or uncorrelated variance. As with time to maturation, this is likely the result of the asymmetry in biological performance in high and low extreme temperatures, exaggerated by autocorrelation. At mean 16°C, thermal variance, either with or without autocorrelation, had no significant effect on time until maturation. Again, this result is similar to the time until maturation results.

Although treatments at 20°C did display negative effect of autocorrelated thermal variance which was absent in treatments at 16°C, no significant interaction effect was found. The interaction effect between different mean temperatures on biological performance is a result of

the asymmetrical shape of thermal performance curve. Therefore the lack of interaction effect may be an evidence that the shape of the section of the thermal performance curves relevant to both means is closer to symmetrical, as a result of both means being located far away from the peak region in thermal performance curve.

2.4.4. Summary

Overall, only autocorrelated treatments at mean 20°C had a significant reduction in biological performance with regards to offspring production and development of *C. elegans*. The lack of significant negative effect (i.e. decrease in offspring size, smaller body length at a given age) in uncorrelated treatments may be due to the shape of the thermal performance curve involved in the measured variable. In the case of body growth and developmental time, both means may have been too far from the peak of the curve to observe the expected negative effect of thermal variance. In the case of offspring production, it is possibly due to the fact that increase in temperature differently affects the two aspects contributing to offspring production, in addition to the fact that one aspect is more visible in a short term experimental setup. At mean 20°C, a temperature closer to the peak of thermal performance curve for both offspring production and individual development, autocorrelation had a negative effect on *C. elegans* performance. However, this effect is absent at mean 16°C.

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Appendix

Appendix 1. MATLAB codes used to generate the temperature series

The code below was used to generate the temperature series used. When a set of series shared a mean and standard deviation, differing only in the degree of autocorrelation, each of those series were generated from a shared set of randomly generated elements, differing only in how they were arranged. All MATLAB code was written by Dr. Kim Cuddington.

Makeseries.m: this code generates a temperature series with given mean, standard deviation and autocorrelation. To start, a matrix containing the intended mean, standard deviation, and autocorrelation is required. For example, the following command will generate 5 temperature series (mean 20 constant, mean 16, standard deviation 5, and no autocorrelation, and three temperature series which all have mean 16, standard deviation of 2.5, and three varying degrees of autocorrelation: 0.0, 0.5, and 0.9) stored in a matrix named ser:

```
>> request_m = {20 0 0 ; 16 2.5 0 ; 16 5 [0 0.5 0.9]};  
>> ser = MakeSeries(request_m)
```

Series made as a group (e.g. made from the matrix 16 5 [0 0.5 0.9]) will be made from the same set of elements, where elements will be rearranged until the desired degree of autocorrelation is achieved.

```
function ser = MakeSeries(request_matrix)
```

```
ser = [];
```

```
loopnum = size(request_matrix);  
loopnum = loopnum(1);
```

```
for i = 1:loopnum % converts the request_matrix into number of 1 x 3 matrices, and generates  
a series for each of them
```

```
    [msend stdevsend arsend] = request_matrix{i,:};  
    sends = [msend stdevsend arsend];
```

```

newfriends = wonAR(sends); % Uses function wonAR to generate the sequences
ser = horzcat(ser, newfriends);
end;

```

wonAR.m: This code generates Autoregressive (1) series of a given length, by generating normally distributed random variables with given mean and standard deviation. Once the variables are produced, they are arranged by the code until desired degree of autocorrelation is achieved.

```

function seri = wonAR(row)

```

```

M = [];

```

```

randn('state',sum(100*clock)) % generates normally distributed random variables
leg=216;simleg=108 ; % length of series
betaco=[row(3:end)]; % autocorrelation
lbet=length(betaco);
varsq=row(2); % standard deviation of series
mr=row(1); % mean of series

```

%The code below attempts to generate a series with intended autocorrelation from previously generated random elements, which it will continue doing until the autocorrelation of the series matches the intended autocorrelation.

```

for auto=1:lbet %2
    tol=0.01;spectral=betaco(auto); % specifies error tolerance. The code will keep generating the
    series until the tolerance is met.
    acoef=betaco(auto);
    slope=[-99 -99];
    while (((slope(2)<(spectral-tol))|(slope(2)>(spectral+tol))))|mn<4)%3;
        gs(1)=randn;
        crand=randn(1,leg);
        for cnts=2:leg %4,

            gs(cnts) =(acoef).*gs(cnts-1)+crand(cnts);
        end %4;
        fnoise=(gs(1:simleg));
        meanxn=mean(fnoise); varxn=(std(fnoise));
        noise=(((fnoise-meanxn)./(varxn))*varsq)+mr;

        z3=noise;
        if spectral==0 %5
            standnoise(auto,:)=z3;
            z=z3;

```

```

        else %5
            z=mimicry(standnoise(1, :),z3); % Calls for function mimicry, then arranges the elements
in standnoise to be in the same rank order as z3.
        end %5;
        mn=min(z);
        ac=corrcoef(z(2:end),z(1:end-1));
        slope(2)=ac(1,2);
    end; %3
    zseries(auto,:)=z;
    zslopes(auto)=ac(1,2);

end;%2
aset=zseries;
tempset=uint16(zseries*10);
for a=1:lbt;
    ac=corrcoef(zseries(a,2:end),zseries(a, 1:end-1));
    alpha(a)=ac(2,1);
end;
seri=[M tempset'];

```

CheckSer %calls for code CheckSer.m, which checks if the series generated has the intended mean, standard deviation, and autocorrelation.

CheckSer.m: This code checks the generated temperature series, then calculates mean, standard deviation, and autocorrelation.

```

loopnum = size(ser);
loopnum = loopnum(2);

stats = [];

for i = 1:loopnum
    dataset = double(ser(1:end, i));
    calcmean = mean(dataset);
    calcstd = std(dataset);
    autocorrs = corrcoef(dataset(1:end-1), dataset(2:end));
    calcautocorr= autocorrs(2);
    calcmin = min(dataset);
    calcmax = max(dataset);
    stats = horzcat(stats, [calcmean;calcstd;calcautocorr;calcmin;calcmax]);
end;

stats

```

psdslop5.m: This code estimates the spectral slope of a given series.

```
function [specSlope] = psdslope(x)
%PSDSLOPE Power Spectral Slope estimate.
% [intercept specSlope] = psdslope(X) estimates the Power Spectral Density of
% signal vector X using Welch's averaged periodogram method with no overlap and
% no windowing. By default, ignores first 3 points of spectrum when calculating slope

nfft = length(x);
window = hanning(nfft);
noverlap = 0;
Fs = 1;
p = [];
dflag = 'none';
% compute PSD
x = x(:); % Make sure x is a column vector
window = window(:);
n = length(x); % Number of data points
nwind = length(window); % length of window
if n < nwind % zero-pad x if it has length less than the window length
    x(nwind)=0; n=nwind;
end
k = fix((n-noverlap)/(nwind-noverlap)); % Number of windows
% (k = fix(n/nwind) for noverlap=0)

index = 1:nwind;
KMU = k*norm(window)^2; % Normalizing scale factor ==> asymptotically unbiased

Spec = zeros(nfft,1); Spec2 = zeros(nfft,1);
for i=1:k
    if strcmp(dflag,'none')
        xw = window.*(x(index));
    elseif strcmp(dflag,'linear')
        xw = window.*detrend(x(index));
    else
        xw = window.*detrend(x(index),0);
    end
    index = index + (nwind - noverlap);
    Xx = abs(fft(xw,nfft)).^2;
    Spec = Spec + Xx;
    Spec2 = Spec2 + abs(Xx).^2;
end
```

```

% Select first half
if ~any(any(imag(x)~=0)), % if x is not complex
    if rem(nfft,2), % nfft odd
        select = (1:(nfft+1)/2)';
    else
        select = (1:nfft/2+1)';
    end
    Spec = Spec(select);
    Spec2 = Spec2(select);
else
    select = (1:nfft)';
end
freq_vector = (select - 1)*Fs/nfft;

Spec = Spec*(1/KMU); % normalize

%ignore first N points of spectrum for slope measurement
N = 10;
r = length(Spec)-(N-1);
X = [ones(r,1), log(freq_vector(N:length(freq_vector)))];
specSlope = X \ log(Spec(N:length(Spec)));

```

Mimicry.m: This function takes 2 vectors, and arranges one in the same rank order as the other.

```

function z = mimicry (x,y)
% z = mimicry(x,y)
% x, y: 2 real vectors of length T
% z: real vector of length T in which the elements of x
% occur in the same rank order as the elements of y
% 10 September 1995
[xsort,xindex] = sort(x) ;
[ysort,yindex] = sort(y) ;
[zsort,zindex] = sort(yindex) ;
z = xsort(zindex);
return

```

Appendix 2. Raw temperature data

Table 3. Raw data for input and output temperature sequences used in the *C. elegans* offspring production experiment.

Date (yyddmm)	Incubator	Temperature(°C)	Standard deviation	Autocorrelation	Input Mean	Input Standard deviation	Input Autocorrelation	Output Mean	Output Standard deviation	Output Autocorrelation
130722	i4	16	5	0	159.97	50.05	-0	160.88	45.43	0.123
130722	i3	16	5	9	159.97	50.05	0.89	159.17	50.02	0.897
130727	i3	16	5	0	160.03	49.99	0.01	161.08	46.04	0.105
130727	i6	16	5	9	160.03	49.99	0.89	159.26	49.81	0.904
130731	i4	16	5	0	159.96	50	0.01	157.73	48.91	0.171
130731	i6	16	5	9	159.96	50	0.9	159.16	49.92	0.91
130803	i5	20	5	0	200	49.98	-0	200.79	45.97	0.061
130803	i1	20	5	9	199.97	49.99	0.89	199.22	49.83	0.895
130803	i3	20	5	9	199.97	49.99	0	200.78	46.16	0.087
130803	i4	20	5	9	200	49.98	0.9	198.03	53.17	0.878
130808	i3	20	5	0	199.97	50	0	200.7	46.2	0.085
130808	i5	20	5	0	200	49.98	-0	200.93	45.57	0.063
130808	i1	20	5	9	199.97	50	0.89	199.22	49.91	0.895
130808	i4	20	5	9	200	49.98	0.9	199.3	49.97	0.912
130820	i3	20	5	9	200.02	25.04	0.89	197.62	31.41	0.626
130820	i5	20	5	9	200.02	50.01	0.9	199.19	49.95	0.908
130908	i6	20	5	0	200.05	49.98	0	200.83	46.81	0.093
130908	i3	20	5	9	200.05	49.98	0.89	199.19	49.66	0.903
130916	i4	16	5	9	160.02	50	0.9	158.35	51.94	0.879
131018	i5	16	5	0	160.019	50.01	-0	160.75	45.807	0.132
131018	i4	16	5	9	160.019	50.01	0.89	158.25	52.014	0.843
131018	i1	20	5	0	200.019	50.01	-0	200.185	47.123	0.099
131018	i6	20	5	9	200.019	50.01	0.89	199.25	49.953	0.902
131021	i5	16	5	0	160.065	50	0	160.991	45.666	0.128
131021	i4	16	5	9	160.065	50	0.9	159.519	49.951	0.913
131021	i6	20	5	9	200	50	0.9	199.222	50.101	0.911
131028	i3	16	5	0	159.981	50	-0	160.361	46.481	0.108
131028	i4	16	5	9	159.981	50	0.9	159.389	49.891	0.907
131028	i1	20	5	0	200	50	0	200.269	46.998	0.075
131028	i5	20	5	0	199.981	50	-0	200.241	46.578	0.103
131114	i6	16	5	0	160.028	49.97	0.01	162.102	43.939	0.173

131114	i5	16	5	9	160.028	49.97	0.89	159.176	49.789	0.904
131114	i3	20	5	0	200.028	49.97	0.01	200.62	46.797	0.11
131114	i4	20	5	9	200.028	49.97	0.89	199.269	49.885	0.903

Table 4. Raw data for input and output temperature sequences used in the *C. elegans* body length and maturation time experiments

Date (yyddmm)	Incuba tor Numb er	temp eratu re	Standar d deviatio n	autoc orrelat ion	Input Mean	Input Standard deviation	Input Autocorr elation	Output Mean	Output Standard deviation	Output Autocorre lation
131018	i5	16	5	0	160.0185	50.00897	-0.002	160.75	45.80681	0.132
131021	i5	16	5	0	160.0648	50.00061	0.002	160.907	45.66569	0.128
131028	i3	16	5	0	159.9815	50.00411	-0.005	160.3611	46.48112	0.108
131104	i1	16	5	0	160.0185	49.99532	0.001	161.2593	45.42394	0.145
131104	i6	16	5	0	159.9815	50.00411	-0.005	161.5	44.65663	0.142
131114	i6	16	5	0	160.0278	49.97241	0.005	162.1019	43.9392	0.173
131121	i5	16	5	0	159.9815	50.00411	-0.005	160.8704	45.533	0.123
131018	i4	16	5	9	160.0185	50.00897	0.887	158.25	52.01404	0.843
131021	i4	16	5	9	160.0648	50.00061	0.900	159.5185	49.95072	0.913
131028	i4	16	5	9	159.9815	50.00411	0.895	159.3889	49.89088	0.907
131104	i3	16	5	9	160.0185	49.99532	0.906	159.713	49.33597	0.923
131104	i4	16	5	9	160.0185	49.99532	0.906	159.4722	49.41763	0.922
131114	i5	16	5	9	160.0278	49.97241	0.888	159.1759	49.78906	0.904
131121	i1	16	5	9	160.0185	49.99532	0.906	159.287	49.85644	0.919
131018	i1	20	5	0	200.0185	50.00897	-0.002	200.1852	47.12345	0.099
131028	i1	20	5	0	200	49.9972	0.000	200.2685	46.99763	0.075
131028	i5	20	5	0	199.9815	50.00411	-0.005	200.2407	46.57802	0.103
131114	i3	20	5	0	200.0278	49.97241	0.005	200.6204	46.79658	0.110
131121	i3	20	5	0	200	49.9972	0.000	199.9907	47.61518	0.065
131018	i6	20	5	9	200.0185	50.00897	0.887	199.25	49.95271	0.902
131021	i6	20	5	9	200	49.9972	0.902	199.222	50.10071	0.911
131114	i4	20	5	9	200.0278	49.97241	0.888	199.2685	49.88465	0.903
131121	i4	20	5	9	200.0185	50.00897	0.887	199.2593	49.96566	0.900

Table 5. Raw input temperature series (mean 20°C, standard deviation 5°C and autocorrelation of 0.9) and output series (recorded every minute) sampled to match the corresponding time.

time(hh:mm:ss)	Input temperature (C°/10)	Output temperature (C°*10)
0:40	179	180
1:20	200	197
2:00	205	205
2:40	203	202
3:20	178	178
4:00	202	198
4:40	222	221
5:20	241	240
6:00	249	248
6:40	259	257
7:20	297	297
8:00	268	268
8:40	263	262
9:20	258	257
10:00	278	277
10:40	304	303
11:20	286	286
12:00	252	252
12:40	246	245
13:20	243	242

14:00	262	261
14:40	255	256
15:20	214	216
16:00	195	193
16:40	164	165
17:20	144	143
18:00	148	146
18:40	190	187
19:20	158	161
20:00	145	145
20:40	138	138
21:20	141	140
22:00	127	128
22:40	128	126
23:20	106	106
24:00:00	94	93
24:40:00	41	46
25:20:00	127	116
26:00:00	158	158
26:40:00	151	153
27:20:00	161	158
28:00:00	153	153

28:40:00	144	143
29:20:00	148	146
30:00:00	166	163
30:40:00	150	151
31:20:00	122	123
32:00:00	147	143
32:40:00	152	151
33:20:00	176	173
34:00:00	191	191
34:40:00	186	187
35:20:00	216	212
36:00:00	234	232
36:40:00	212	212
37:20:00	191	191
38:00:00	180	180
38:40:00	175	175
39:20:00	173	172
40:00:00	177	176
40:40:00	202	200
41:20:00	213	212
42:00:00	247	247
42:40:00	258	257

43:20:00	285	286
44:00:00	320	318
44:40:00	344	342
45:20:00	271	275
46:00:00	237	236
46:40:00	246	242
47:20:00	225	226
48:00:00	243	240
48:40:00	215	217
49:20:00	192	192
50:00:00	177	177
50:40:00	175	173
51:20:00	188	186
52:00:00	195	193
52:40:00	174	175
53:20:00	196	192
54:00:00	176	177
54:40:00	172	172
55:20:00	148	148
56:00:00	142	141
56:40:00	165	162
57:20:00	156	156

58:00:00	167	165
58:40:00	190	187
59:20:00	210	208
60:00:00	207	207
60:40:00	216	215
61:20:00	250	248
62:00:00	242	242
62:40:00	223	223
63:20:00	230	227
64:00:00	225	225
64:40:00	251	250
65:20:00	227	228
66:00:00	211	211
66:40:00	206	206
67:20:00	179	180
68:00:00	188	186
68:40:00	192	191
69:20:00	196	195
70:00:00	208	207
70:40:00	203	203
71:20:00	216	215
72:00:00	230	228

Table 6. Raw input temperature series (mean 20°C, standard deviation 5°C and autocorrelation of 0.0) and output series (recorded every minute) sampled to match the corresponding time. This series was generated at the same time as the series presented in table 5, and therefore is made of the same elements.

time(hh:mm:ss)	Input temperature (C°/10)	Output temperature (C°*10)
0:40	196	196
1:20	239	237
2:00	232	232
2:40	142	148
3:20	197	190
4:00	197	198
4:40	215	212
5:20	222	221
6:00	214	215
6:40	201	201
7:20	217	215
8:00	211	211
8:40	248	247
9:20	174	180
10:00	205	200
10:40	155	158
11:20	172	167
12:00	197	195
12:40	187	188

13:20	191	190
14:00	222	218
14:40	236	236
15:20	186	190
16:00	200	196
16:40	179	181
17:20	180	178
18:00	251	250
18:40	222	223
19:20	149	153
20:00	226	222
20:40	218	220
21:20	193	193
22:00	217	213
22:40	172	176
23:20	221	217
24:00:00	186	188
24:40:00	233	230
25:20:00	203	206
26:00:00	152	153
26:40:00	211	207
27:20:00	201	202

28:00:00	200	200
28:40:00	228	225
29:20:00	192	195
30:00:00	194	192
30:40:00	224	221
31:20:00	199	201
32:00:00	210	207
32:40:00	185	187
33:20:00	190	187
34:00:00	213	211
34:40:00	190	192
35:20:00	178	177
36:00:00	195	192
36:40:00	198	197
37:20:00	256	255
38:00:00	182	188
38:40:00	188	183
39:20:00	193	192
40:00:00	157	158
40:40:00	214	212
41:20:00	199	201
42:00:00	203	202

42:40:00	157	158
43:20:00	167	163
44:00:00	213	212
44:40:00	221	221
45:20:00	247	246
46:00:00	203	205
46:40:00	185	183
47:20:00	180	177
48:00:00	216	213
48:40:00	195	197
49:20:00	198	196
50:00:00	185	186
50:40:00	201	197
51:20:00	220	218
52:00:00	199	201
52:40:00	214	212
53:20:00	200	201
54:00:00	222	220
54:40:00	183	185
55:20:00	203	198
56:00:00	251	251
56:40:00	197	200

57:20:00	223	218
58:00:00	204	205
58:40:00	233	231
59:20:00	197	198
60:00:00	176	176
60:40:00	218	216
61:20:00	186	188
62:00:00	266	265
62:40:00	248	248
63:20:00	172	176
64:00:00	201	196
64:40:00	151	155
65:20:00	167	162
66:00:00	177	176
66:40:00	210	208
67:20:00	150	155
68:00:00	178	173
68:40:00	210	208
69:20:00	203	203
70:00:00	168	170
70:40:00	196	192
71:20:00	185	186

72:00:00

168

167
