# Impacts of temperature variation on duckweed population growth and distribution in a changing climate

by

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## Author's Declaration

This thesis consists of material all of which I authored or co-authored: see Statement of Contributions included in the thesis. This is a true copy of the thesis, including any required final revisions, as accepted by my examiners.

I understand that my thesis may be made electronically available to the public.

## Statement of Contributions

I, Debora Andrade Pereira, am the sole author for Chapters 1 and 5, which were written under the supervision of Dr. Kim Cuddington and are not intended for publication.

Chapters 2 to 4 are in the form of independent manuscripts. As the principal author of these chapters, I conceived study designs, conducted data analysis, and wrote the manuscripts. I use pronoun "we" instead of "I" to reflect the collaborative aspect of this research, which included my supervisor who oversaw all stages of my work and undergraduate lab technicians who assisted in experiments.

Chapter 2 was published in 2024, while the other two subsequent chapters will be submitted for publication momentarily. The research in Chapter 2 was conducted at the University of Waterloo under the supervision of Dr. Kim Cuddington. Dr. Kim Cuddington and I (Debora Andrade-Pereira) co-authored the manuscript. I was responsible for model development and figure preparation. Both authors reviewed the model analyses and the manuscript. The Version of Record of this article is published in Plant Ecology journal, and is available online at https://doi.org/10.1007/s11258-024-01436-3. First published in Plant Ecology journal, 225(8), 839-850, 2024 by Springer Nature. Reproduced with permission from Springer Nature.

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## Abstract

Understanding the impacts of climate change on aquatic plants involves examining how temperature fluctuation patterns influence their temperature-dependent vital rates and distribution. Duckweeds, small aquatic plants with both economic significance and ecological concern, can pose challenges due to overgrowth and the spread of invasive species. The impact of climate-induced temperature changes on aquatic plants remains poorly understood, as many studies use constant conditions that do not account for natural variability in temperature. Research focused on increased average temperatures has shown general ectotherm responses tied to geographic location, such as enhanced growth in temperate regions. However, when temperature fluctuations are considered, responses differ from those under constant conditions due to nonlinear and asymmetrical thermal performance. Increased autocorrelation, with prolonged sequences of unusually high or low temperatures, can affect population growth rates, while nighttime warming alters diel temperature variation and potentially influences time-sensitive processes like photosynthesis and respiration. This thesis investigates the thermal performance and distribution of duckweed species under varying temperature regimes associated with climate change, incorporating both controlled experiments and predictive modeling.

The second chapter uses a Maxent species distribution model to predict the potential range expansion of *Landoltia punctata* (dotted duckweed), an invasive, herbicide-resistant species. Habitat suitability is modeled under current and future climate scenarios, using satellite-derived water temperature data and constraining model features to match the shape of thermal performance curves obtained from laboratory experiments. Results indicate high suitability for this species in Western Europe and Southern Canada, with the Great Lakes region becoming increasingly suitable in the future due to climate warming. These projections underscore the importance of climate-informed management strategies to mitigate the ecological impact of invasive species.

The third chapter investigates how diel temperature variability and climate change affect the reproduction of *Lemna minor* (common duckweed) during spring and summer. Experimental results highlight the importance of temperature variance as opposed to the timing of warming. While increased mean spring temperatures enhance duckweed performance, reduced temperature variance during high summer temperatures in regions such as Canada helps mitigate the negative impacts of otherwise excessively hot fluctuating conditions. These findings emphasize the varying effects of climate change on duckweed's thermal performance across different seasons.

The fourth chapter examines the effects of temperature autocorrelation on both common and dotted duckweed reproduction and survival. Experiments show that strongly autocorrelated sequences result in mortality due to heat stress when hot temperature sequences begin with elevated heat. In contrast, autocorrelation has limited impacts under cooler average conditions, likely due to slower physiological responses. These findings align with broader predictions of increased extinction risks for ectotherms under persistent and extreme temperature patterns caused by climate change.

This work is a step towards a more realistic understanding of aquatic plant responses to climate change by considering thermal performance responses, diverse temperature fluctuation patterns, and water temperatures. Our results can be used in population dynamics models to make more realistic predictions of climate change responses. The experimental and modeling findings in this thesis advance our understanding of aquatic plant responses to climate change and support the development of informed strategies to manage their ecological impacts and sustainable production in a warming world.

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# Chapter 1
# General introduction

Climate change is altering not only mean temperatures but also the pattern of variation in these temperatures. Changes to the timing and magnitude of temperature variation can impact the thermal performance and distribution of plants. As temperatures rise and fluctuations are increasingly altered by climate change, understanding general patterns of plant responses is important for making ecological predictions and decisions. However, many studies of the impacts of climate change on the physiology and ecology of organisms do not include treatments with realistic temperature fluctuations. In addition, such studies may not explore a wide range of mean temperatures in order to understand the thermal tolerance of organisms. These omissions could lead to over or under estimations of performance under climate change conditions. In this thesis, I investigate the effects of changes in mean temperatures, temporal autocorrelation, and night-warming on the population growth and distribution of duckweeds, a cosmopolitan aquatic floating plant. By doing so, I provide a better understanding of responses to climate variability for this plant group.

In this chapter, I begin with a literature review on plant thermal responses in a changing climate. This includes an introduction of thermal performance curves as a tool to understand long-term temperature increases and the importance of adding variance to thermal performance predictions in a warming climate. Next, I discuss how temperature fluctuations and the timing of warming affect plant population growth, focusing on night-warming and temporal autocorrelation. I then introduce duckweeds, emphasizing their dual role as economically significant plants and posing ecological challenges. I detail how climate change-driven temperature variations impact the life history and distribution of duckweeds. The chapter ends with a review of the main findings and contributions of each subsequent chapter of the thesis.

## 1.1 Plant thermal responses in a changing climate

As our climate becomes both warmer and more variable, understanding the impacts of climate change on plants requires examining temperature fluctuation patterns and their effects on vital rates and distribution. Previous studies on climate change impacts have primarily focused on long-term average temperature projections, such as enhanced growth in temperate regions as mean temperatures move towards the values that produce optimum performance in some species (Estay, Lima, & Bozinovic, 2014). Floating aquatic plants, for example, are predicted to benefit from climate warming (Lind, Eckstein, & Relyea, 2022) and result in mat formation and negative impacts to submerged organisms

(Ceschin, 2020; Lind, Eckstein, & Relyea, 2022). However, several climate change experiments focus on average temperature increases by exposing organisms to ambient versus future constant temperatures (Morash et al., 2018). However, temperature fluctuations can cause different responses than constant conditions due to the nonlinear and asymmetrical nature of thermal responses.

### 1.1.1 Thermal performance curves to gain insight into long-term temperature increases

Thermal performance curves are one tool we can use to understand the effects of climate warming and variability on organisms (Sinclair, 2016). They can be used to understand the effects of long-term average warming because they describe how constant temperatures affect performance metrics (Huey & Kingsolver, 1989), such as reproduction or photosynthetic rates. Because these curves are usually asymmetric (Huey & Kingsolver, 1989), high temperatures have a disproportionately negative effect compared to colder temperatures (Roitberg et al., 2016). Although thermal performance curves can present genetic variability among individuals within a plant species, those measured under constant temperatures at the population level generally reflect species-level niche breadth reasonably well (Wooliver et al., 2022). For this reason, thermal performance curves could also be used to inform species distribution models by constraining the shape of occurrence responses to environmental factors such as temperature to biologically meaningful ranges and shapes (e.g., unimodal) (Kearney & Porter, 2009). However, plants are underrepresented in thermal performance studies (Wooliver et al., 2022). Therefore, further studies are needed that incorporate thermal performance curves into research focused on the impact of climate change on plant performance and distribution.

### 1.1.2 Adding variance to thermal performance predictions in a warming climate

Temperature fluctuations can significantly impact biological responses more than changes in mean temperature alone (Dillon et al., 2016; Vasseur et al., 2014), especially for ectotherms such as plants, whose body temperature depends on ambient temperatures. Modelling studies show that temperature variance decreases performance across the thermal tolerance and reduces the optimum temperature in relation to constant temperatures (Denny, 2019; Vasseur et al., 2014), as demonstrated in experiments (e.g., Paaijmans et al., 2013). Experimental studies have shown that during the growing season, consistently large daily temperature ranges, which may reflect increased temperature variance, negatively affect the photosynthetic performance of wetland plants compared to narrower temperature ranges (Xiao et al., 2019).

Due to the non-linear and concave-down nature of thermal performance curves, performance under fluctuating conditions is determined by Jensen's inequality (Jensen, 1906). This means that the average performance after exposure to temperature fluctuations with a given average temperature differs from the performance after exposure to just that average temperature. Thus, at specific average temperatures, increased variance can either disproportionally increase or decrease performance in comparison to mean temperatures, depending on how close these average temperatures are from the optimum (Ruel & Ayres, 1999). For instance, while mean temperatures alone might benefit temperate species, increased temperature variance during summer could lead to heat stress, resulting in negative outcomes for ectotherms in this region due to climate change (Kingsolver, Diamond, & Buckley, 2013). Moreover, as curves are usually left-skewed, hot fluctuating temperatures may have even more disproportionate negative impacts around the optimum (Roitberg & Mangel, 2016).

Phenotypic plasticity plays a role in how plants respond to changing thermal environments. Some species exhibit variation in thermal performance breadth, which may enhance their ability to cope with climate change (Wooliver et al., 2022). One mechanism underlying plasticity is acclimation. However, this response has its limits: while beneficial acclimation can reduce thermal stress, it may fail if individuals cannot survive initial exposures or if repeated stress leads to widespread mortality (Siegle, Taylor, & O'Connor, 2018; Williams et al., 2016).

## 1.2 Temperature fluctuations, timing of warming, and plant population growth

### 1.2.1 Night-warming

In addition to temperature variance, the timing of thermal fluctuations can also influence plant performance. One such temporal pattern of thermal fluctuations is night-warming, where nighttime temperatures rise more than daytime temperatures. This widespread trend results from climate change and is predicted to increase in many areas, including Canada (Cox et al., 2020). With respect to temperature variation, elevated night-time temperatures reduce diel temperature variation.

While changes in temperature variance are known to impact ectotherms (e.g., for floating aquatic plants, see Cuddington et al., 2025, pre-print), studies focused on the time-dependence of certain wild plant physiological processes suggest that night-warming may have additional impacts by altering processes like photosynthesis and respiration. For example, warmer nights can accelerate carbohydrate depletion and induce more photosynthesis the next day, resulting in increased growth

(He, Li, & Peng, 2012; Turnbull et al. 2002, 2004; Wan et al., 2009). I note, however, that an increase in plant growth was not commonly found in agricultural studies studying night-warming effects (e.g., Sadok & Jagadish, 2020), perhaps due to impaired biomass allocation to reproductive organs (Jing et al., 2016). While a semi-aquatic plant species showed negative impacts of night-warming (Wu, Ismail, & Ding, 2017), there are no night-warming studies including floating aquatic plants such as duckweeds.

Aquatic plants may respond differently than terrestrial plants to night-warming, as they tend to lose less water in warm conditions. Agricultural losses during warmer nights are linked to increased transpiration (Sadok & Jagadish, 2020), while some terrestrial plants benefit from increased stomatal density (Fan et al., 2022). In contrast, aquatic plants like duckweeds, which have permanently open stomata, do not face water loss issues (Raven, 2002).

In a literature review of peer-reviewed manuscripts on single-species experiments with wild plants exposed to night-warming treatments, I found that these studies do not separate the effects of changes in temperature variance and mean temperature from the timing of warming (Table 1.1). Moreover, most of the growth chamber studies alternate between single constant daytime and nighttime temperatures, which is not realistic in terms of natural diel temperature fluctuations. This would not be a problem considering that there are field studies. However, these studies generally compare the impact of different warming timings, such as night-warming and daytime warming, to ambient conditions. These scenarios differ in mean temperatures and temperature variances: day-warming has higher mean temperatures and variance, while night-warming has lower variance compared to current conditions. While most of these studies indicate that night-warming benefits plants, with some attributing this to photosynthetic overcompensation, the confounding factors we discussed make it difficult to determine which temperature patterns actually define plant population growth rates.

Table 1.1 Summary of experiments on climate change, night-warming, and wild plant performance. The search used keywords night, warm*, plant*, climate change. Criteria for inclusion: type of publication: peer-reviewed experimental papers; period: 1990-2024; treatments: current temperatures vs. climate change-induced night-warming; organism: single-species wild plants; metrics: photosynthetic performance, biomass, reproduction; sources searched: meta-analysis bibliography of Jing et al. (2016) and Google Scholar.

| **Publication** | **Setting** | **Type of fluctuation included** |
|---|---|---|
| Cheesman & Klaus, 2013 | growth chambers | constant day, constant night |
| Cheesman & Klaus, 2013 | open-top chambers | ambient fluctuations; warmed nights |
| Chen et al., 2017 | growth chambers | constant day, constant night |
| He, Li, & Peng, 2012 | field | ambient fluctuations; warmed nights |
| Patterson, 1990 | growth chambers | constant day, constant night |
| Prieto et al., 2009 | field | ambient fluctuations; warmed nights |
| Su, Han, & Chen, 2020 | growth chambers | constant day, constant night |
| Turnbull et al., 2002 | mesocosm | constant day, constant night (small variance) |
| Turnbull et al., 2004 | mesocosm | constant day, constant night (small variance) |
| Wan et al., 2009 | field | ambient fluctuations; warmed nights |
| Wu, Ismail, & Ding, 2017 | field | ambient fluctuations; warmed nights |

Seasonality complicates our ability to draw general conclusions about night-warming impacts. For instance, an invasive Asteraceae species showed increased biomass in winter but decreased in summer, suggesting that night-warming may not facilitate long-term invasion (Chen et al., 2017). Similarly, spring and summer could yield different outcomes. Milder spring conditions might promote plant growth through photosynthetic overcompensation, while hot summer daytime temperatures combined with night-warming could have negative effects due to insufficient recovery time, as seen in other ectotherms (Bai et al., 2019; Ma et al., 2021; Speights, Harmon, & Barton, 2017; Zhao et al., 2013). This may explain reduced yields in some crops during hot summers with night-warming (Narayanan et al., 2015). Thus, night-warming experiments that isolate seasonal, statistical, and timing aspects of temperature changes are essential.

### 1.3 Temporal autocorrelation

Temporal autocorrelation is another fluctuation pattern altered by climate change (Di Cecco & Gouhier, 2018). Autocorrelation describes the organization of individual temperature observations in

time around the average. For example, white noise has zero autocorrelation. This means that the probability that a given temperature is above or below the mean value is independent of whether the previous temperature was above or below that mean. Environmental factors, such as temperature or precipitation, tend to have strong positive autocorrelation. In sequences with this characteristic, deviations from the mean are grouped together, so that if a temperature is above the mean the following temperature will likely be above the mean as well. This variation, known as environmental noise, can be classified by colours indicating the strength of autocorrelation where white noise has zero autocorrelation, red noise is positively autocorrelated and blue noise is negatively autocorrelated (Cuddington & Yodzis, 1999; Halley, 1996). Long-term changes in autocorrelation strength can be estimated using spectral analysis on time-series. This analysis decomposes signals in the time domain to the frequency domain (Platt & Denman, 1975). The decomposition can be used to identify the prevalence of long-term fluctuations which indicate strong positive autocorrelation (e.g., Di Cecco & Gouhier, 2018).

Strong positive autocorrelation can impact organisms. For example, a sudden exposure to a cluster of unfavorable temperatures may negatively affect demographic rates (Ma et al., 2018), such as seen in tree die-offs during heat waves (Breshears et al., 2021). Some studies suggest that while temperature variance primarily influences variability in plant population dynamics, autocorrelation affects population structure (Gascoigne et al., 2025). More generally, the clustering of favourable and unfavourable conditions can alter the risk of invasive species establishment (Cuddington & Hastings, 2015).

### 1.4 Duckweeds: a group of economically important and ecologically concerning species

In my research, I use duckweeds as a model aquatic plant to investigate temperature-driven impacts of climate change. Duckweeds are among the smallest angiosperms known. This group of aquatic plants primarily reproduces asexually and is known for having rapid growth rates, among the highest among plants (Ziegler et al., 2015). They are ideal for ecological studies because of their fast and predominantly asexual reproduction and cosmopolitan distribution (Laird & Barks, 2018). Research fields such as plant biology, toxicology, ecology, and evolution have benefited from using duckweeds as a model system (Laird & Barks, 2018).

Duckweeds belong to the Araceae family (subfamily Lemnoideae), and are distributed in five genera (Landoltia, Lemna, Spirodela, Wolffia, and Wolffiella) (Cao, Fourounjian, & Wang, 2020). Their size varies from 0.5 to 2 cm, and they have a simple body structure, each frond is an individual plant composed of leaf-like stems with minimal cellular differentiation (Landolt, 1986; Cao, Fourounjian, & Wang, 2020).

Duckweeds have significant economic applications, with potential uses in various sustainable applications, such as phytoremediation, animal nutrition, protein for human consumption, and biofuel production (Appenroth et al., 2015; Liu et al., 2021). On the other hand, duckweeds can pose ecological problems due to overgrowth and invasive spread. These plants can damage ecosystems and recreational areas, and are difficult to manage due to multiple introduction pathways, rapid growth, and resistance to environmental extremes. For example, they can survive in the digestive systems and feces of waterfowl for several days (Paolacci et al., 2023), and so be easily dispersed long distances during migration. Human activities, such as the transport of stowaway plants in international gardening and aquarium trade, have also facilitated the global introduction of invasive duckweed species (Jacono & Pfingsten, 2023; Van den Neucker & Scheers, 2022). The overgrowth of native and invasive duckweed species in nutrient-rich water bodies leads to mat formation, where a nearly solid cluster of fronds covers the water surface. These mats can cause oxygen and light depletion, which produces detrimental effects on ecosystems (Ceschin et al., 2020). Additionally, controlling the spread of duckweed species is highly challenging and costly, as invasive aquatic plant management is estimated to cost billions of dollars annually on a global scale (Cuthbert et al., 2021). Their rapid and predominantly asexual reproduction contributes to the difficulty in controlling their spread. Finally, some duckweed species can withstand extreme environmental conditions, such as high temperatures and drought (Jacono, 2002; Ziegler, Appenroth, & Sree, 2023), as I describe in the next section.

### 1.5 Current knowledge on temperature dependence and climate change effects on duckweeds

Temperature plays a crucial role in determining the distribution of duckweeds (Crawford et al., 2006) by influencing their growth, survival, and reproductive success (Echelpoel, 2020; Mcilraith, Robinson, & Shay, 1989; Rejmánková, 1975). The ideal temperature range for the growth and reproduction of most duckweed species is between 20°C and 30°C, while minimum temperatures for minimal growth range from below 8°C to 20°C (Ziegler, Appenroth, & Sree, 2023). For some

species, reduced growth still occurs at high temperatures between below 30°C and above 34°C (Ziegler, Appenroth, & Sree, 2023).

Predictions using population models indicate that a mean temperature increase of approximately 2°C due to climate change may lead to widespread overgrowth of duckweed biomass during future spring and summer seasons in temperate regions such as the UK and the Netherlands (Feller, Taylor, & Lunt, 2024; Peeters et al., 2013). As average temperatures during the growing season approach the optimum for several duckweed species, uncontrolled duckweed growth may result in reduced biodiversity, fish mortality, and a greater need for management measures (Feller, Taylor, & Lunt, 2024). Moreover, invasive duckweed species are spreading in temperate areas as our climate changes (e.g., Ceschin et al., 2020), resulting in reduced native biodiversity, loss of recreational areas, and clogged pipes (Center for Aquatic & Invasive Plants, University of Florida, 2023; Ceschin et al., 2020; Duenas, 2022). However, experiments using temperature gradients showed that the range expansion of duckweeds slows down compared to conditions with constant temperatures, suggesting that predictions based on average temperatures may overestimate future coverage (Usui & Angert, 2024). This may be due to the fact that exposure to temperature gradients leads to the simultaneous evolution of a reduced tolerance range and a broader optimum range over a short period of about 100 days (Usui & Angert, 2024), resulting in net performance that may be lower than before evolution.

Duckweeds have evolved adaptations to survive cold winters, such as the formation of turions. These starch-rich structures sink and remain dormant, and resurface in spring to germinate (Ziegler, Appenroth, & Sree, 2023). Freezing temperatures were generally considered lethal for species that do not form such dormant turions (Pasos-Panqueva, Baker, & Camargo-Valero, 2024). However, new findings show that some strains can survive winter conditions without these dormant structures (Jewell & Bell, 2023). The diversity in thermal responses is also seen across latitudinal gradients, where duckweed strains adapted to local climates maximize their growth season by delaying turion formation (Hitsman & Simons, 2020).

In response to high temperatures, some duckweed strains of the *Spirodela* genera have shown tolerance to prolonged heat, surviving periods of 40ºC (Jacobs, 1947; Ziegler, Appenroth, & Sree, 2023). However, most studies focus on temperatures within the tolerance range (Pasos-Panqueva, Baker, & Camargo-Valero, 2024). Thus, extreme heat tolerance information is lacking for many duckweed species, such as those in the *Lemna* and *Landoltia* genera.

## 1.6 Research objectives

As this brief review indicates, there is a lack of studies on the effects of climate change on the population growth and distribution of duckweeds, a group of aquatic plants with significant economic potential and ecological risks. This thesis aims to predict the current and future distribution of invasive duckweed species as dependent on current and future long-term climatic trends, and to examine the effects of two short-term climate change patterns, night-warming and temporal autocorrelation in temperatures, on duckweed population growth.

In chapter 2, I use duckweed thermal performance curves and life-history information to create species distribution models predicting the occurrence of invasive duckweed. These models estimate regions of higher climatic suitability for invasive duckweed species, aiding in managing their spread. I introduce a Maxent model to predict the range expansion of *Landoltia punctata*, an herbicide-resistant tropical species approaching the Canadian border. Habitat suitability is modeled under current and future climate scenarios using satellite-derived water temperature data, unlike many studies which use air temperatures for aquatic organisms. Results indicate high suitability in Western Europe and Southern Canada, with the Great Lakes region becoming more suitable due to climate warming. This research underscores the importance of climate-informed management strategies to mitigate invasive species' ecological impact.

In chapter 3, I examine the impacts of night-warming on duckweed population growth rates. Differently from previous studies, I separated effects from the timing of warming from changes in mean temperature and temperature variance, which we know significantly impact aquatic plant growth rates. My findings show that temperature variance is more critical than warming timing. Increased mean spring temperatures enhance duckweed population growth rates, while reduced temperature variance during hot summers mitigates negative impacts. This study highlights the interplay between mean temperature and temperature variance on duckweed performance across seasons, which provides guidance for environmental control, sustainable duckweed applications, and future climate change experiments.

In chapter 4, I analyze the effects of temperature temporal autocorrelation on duckweed reproduction and survival. This is the first experiment on the effects of autocorrelation on plants. By separating effects of autocorrelation from variance and analyzing interactions between autocorrelation and mean temperature changes, I show increased mortality when average temperatures are above

optimal and autocorrelated regimes start hot. Milder starting periods do not affect survival. This suggests alternating scenarios of increased mortality and typical growth with climate change, consistent with predictions of increased extinction risks, heightened invasion risks, and changes in ectotherm population dynamics under extreme temperature patterns.

Overall, my research provides the first risk map for invasive dotted duckweeds in current and future climatic conditions, aiding managers in planning actions in Canada and Europe. The experimental chapters on climate change patterns are also the first studies of their kind to elucidate duckweed responses to increasingly frequent and intense phenomena, such as heat waves and night-warming, offering insights into aquatic plant responses to climate change. Each chapter was conceived and prepared as a separate manuscript for publication. Chapter 2 has been published in Plant Ecology journal (Andrade-Pereira & Cuddington, 2024), and chapters 3 and 4 will be submitted for publication momentarily. As each chapter is an independent publishable unit, there may be some repetition of ideas, especially in the introductory sections.

# Chapter 2
# Range expansion risk for a newly established invasive duckweed species in Europe and Canada

## 2.1 Abstract

*Landoltia punctata* is an invasive aquatic plant that has spread across the United States. Unlike native duckweeds, this species has developed herbicide resistance. As a result, invasion can lead to high management costs and the loss of recreational areas and natural habitats. The species has been recently found in Europe, and is also approaching the northern US border with Canada. We predicted the potential distribution of *L. punctata* in western Europe and Canada using presence-only data from the Global Biodiversity Information Facility as well as other literature records. We fit predictive models to this data using a Maxent approach. Since climate data based on surface lake water conditions are often more relevant to macrophytes than air temperature metrics, our models included both water and air temperature bioclimatic variables related to the life history of the species. Model comparisons confirmed a superior fit of lake temperatures to duckweed distribution records. The best fit model suggests a high habitat suitability for the species in most Western European countries and Western Canada. A moderate emission scenario suggests that in 2041, currently compatible areas will still be suitable, and that the Great Lakes region will become suitable. Preventive measures to avoid future spread of *L. punctata* are recommended in these locations to avoid impacts associated with this and similar duckweed species in Europe and the US.

## 2.2 Introduction

Dotted duckweed (*Landoltia punctata*) is an invasive alien species of concern which has spread in the United States in the last few decades. As the climate warms, this tropically originated species is moving north (Jewitt, 2021). It has been recently observed in US states bordering the Great Lakes region (US Army Corps of Engineers, 2013), and may move north into Canada. In addition, the species has also been recently documented in Europe (European and Mediterranean Plant Protection Organization – EPPO, 2015). The continental spread of dotted duckweed in the US, and subsequent ecological and economic consequences, suggests that an examination of invasion risks for dotted duckweed in Europe and Canada may be beneficial.

Globally, aquatic invasive alien species have cost US$23 billion in 2020, although this estimate is likely higher due to underreporting (Cuthbert et al., 2021). Aquatic weeds have an annual economic cost of US$110 million in the US (Pimentel et al., 2000), although we did not find studies estimating the economic costs of *L. punctata* specifically. For controlling duckweed overgrowth in general, an assessment of repeated herbicide application and water quality monitoring program conducted in duckweed infested Maskinonge River, Ontario, Canada, estimated an approximate cost of US$10,400/ha (cost corrected to November 2023 inflation rates; Warren & Neil, 1992). Unlike its native counterparts, this species has developed resistance to diquat (Koschnick et al, 2006), one of the most frequently used herbicides to control duckweeds (Wersal and Madsen, 2009). Thus, increased costs associated with herbicide research and development, and management costs from mechanical harvesting can be expected.

Once introduced, dotted duckweed can suppress native duckweeds (Center for Aquatic & Invasive Plants, 2023; Koschnick et al, 2006). It may form dense mats which cover the surface of water bodies, negatively affecting ecosystems and aesthetic features of recreational areas (Center for Aquatic & Invasive Plants, University of Florida, 2023). Duckweed mats can reduce light and oxygen levels in the water column, resulting in mortality of fish and other organisms. These mats may also disrupt recreational activities, such as fishing, canyoning, boating, and swimming (Jewitt, 2021).

Previous studies considering the risk of dotted duckweed invasion in Europe concluded that there is a low risk of negative consequences, as this invasive species is ecologically similar to native species, such as common duckweed (*Lemna minor*) (LCH van Valkenburg & Pot, 2008; Branquart, 2016). This assessment may be accurate; however, we note that Europe has been facing severe consequences from the invasion of a similar non-native duckweed species: least duckweed (*L. minuta*). Least duckweed has been clearly capable of quickly spreading (Supplemental Fig. 2.1 in Appendix A) and occasionally replacing the native common duckweed (Paolacci, Jansen, & Harrison, 2018b). Least duckweed invasions have also been associated with mat formations leading to waterway clogging (Duenas, 2022); reduction in light and oxygen resulting in lower plant and invertebrate richness (Ceschin, 2020); and loss of recreational areas (Paolacci, Harrison, & Jansen, 2018b).

Dotted duckweed is native to Southeastern Asia and Australia (Landolt, 1986) and was accidentally introduced to the US state of Missouri around 1930, likely during aquarium supply transportation (Jacono & Pfingsten, 2023). It is considered a noxious weed in Texas and Louisiana, as a species of

high concern in Alabama (Alabama Aquatic Nuisance Species Task Force, 2021), and it has a status of "cautious" in Florida, Minnesota, Oklahoma, and Wisconsin (Foster et al., 2009; Cornell Legal Information Institute, 2009; Florida Invasive Species Council, 2019; Texas Invasive Species Institute, 2021; Midwest Invasive Plant Network, 2022). Dotted duckweed is present in the Mississippi River, about 110 km from the Great Lakes basin, and there is a possibility of transfer to this region via aquatic pathways (US Army Corps of Engineers, 2013; Fig. 2.1).

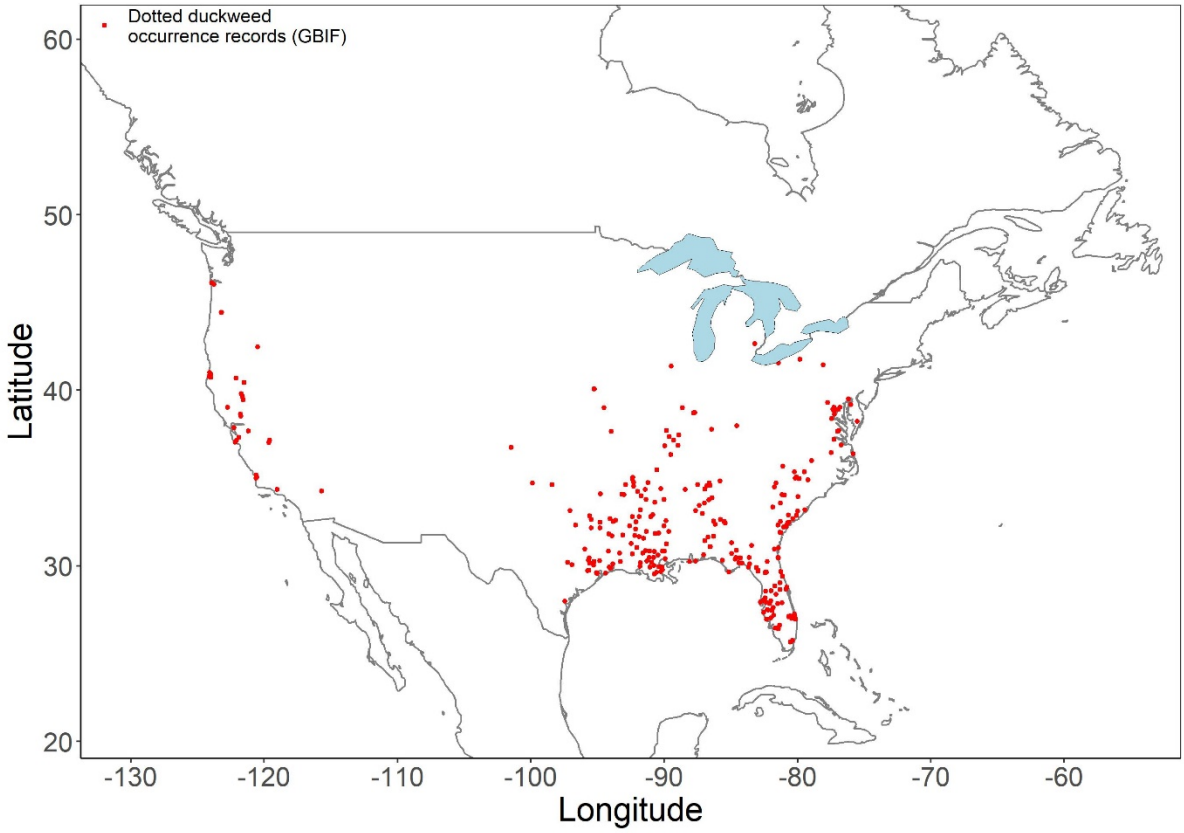



Figure 2.1 Dotted duckweed occurrence records in the US.
Note the proximity of several occurrence points to the Great Lakes region. Species occurrence retrieved from GBIF.org on 2024-03-18 (https://doi.org/10.15468/dl.7uqs9k).

In Europe, this new invader was recently identified in several western countries. For instance, in 2022 it was found for the first time in a yearly survey as a stowaway plant in Belgian garden shops (Neucker & Scheers, 2022). Previous studies suggested a low invasion risk due to frost sensitivity (van Valkenburg & Pot, 2008; Branquart, 2016). However, more recent investigations indicate that

both dotted and common duckweed resting fronds may have the ability to overwinter in between ice layers (Ziegler, Appenroth, & Sree, 2023). In fact, common duckweed overwintering capability was demonstrated in Quebec, Canada (Jewell & Bell, 2023). Although dotted duckweed overwintering in natural systems has not been documented yet, frost sensitivity may not be a restrictive factor.

Duckweed species can be spread by waterfowl, boats, aquarium trade, and accidentally in horticultural imports of water garden species (Landolt, 1986, Jewitt, 2021; Van den Neucker & Scheers, 2022; Jacono & Pfingsten, 2023). Duckweed fronds may be carried on waterfowl body parts, but in addition, living duckweed fronds in duck feces were recently found to survive for several days and subsequently reproduce. Considering the high mobility of ducks over both short distances (i.e. between water bodies) and long distances (i.e. migration), along with the high frequency of duck fecal events, endozoochory may be a major duckweed spread mechanism (Paolacci et al., 2023). The usual pathways for *L. punctata* introduction, namely aquarium trade, as a stowaway plant, and animal and boat transportation indicate the potential for multiple introductions and increased propagule pressure which makes widespread invasions such as that seen in the US more likely (Lockwood, Cassey, & Blackburn, 2009).

As with other duckweeds, temperature is a key factor determining dotted duckweed distribution (Crawford et al., 2006), as this factor affects growth, survival, and reproductive success (Rejmánková, 1975; Mcilraith, Robinson, & Shay, 1989; Echelpoel, 2020). Dotted duckweed, least duckweed, and common duckweed have similar habitat preferences (e.g., there are indistinguishable responses to environmental conditions found in Echelpoel, Boets, & Goethals, 2016), such as stagnant, nutrient-rich waters, temperature optima between 25-29 °C, and intermediate levels of annual precipitation (Jacono, 2002; Paolacci, Jansen, & Harrison, 2018b). Although there are some exceptions related to documented opportunistic behaviour resulting from environmental changes (Njambuya, Stiers, & Triest, 2011; Gérard & Triest, 2014; Paolacci, Jansen, & Harrison, 2016, 2018a). In experiments, we did not find thermal performance divergences between dotted and common duckweed (Supplemental Fig. 2.2 in Appendix A). Due to these ecological similarities, it is possible that invasive dotted duckweed may achieve a range as large as its native counterpart common duckweed.

Recent findings of duckweed frost resistance, dotted duckweed herbicide resistance, and multiple introduction pathways (e.g., waterfowl dispersal by endozoochory and as a stow-away plant) lead us to hypothesize that dotted duckweed has a potential range similar to that of least duckweed and

common duckweed in Canada and in Europe. In addition to current threats posed by invasive species such as dotted duckweed, future invasion scenarios will depend on climate change intensification. The northward expansion of this species in the US is attributed to climate warming (Jewitt, 2021). Secondly, we predict that dotted duckweed range expansion to more Northern regions of Europe and Canada will become more likely in future climate scenarios. To have insights into the attainable geographical range for this species, we developed potential distribution maps in Europe and Canada using relevant selected dotted duckweed life history and climate information.

## 2.3 Methods

To model current and future habitat suitability for dotted duckweed in Canada and Europe, we used maximum entropy distribution models (Maxent). Maxent models are widely used since they require only species occurrence and climate data and can have good prediction power (Elith et al., 2006, Elith et al., 2011). We retrieved occurrence records of dotted duckweed from the Global Biodiversity Information Facility (GBIF, https://www.gbif.org/) and from published papers (Supplemental Table 2.1 in Appendix A). To find predictive correlates of habitat suitability, we used both air temperature metrics obtained from the WorldClim database and satellite-derived lake water temperatures, which better represent duckweed habitat conditions than the customarily used air temperature-derived variables (Armitage, 2023). We compared Maxent models using air versus lake water temperatures. Finally, future climate scenarios based on air temperatures projected by the Intergovernmental Panel on Climate Change (IPCC) were used to evaluate future habitat suitability for dotted duckweed in Canada and Europe.

### 2.3.1 Species information

Dotted duckweed is among the smallest flowering plants known (Tippery & Les, 2020). It has the typical simplified body plan of duckweeds, with floating fronds consisting of both leaf and stem cells. The species is characterized by several roots attached to the abaxial side of the fronds. Flowering is rare, as duckweeds preferentially reproduce asexually. As with other duckweeds, reproductive rates are among the highest in the plant kingdom (Ziegler et al., 2015).

### 2.3.2 Types of climatic data

We used both air temperature and water temperature climatic variables to create habitat suitability models. We also predicted future distribution scenarios based on future temperature variables. All

climatic layers used in this study have a spatial resolution of 5 arc minutes (~10 km) and were aligned and cropped to have corresponding extent and dimensions. Commonly used current air temperatures were obtained from the WorldClim database (version 2.1, https://www.worldclim.org; Merow, Smith, & Silander Jr, 2011; Fick & Hijmans, 2017). Lake water temperature predictors were calculated by Armitage (2023) and are based on GloboLakes, a global database of satellite-calculated daily temperatures of lake surfaces (www.laketemp.net/home_GL). The author adjusted the relationship between these lake temperatures and air temperatures using machine learning models based on various atmospheric and geographical factors. Bioclimatic layers were then developed in a similar fashion to air temperature bioclimatic variables.

To predict habitat suitability under a climate change scenario, we used air temperatures from the Intergovernmental Panel on Climate Change (IPCC) climatic predictions for 2041-2060 under a moderate scenario (ssp245), which considers relatively slow carbon emissions across time. We selected two different global circulation models: ACCESS-CM2 (Australian Community Climate and Earth System Climate Simulator) and MIROC6 (Model for Interdisciplinary Research on Climate). These future temperature predictions are based on the current air temperature variables, version 2.1, and are also available from the WorldClim database.

### 2.3.3 Model formulation

Ambient temperatures are a major factor impacting ectothermic organisms such as plants, as their body temperatures depend on external heat sources (Huey & Kingsolver, 1989). As previously mentioned, temperature changes affect duckweed growth, survival, and distribution (Rejmánková, 1975; Mcilraith, Robinson, & Shay, 1989; Echelpoel, 2020). In order to be able to compare our Maxent model predictions to known responses to temperature, and to maximize the interpretability of our models, we constrained both the shape of the predicting functions and the number of predictors. To begin this process, rather than using all 19 available climate-based predictors in the air and lake temperature databases, we pre-selected biologically relevant predictors within each data type (e.g., temperature and precipitation mean and variance metrics). The candidate predictors were selected based on known factors impacting dotted duckweed life history.

To describe the thermal-dependency of dotted duckweeds, we selected the bioclimatic predictors BIO 1 (mean annual temperature), BIO 2 (mean diurnal range, reflecting the average daily temperature variation), BIO 7 (annual range of temperature), and BIO 3 (isothermality, calculated as

the ratio BIO 2/BIO 7). BIO 1 and BIO 7 were previously used in Maxent models describing the distribution of similar duckweed species, and are also known to be good predictors of duckweed thermal performance (see Armitage, 2023). The bioclimatic variable BIO 10 (mean temperature of the warmest quarter) was included to reflect dotted duckweed optimal growth in warm months. The optimal temperature for dotted duckweed photosynthesis is 25 °C (Wisconsin Department of Natural Resources, 2018), which more closely corresponds to summer average temperatures in Europe and southern Canada (around 20 °C; Copernicus, 2023, Environment Canada, 2023) in comparison with other seasons of the year. Conversely, we included the mean temperature of the coldest quarter (BIO 11) as a potential limitation to the northern distribution of dotted duckweed.

As previously discussed, duckweeds are rare in places with high or very low precipitation (FNA, 2000). The bioclimatic variables BIO 12 (mean annual precipitation) and BIO 15 (precipitation seasonality, given by the coefficient of variation of monthly precipitation values) were selected to determine if habitat suitability reflected a need for moderate levels of precipitation. However, we note that dotted duckweeds display drought tolerance, and are found in dry regions of Australia. The species can produce drought-resistant seeds (Jacono, 2002). The bioclimatic variable BIO 17 (precipitation of driest quarter) was added to determine if dotted duckweed can be found in drier regions. We also selected BIO 18 (precipitation of the warmest quarter) to evaluate potential effects of precipitation during the growth season.

After selecting variables potentially relevant to dotted duckweed distribution, we constrained the functional forms used to predict occurrence from climate variables to linear and/or quadratic features that might reflect known biological responses and/or life history characteristics which depend on climatic factors, such as unimodal thermal dependence (Merow et al. 2013).

### 2.3.4 Species occurrence data

The majority of the occurrence records used in our models were collected from the Global Biodiversity Information Facility (https://doi.org/10.15468/dl.7uqs9k). Fifteen European records described in research papers were also added (Supplemental Table 2.1 in Appendix A). We removed duplicate records, records missing geographic coordinates and records without matching data in our climatic variable datasets, which resulted in 1,121 observations. To reduce bias from heavily sampled regions, we thinned the data by randomly selecting a single data point per 5 arc minutes in the training region. In the testing region, we randomly selected two data points per cell to allow for a

reasonable testing dataset size greater than 30 observations. This sampling was performed using the gridSample function in R package dismo (Hijmans, 2011). Our final thinned dataset was composed of 700 observations.

### 2.3.5 Maxent species distribution models

For each of the two climatic datasets used (current air temperatures and lake water temperatures), we created and compared candidate Maxent models (Maxent 3.4.4: https://biodiversityinformatics.amnh.org/open_source/maxent; Phillips et al. 2017) using the R (R Core Team, 2021) package ENMeval package (Muscarella et al., 2014). We first divided our thinned dataset into training and testing datasets. The training dataset consisted of global records except Europe (668 observations), while our testing dataset was composed of European records (32 observations). Using records from both native and invaded regions has improved model transferability when modeling the distribution of invasive species (Mainali et al., 2015). We selected 700 background points from a study area encompassing 600 km around occurrence records using a 5 arc minute spatial resolution. This moderate sampling range reconciles the need for selecting a region broad enough to capture the diversity of environmental conditions for a globally distributed species while avoiding false-positive predictions where the species is not present (Amaro et al., 2023). Additionally, similar buffer sizes have been used in the literature to model the distribution of duckweeds, as such extents can well represent bird dispersion (Cobos & Peterson, 2023). The number of background points corresponds to the number of observations in the training or test region, as this approach produces the most accurate predictions of invasive species distributions (Barbet-Massin, 2012). To account for spatial autocorrelation, we performed cross validation by dividing the training data into 4 spatial blocks (Roberts et al., 2017).

We performed preliminary model fits using air and lake water temperature predictors to obtain variable contribution, permutation importance, and correlation. Correlation between predictors was determined using Pearson's correlation coefficients (Table 2.1). We first selected the most important predictors by excluding variables with permutation importance <5%. We based our selection on permutation importance because when there are highly correlated environmental variables, Phillips (2006) notes that percent contributions should be interpreted with caution. For the lake temperature dataset, precipitation seasonality (BIO 15) explained the most variance (Table 2.1). In comparison, the annual range of temperature (BIO 7) had both a high percent contribution and permutation

importance. Following this, mean temperature of coldest quarter (BIO 11), mean annual temperature (BIO 1), and mean temperature of warmest quarter (BIO 10) had the highest permutation importance. For both air and lake water temperature predictor analyses, we pre-selected mean annual temperature (BIO 1) regardless of its contribution, as we used this variable to compare suitability curves with thermal responses obtained in the lab. We also ensured that at least one of the pre-selected variables conveys information on precipitation.

To maximize the inclusion of potentially important variables which would otherwise be excluded due to collinearity, we organized the most important predictors into two groups. We assigned each variable to group 1 and 2, respectively, in the following order: highest permutation importance and correlation coefficient below 0.8 (Elith et al., 2010). We added BIO 15 to both groups, as this is the only pre-selected variable related to precipitation. The first group included precipitation seasonality (BIO 15), annual temperature variation (BIO 7), and mean annual temperature (BIO 1), while the second group included precipitation seasonality (BIO 15), mean temperature of coldest quarter (BIO 11), and mean temperature of warmest quarter (BIO10).

Table 2.1 Variable percent contribution and Pearson correlation coefficients of preselected variables for the preliminary model fit.
The variables designated for subsequent model fits were selected based on highest permutation importance and lowest correlation values. Selected predictors were annual temperature variation (BIO 7), precipitation seasonality (BIO 15), and mean annual temperature (BIO 1), and precipitation seasonality (BIO 15), mean temperature of coldest quarter (BIO 11), and mean temperature of warmest quarter (BIO10) in the second predictor group. Results are shown for the lake water based predictors, as the preliminary model exercise using air temperatures resulted in the same pre-selection of variables.

| Preliminary variables | Variable contrib. | Permut. Import. | BIO 15 | BIO 7 | BIO 11 | BIO 3 | BIO 10 | BIO 2 | BIO 1 | BIO 12 | BIO 18 | BIO 17 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **BIO 15** | **40.89%** | **7.44%** | **1** | **-0.13** | **0.22** | **0.06** | **0.21** | **0.02** | **0.24** | **0.22** | **0.02** | **-0.50** |
| **BIO 7** | **22.92%** | **30.17%** | | **1.00** | **-0.84** | **-0.82** | **-0.32** | **0.22** | **-0.69** | **-0.55** | **-0.42** | **-0.29** |
| **BIO 11** | **10.93%** | **23.31%** | | | **1.00** | **0.81** | **0.76** | **0.04** | **0.97** | **0.43** | **0.30** | **0.16** |
| BIO 3 | 10.05% | 2.55% | | | | 1.00 | 0.40 | 0.24 | 0.69 | 0.56 | 0.41 | 0.30 |
| **BIO 10** | **4.53%** | **13.81%** | | | | | **1.00** | **0.15** | **0.90** | **0.10** | **0.03** | **-0.04** |
| BIO 2 | 3.72% | 2.77% | | | | | | 1.00 | 0.08 | -0.12 | -0.09 | -0.14 |
| **BIO 1** | **2.84%** | **16.53%** | | | | | | | **1.00** | **0.32** | **0.21** | **0.08** |
| BIO 12 | 2.24% | 1.97% | | | | | | | | 1.00 | 0.84 | 0.76 |
| BIO 18 | 1.18% | 0.05% | | | | | | | | | 1.00 | 0.51 |
| BIO 17 | 0.69% | 1.41% | | | | | | | | | | 1.00 |

We then created several candidate Maxent models using these two groups of three climatic variables and a range of regularization parameters to produce models with different levels of complexity. These models were evaluated using the Area Under the Curve (AUC) approach, which gives the probability of telling apart a given presence observation in the testing dataset from a random background point in the study area (Merow, Smith, & Silander Jr, 2013). We also estimated the True Skill Statistic (TSS), which compares model predictions to a validation dataset. It is calculated as the proportion of presences correctly predicted (sensitivity) + the proportion of absences correctly predicted (specificity) -1 (Allouche, Tsoar, & Kadmon, 2006; Ruete & Leynauld, 2015). This commonly used metric in species distribution models (e.g. Ahmadi et al., 2023; Li et al., 2023) provides additional input in relation to AUC, as it accounts for missed positive cases and incorrectly labeled negative cases as positive. For each selected maxent model, the AUC and TSS were estimated for 2 datasets: the training data which consisted of global records excluding Europe, and the test data including European records.

The Akaike information criterion (AIC) was also calculated for each model. The permutation importance of each predictor was determined by randomly rearranging the values of variables across training points and assessing the subsequent reduction in AIC values. Finally, we analyzed if tolerance ranges from response curves are realistic considering climate data in occurrence regions. For BIO 1 (mean annual temperatures), we verified the adequacy of response curves to experimentally-obtained performance curves reflecting the life history of dotted duckweeds.

## 2.4 Results

### 2.4.1 Distribution models

In order to predict current and future distribution scenarios for dotted duckweed, we selected the best model for each set of the two climatic data types: lake and air temperatures. Selection was based on the lowest AICc (Akaike Information Criterion corrected for small sample sizes) among candidate models fit with linear or both linear and quadratic terms, and several options of regularization parameters (see Supplemental Tables 2.2-2.5 in Appendix A for information on all candidate models). For all cases, the selected model was fitted with both linear and quadratic terms, 0.5 regularization multipliers and 5-6 parameters (Table 2.2).

Table 2.2 The best models for each of the climatic data types (current lake water and current air temperatures).
Each of these models was selected amongst 16 candidate models (Supplemental Tables 2.2-2.5, Appendix A). Model features (linear or linear and quadratic together), regularization multipliers, Area Under the Curve (AUC) metric for the data used in training, average AUC considering the 4 testing spatial blocks, AICc values, and number of parameters used in each selected model. The selected models have the lowest AICc.

| Predictor group | Model | Features | Regularization multiplier | Training AUC | Mean testing AUC | AICc | Number of parameters |
|---|---|---|---|---|---|---|---|
| BIO 1,7,15 | Lake temp. | LQ | 0.5 | 0.81 | 0.75 | 17,802.42 | 6 |
| | Air temp. | LQ | 0.5 | 0.83 | 0.77 | 17,575.61 | 6 |
| BIO 10,11,15 | Lake temp. | LQ | 0.5 | 0.80 | 0.75 | 17,748.51 | 5 |
| | Air temp. | LQ | 0.5 | 0.81 | 0.75 | 17,573.02 | 6 |

Selected models had moderate to good predictive power. The global training data had AUC ranges of 0.81-0.82 and TSS of 0.48-0.49 when applying the maximum training sensitivity plus specificity threshold (BIO 1,7,15: lake=0.54, air=0.48; BIO 10,11,15: lake=0.58, air=0.58). The European test data had AUC ranges of 0.68-0.86 and TSS of 0.35-0.74 when considering this same threshold method (Table 2.3). The model using lake water temperatures and the predictor group BIO 1, 7, and 15 had the highest predictive power (test region metrics: AUC=0.86; TSS=0.74), while the model using air temperatures and predictors BIO 10, 11 and 15 had the lowest predictive power. Model transferability was low for both models using BIO 10, 11 and 15 predictors, with TSS values of 0.35-0.49 in the test region (Table 2.3).

Table 2.3 Accuracy metrics for models based on different temperature variables.
The training dataset is composed of global dotted duckweed records except European observations; the testing dataset is comprised of European observations from GBIF and literature records.

| Predictor group | Model | TSS | | AUC | |
|---|---|---|---|---|---|
| | | Train | Test | Train | Test |
| BIO 1,7,15 | Lake temp. | 0.48 | **0.74** | 0.81 | **0.86** |
| | Air temp. | **0.49** | 0.54 | **0.82** | 0.78 |
| BIO 10,11,15 | Lake temp. | **0.49** | 0.49 | 0.81 | 0.77 |
| | Air temp. | **0.49** | 0.35 | 0.81 | 0.68 |

The percentage contribution and permutation importance for each climatic variable was obtained across models. Overall, BIO 15 (precipitation seasonality) and BIO 11 (mean temperature of the coldest quarter) variables have the highest percentage contribution and permutation importance across models. In models using air predictors, BIO 1 (mean annual temperature) has the highest permutation importance. BIO 10 (mean temperature of warmest quarter) is the least important variable across models.

Table 2.4 Percentual contribution and permutation importance for predictors used across models. Values for variables with the highest percent contribution and permutation importance are in bold.

| | Models | | | |
|---|---|---|---|---|
| | Lake temperature | | Air temperature | |
| Climatic variables | Variable contribution | Permutation importance | Variable contribution | Permutation importance |
| Predictor group 1 | | | | |
| BIO 15 (precipitation seasonality) | **53.60%** | **40.31%** | **45.92%** | 25.62% |
| BIO 7 (annual range of temperature) | 27.97% | 26.74% | 29.16% | 27.69% |
| BIO 1 (mean annual temperature) | 18.43% | 32.95% | 24.92% | **46.67%** |
| Predictor group 2 | | | | |
| BIO 11 (mean temp. of coldest quarter) | **49.29%** | **50.06%** | **50.02%** | **60.43%** |
| BIO 15 (precipitation seasonality) | 49.04% | 42.77% | 46.60% | 29.68% |
| BIO 10 (mean temp. of warmest quarter) | 1.67% | 7.16% | 3.38% | 9.90% |

### 2.4.2 Response curves

The fitted response curves for the climatic variables are either unimodal or described by a decreasing function (Fig. 2.2). Overall, these responses are realistic, as their ranges are within climate data from occurrence records, as indicated by optimal suitability values and range (Table 2.5). In the first group of predictors, BIO 7 (annual range of temperature) responses are the most divergent among models, as the lake-based temperature models predict a narrower response curve that reflects a lower range of suitable temperature variation in temperatures in comparison to the air temperature model curve. In the second group of predictors, BIO 10 (mean temperature of warmest quarter) curve for models based on lake temperatures predicts optimal suitability in lower temperatures than predicted by air temperature models.

Table 2.5 Suitability values given by response curves of each Maxent model.

| | Predictor group 1 | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | BIO 1 (°C) | | | BIO 7 (Δ°C, annual) | | | BIO 15 (CV) | | |
| Model | Min | Opt | Max | Min | Opt | Max | Min | Opt | Max |
| Lake temp. | 0.69 | 17.89 | 30.05 | 4.74 | 16.01 | 34.90 | -2.28 | -2.28 | 144.72 |
| Air temp. | -12.99 | 16.24 | 38.68 | -1.04 | 21.21 | 56.92 | -5.20 | -5.20 | 143.96 |
| | Predictor group 2 | | | | | | | | |
| | BIO 15 (CV) | | | BIO 10 (°C) | | | BIO 11 (°C) | | |
| Model | Min | Opt | Max | Min | Opt | Max | Min | Opt | Max |
| Lake temp. | -2.28 | -2.28 | 143.96 | 5.50 | 5.50 | 33.51 | -4.75 | 13.66 | 29.64 |
| Air temp. | -5.20 | -5.20 | 143.96 | -6.07 | 18.35 | 43.28 | -22.12 | 11.76 | 37.78 |

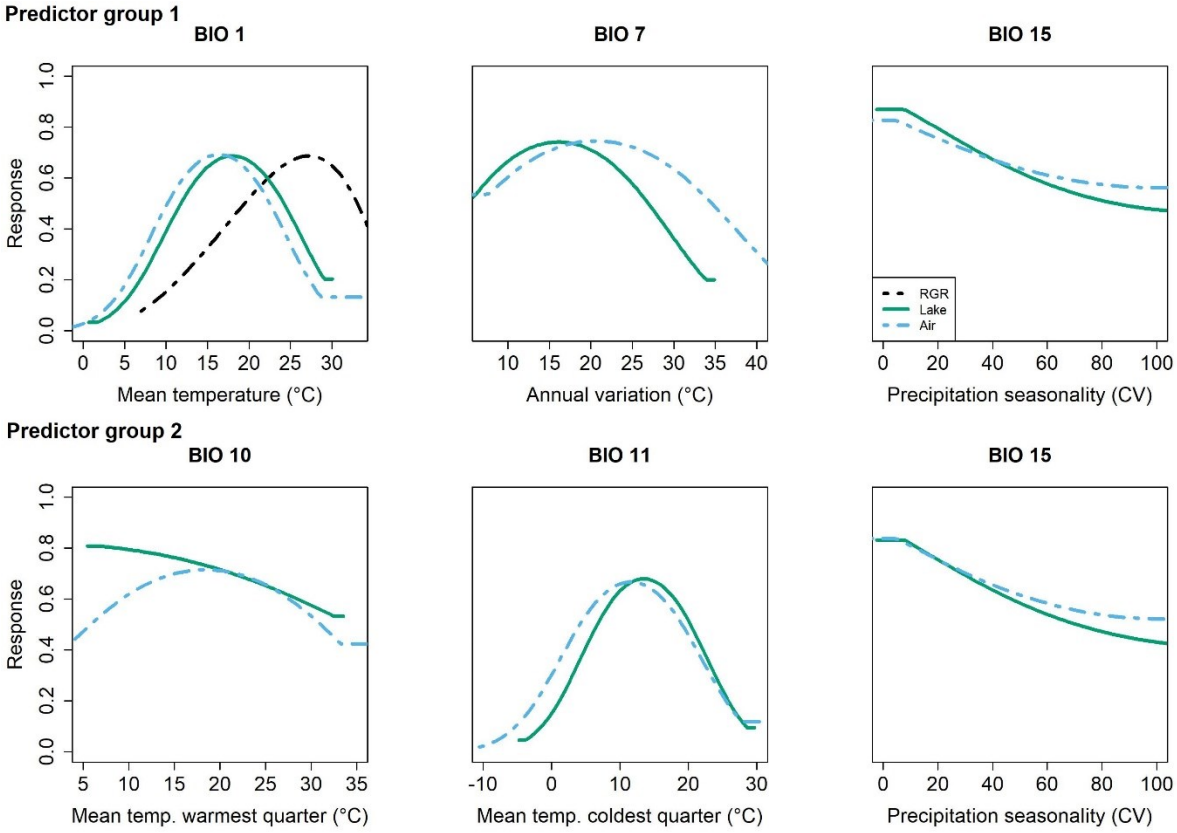



Figure 2.2 Response curves obtained from the selected Maxent models for BIO 1 (mean annual temperature), BIO 7 (annual range of temperature), BIO 15 (precipitation seasonality), BIO 10 (mean temperature of coldest quarter), and BIO 11 (mean temperature of the coldest quarter).
For BIO 1, a thermal performance curve for dotted duckweed was added for comparison with Maxent response curves. Performance is based on relative growth rates (RGR) obtained by counting final populations of individual fronds across several constant temperatures after a 5-day experiment period. The curve is based on the Ratkowsky model (Ratkowsky, 1983). Plots for BIO 15 show coefficients of variation from 0-100, although values higher than 100 are found in a few environmental raster cells likely due to precipitation anomalies (O'Donnel & Ignizio, 2012).

BIO 1 (annual mean temperature) response curves from both water and air models based on current temperatures are very similar. A thermal performance curve for dotted duckweed obtained in laboratory conditions was scaled to maximum BIO 1 response values to enable a comparison with response curves in a similar fashion as in Armitage (2023). This curve was then superimposed to BIO 1 response curves. The response curves fit using lake and air temperatures are similarly following the overall shape of a thermal performance curve obtained in the laboratory for this species. Additionally,

the response curve using lake temperatures approaches slightly more the laboratory-obtained thermal performance curve in comparison to curves fit using air temperature conditions (Fig. 2.2).

### 2.4.3 Current and future habitat suitability prediction maps

Overall, our models indicate several currently unoccupied regions which are suitable for dotted duckweed in Europe and Canada. Such areas include most of western Europe and some western areas in Canada. The lake and air temperature models based on BIO 1 (annual mean temperature), BIO 7 (temperature annual range), and BIO 15 (precipitation seasonality) have the lowest prediction errors and are more conservative than models based on other variables. Consequently, these models were used to create prediction maps (Fig. 2.3; refer to Supplemental Fig. 2.3-2.4 in Appendix A for maps based on other models). For both Europe and Canada, the models based on lake water temperatures predict a larger suitable area for dotted duckweed in relation to models using air temperatures.

Future dotted duckweed habitat suitability in a moderate emission scenario is very similar when using ACCESS-CM2 and MIROC6 projections. Both predictions indicate that Western Europe will continue being highly suitable, while some areas in eastern Canada that are currently unsuitable will become suitable, such as the Great Lakes region (Fig. 2.3).

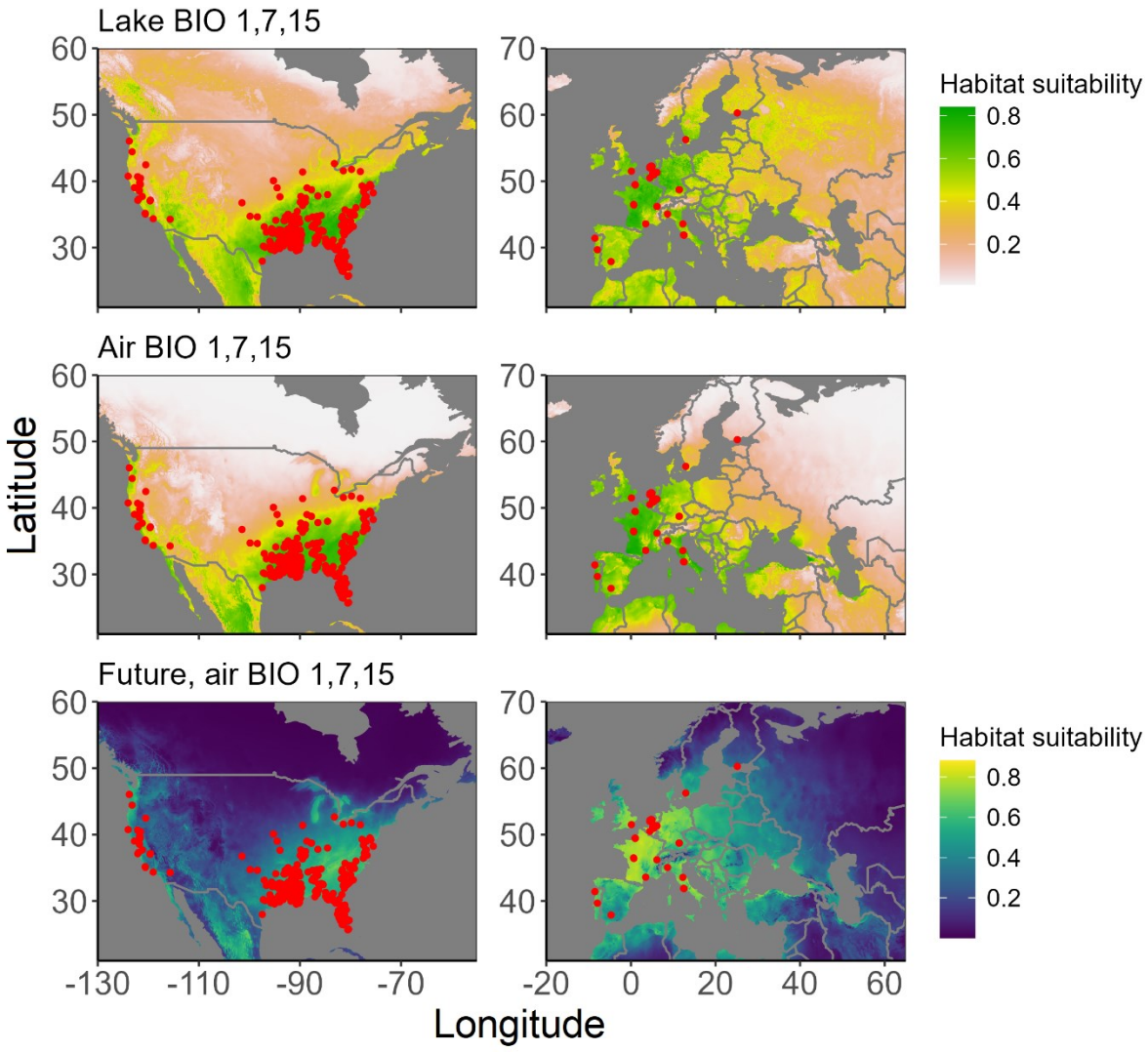



Figure 2.3 Predicted habitat suitability for dotted duckweed in western Europe and North America. Habitat suitability is in a scale of 0 to 1 and results are shown for the best models based on predictor group 1: BIO 1 (mean annual temperature), BIO 7 (annual range of temperature), and BIO 15 (precipitation seasonality) environmental variables from Armitage (2023) (lake water temperatures) and https://worldclim.org (current air temperature models: WorldClim version 2.1 climate data for 1970-2000; future environment predictions: CMIP6 downscaled future climate projections, ACCESS-CM2). Maps based on other predictors are available in Supplemental Fig. 2.3, Appendix A. Red dots are dotted duckweed occurrence records.

## 2.5 Discussion

Our models provide evidence that most areas in Western Europe and some western and eastern areas of Canada are currently suitable habitats for dotted duckweed. Our future predictions indicate that Europe will continue being highly suitable for this species, and that the Great Lakes region in Canada will become a suitable habitat for this invasive species.

The best lake water temperature model has a lower prediction error than the air temperature models across all performance metrics in the test region. These results are in accordance with previous research that predicted the distribution of common duckweed and greater duckweed using the same satellite-measured lake water temperature climatic layers (Armitage, 2023). Al-Chokhachy et al. (2013) also found that stream water temperatures improve the performance of models predicting fish distribution. In conjunction with these other studies, our research suggests that using water temperature-corrected predictors more closely reflects thermal niches and leads to more accurate distribution predictions than models based on air temperatures.

In Armitage's (2023) study, response curves for mean annual temperatures (BIO 1) were found to be similar to thermal performance curves for common and greater duckweed. In our research, model tuning also resulted in response curves that approximate the shape, but not the optimum, of dotted duckweed thermal performance curves. Thus, the distribution predictions obtained herein and by Armitage (2023) may reflect the thermal physiology of the studied species. The superiority of these predictions in relation to air temperature-based models can be illustrated by observing duckweed range limits in Armitage (2023) and in our research. In both studies, lake water temperature-based models better reflect observed margins (e.g., predictions matching observed records in northern locations such as Sweden and Finland) in comparison to models based on air temperatures. Differently from models based on air temperature, models based on water temperatures also predict high suitability in some western Canadian regions, which match the distribution of ecologically similar common duckweed, as seen in Armitage (2023).

Unlike Armitage (2023), our models based on water versus air temperatures do not result in different annual mean temperature (BIO 1) response curves: both models similarly approach the shape of dotted duckweed thermal performance curves. The response curves for BIO 15 (precipitation seasonality) are also very similar in these two models. Thus, for dotted duckweed, BIO 7 (annual range of temperature) seems to be behind the superior performance of the model based on lake water

temperature. BIO 7, with a permutation importance of ~27% across models, has a narrower curve and lower optimum in models using lake water temperatures. Considering that the model based on lake water climate factors is the best predictor, we can conclude that dotted duckweed optimal habitats are characterized by lower annual temperature variation than suggested by models based on air temperature.

Precipitation seasonality (BIO 15) is more important for the model using lake temperature than it is for the models using air temperature, as shown by permutation importance percentages. Both models predict optimal suitability when precipitation variation is low, which reflects well the need for moderate precipitation levels reported in the literature for duckweeds. This prediction aligns with the species' absence in arid areas of both Australia and the United States, which indicates that the models are extrapolating well environmental constraints found in the native region.

Models using the second set of predictors (BIO 10, BIO 11, and BIO 15) have limited predictive power in the test region. We observed some similar patterns in their response curves when compared to the first set of predictors discussed earlier. As seen with BIO 1, curves for BIO 11 (mean temperature of coldest quarter) are not very dissonant between lake and air temperature models. The same applies to BIO 15 (precipitation seasonality) response curves. The notable permutation importance of BIO 11 (50-60%) highlights its role in limiting dotted duckweed range, contrasting with the lesser importance assigned to BIO 10 (mean temperature of the warmest quarter).

Taken together, the divergences identified in models fit using lake water temperature-corrected variables lead to predictions of a wider suitable habitat for dotted duckweed in Europe and Canada than estimated by air temperatures. Our model with the highest predictive performance based on lake water temperature and BIO 1, 7, and 15 suggests that the countries at highest risk for dotted duckweed establishment in western Europe are Portugal*, Ireland, France*, Belgium*, Netherlands*, Italy*, Switzerland*, and Denmark. Such territories are practically 100% suitable for this invasive species. In other countries such as Spain, UK*, Germany, Austria, and Sweden*, and Finland* (in the latter two countries, suitability is restricted to the south), dotted duckweeds can find a mix of either moderately suitable or highly suitable habitats (N.B., countries highlighted with an asterisk have already been invaded by dotted duckweed). Unsuitable regions in Europe include semi-arid areas in Spain and the Alps mountain range.

In Canada, current suitability is mainly restricted to the province of British Columbia and a few maritime locations. In British Columbia, highly suitable areas include the southern coast and the central interior region. These areas are characterized by more moderate precipitation levels and milder temperatures than mountainous or northern regions of the province (Wang et al., 2016). Additionally, dotted duckweed was observed in Portland, Oregon, US, in 2007, at about the same longitude and around 240 km south from this Canadian region. Thus, risk of invasion is increased in highly suitable habitats in western Canada predicted by our model because of the presence of this invasive species relatively close to the US-Canada border. British Columbia was also shown to be highly suitable to common duckweed in previous studies using lake water temperature-based models. Moreover, the current distribution range of common duckweed includes these and other northern regions in Canada (Armitage, 2023). Therefore, it is plausible to consider that these areas can be invaded by dotted duckweed because these species have very similar thermal performance curves (Supplemental Fig. 2.2, Appendix A).

Future distribution predictions based on a moderate emission scenario indicate that most currently suitable areas will still be viable for dotted duckweeds in 2041. Moreover, new suitable areas will become available both in Europe and in Canada. Regions in Central and Eastern Europe will become more viable than nowadays, while the Great Lakes region will become suitable in Canada. Because this species is currently abundant across the East of the US, including observations very close to the Canadian border (about 110 km to the Canadian Great Lakes region), climate change preparedness plans for the region should consider this invasion risk in the near future.

One limitation of our study is the lack of future climate predictions for lake water temperature. Predictions based on water temperatures could yield more realistic species distributions across climate change emission scenarios. Such data might also be valuable if applied to other invasive species, such as the new six invasive aquatic plants among the recently released list of the 10 prohibited invasive species in Ontario, Canada (Ontario Newsroom, 2023).

Our research predicts that dotted duckweed has the potential to achieve wide distribution in North America and West Europe. This wide distribution may lead to significant ecological and economic impacts. Previous studies predict low invasion impacts for dotted duckweed in Europe due to life history similarities with its native counterpart common duckweed. However, least duckweed (*L. minuta*), which is another ecologically similar duckweed species, has achieved widespread

distribution in Europe. Likewise, dotted duckweed has spread across the US in the last few decades. Thus, we suggest that dotted duckweed has the potential to be as disruptive as least duckweed in western Europe.

In addition to ecological damage, waterway clogging, and loss of recreational areas, aquatic invasive species such as dotted duckweed can invade aquaculture breeding tanks and result in economic loss in this industry (e.g., see impacts of invasive tunicates for the mussel aquaculture industry in Canada; Fisheries and Oceans Canada, 2016). In fact, one data record describes dotted duckweed growing as "very vital monocultures found in outdoor breeding tanks" in Belgium (van Valkenburg & Pot, 2008). Aquaculture is a major industry in western Europe, worth €4.2 billion in 2021 (Eurostat, 2021). Thus, considering risks to both natural environments and economic activities, environmental managers both in European countries with a recent dotted duckweed invasion history and in uninvaded countries are recommended to develop surveillance systems and/or early containment programs.

The potential for widespread distribution found in our work highlights the importance of prevention or early containment as the only feasible measures to avoid or reduce negative consequences of invasive dotted duckweed spread. The large regions of potentially suitable habitat predicted by our model indicate the potential for invasion scenarios in Europe and some Canadian regions similar to the observed for least duckweed in Europe and dotted duckweed in the US. Future studies could also focus on other geographic areas currently reporting dotted duckweed invasions, such as eastern Europe, the Middle East, and South America.

Once dotted duckweed spreads, management becomes virtually impossible, as it reproduces asexually and has one of the fastest growth rates seen in higher plants (Ziegler et al., 2014). In addition, there are multiple introduction pathways, such as aquarium and garden trade (Neucker & Scheers, 2022), and deposition through waterfowl feces (Paolacci et al., 2023). Herbicide resistance developed by dotted duckweed (Koschnick et al, 2006) is an additional factor that would hinder management actions. Potential preventive measures include the development of regulations and educational awareness to the garden and aquarium industries.

# Chapter 3
# The influence of diel temperature variability on duckweed growth rates

## 3.1 Abstract

Night-warming, driven by climate change, is affecting plant growth rates globally. This phenomenon involves changes in diel mean temperature, temperature variance, and warming timing, all with potential to impact plants. Previous research has not separated these aspects or included floating aquatic plants, which are predicted to overgrow and harm ecosystems due to mat formation amplified by climate warming. Both positive and negative effects have been suggested as resulting from changes in the timing of warming, and seasonality also seems to play a role. Duckweeds, fast-growing cosmopolitan aquatic plants, have economic potential but pose ecological risks. We conducted night-warming experiments simulating current and future spring and summer conditions, measuring duckweed growth rate and biomass. Controls for mean temperature and variance were included, guided by thermal performance curves. Our findings indicate that warming timing is not crucial; plants respond positively to favourable mean temperature increases in spring, while reduced temperature variance keeps current growth levels in future warmer summers. Future night-warming plant research is recommended to separate statistical and temporal aspects of climate change. Future duckweed control management should consider that both future springs and summers have conditions allowing uncontrolled plant overgrowth.

## 3.2 Introduction

Recent studies highlight a potentially important climate change pattern in many temperate regions: disproportionately warmer nights compared to days, known as “night-warming” (Turnbull, 2002; Cox et al., 2020; IPCC, 2023). This phenomenon has been observed in historical climate data and is projected for future climates (Cox et al., 2020; Wang et al., 2023). This change in the daily sequence of temperatures has the potential to affect plant productivity, abundance, and distribution (Alward et al., 1999; Turnbull, 2002). We examine how future climate change temperature regimes in Ontario, characterized by an increase in night-warming, will potentially influence the population growth and biomass of common duckweed, and compare these effects to current spring and summer conditions.

Many climate change experiments focus solely on increases in average temperature (Morash et al., 2018). Similarly, over 50% of marine and 24% of freshwater ecosystem ecology experiments use

constant temperature regimes (Thompson et al., 2013), thereby overlooking realistic temperature variability patterns, such as night-warming. It is documented that nighttime warming affects plants by altering phenology, plant growth, biomass allocation, crop yield, carbon and nitrogen cycles, and the production of essential oils and nectar (Gaston, Gardner, & Cox, 2023). Even the few existing climate change experiments on wild plants which include night-warming often focus on constant daytime and nighttime temperatures, and do not separate effects of the timing of warming from other factors which we know affect plant performance, such as mean temperature warming and changes in temperature variance (General Introduction, Table 1.1).

These night-warming studies are mainly focused on terrestrial plants, use moderately warm temperature treatments, and suggest that night-warming affects plant physiology independently of changes in overall mean and temperature variance. This would be due to the time-dependence of photosynthesis and respiration, where increased carbohydrate consumption during warmer nights leads to higher photosynthetic activity the next day, promoting greater plant growth (He, Li, & Peng, 2012; Paul & Foyer, 2001; Su, Han, & Chen, 2020; Turnbull et al., 2002, 2004; Wan et al., 2009). However, this observed benefit of night-warming is not universal, as night-warming depends on factors such as water availability, climatic region, season, and species-specific physiological traits (Cox & Gaston, 2024; Fan et al., 2022). For example, many agricultural studies report negative effects of night-warming (e.g., Cheng et al., 2010; Hein et al., 2020; Impa et al., 2019, 2021; Loka & Oosterhuis, 2010; Peraudeau et al., 2015; Prasad & Djanaguiraman, 2011), potentially due to increased respiration reducing carbon available for plant and seed growth (Sadok & Jagadish, 2020).

Another factor making it difficult to draw conclusions regarding nighttime warming effects is that seasonal variations may play a role. For instance, an invasive Asteraceae species showed increased biomass during the winter in China, but the opposite occurred during the summer, indicating that invasion may not be promoted by night-warming in the long term (Chen et al., 2017). Same trends were observed for shrubs in the Mediterranean region (Prieto, 2009). Across ectotherms in general, we lack data on how daily temperature changes affect organisms around high average temperatures, making it hard to predict climate change impacts related to heat stress (Kefford et al., 2022; Kingsolver, Diamond, & Buckley, 2013).

As in the spring, the timing of warming is suggested to have specific effects in the summer. Differently than the spring though, the combination of hot summer daytime temperatures and

nighttime warming may result in net negative effects due to the lack of recovery from high daytime temperatures, as demonstrated in other ectotherms (Bai et al., 2019; Ma et al., 2021; Speights, Harmon, & Barton, 2017; Zhao et al., 2013). Although we did not find plant studies specifically focusing on night-warming effects combined with hot daytime temperatures, there are indications that there may be detrimental effects. For example, one study found reduced heat responses in a model plant during the night (Dickinson et al., 2018). This impact may explain the reduced yield in some agricultural crops with nighttime warming during hot summers (e.g., Narayanan et al., 2015).

Some plant groups, like floating aquatic plants, are underrepresented in night-warming studies. These plants are important to study because research suggests climate warming will benefit them over submerged plants due to mat formation (Lind, Eckstein, & Relyea, 2022; Netten et al., 2010), resulting in negative ecosystem impacts (Ceschin, 2020; Lind, Eckstein, & Relyea, 2022). The single climate change study on a semi-aquatic wild plant species (Wu, Ismail, & Ding, 2017) showed detrimental effects of night-warming, though confounding factors related to changing mean temperature and variance were present. This lack of night-warming studies may not be problematic if aquatic plants respond similarly to terrestrial plants. However, there are reasons to suspect differences between terrestrial and aquatic plants in their response to night-warming. For example, while some terrestrial plants show improved performance due to increased stomatal density following warmer nights (Fan et al., 2022), water loss is not an issue for aquatic plants living in permanent water bodies. Many aquatic plants, such as duckweeds, have permanently open stomata (Raven, 2002).

While aquatic environments are usually less variable than terrestrial ones, the magnitude of surface water fluctuations vary significantly depending on lake volume (Martinsen, Andersen, & Sand-Jensen, 2019), therefore in small ponds where duckweed is often found, changes in temperature variance may affect these floating plants. The significance of temperature variability in population growth rates has been investigated in a few studies (Vasseur et al., 2014; Di Cecco & Gouhier, 2018). Increased temperature fluctuations can cause disproportionate deviations from growth rates predicted by constant temperatures due to nonlinear and asymmetrical thermal responses (Ruel & Ayres, 1999). Temperature variations can influence biological reactions more than changes in average temperature alone (Dillon et al., 2016; Vasseur et al., 2014), especially for ectotherms, whose body temperature relies on ambient conditions. For example, experimental studies have shown that during the growing season, consistently large daily temperature ranges, which may reflect increased temperature

variance, negatively affect the photosynthetic performance of wetland plants compared to smaller temperature ranges (Xiao et al., 2019).

Thermal performance curves help gain insight into temperature variability effects by showing how species performance in traits like growth, reproduction, or photosynthesis varies with temperature. These typically asymmetric curves show performance increasing slowly to the optimal range, then rapidly declining beyond it (Huey & Kingsolver, 1989). While responses to constant temperature changes follow this shape, temperature variation leads to diverse responses. For example, due to steep declines after optimal temperatures, impacts are greater when mean temperatures are at or above thermal optima (e.g., Slein et al., 2023). Jensen's inequality (Jensen, 1906) predicts that for nonlinear functions like these curves, average performance with varying temperatures differs from performance at a constant average temperature. Generally, temperature variation reduces average performance, lowers the temperature of maximum performance, and narrows the range of viable temperatures (Denny, 2019). Changes in mean and variance of temperatures can have a synergistic impact, amplifying climate change effects on organisms (Payne & Smith, 2017; Kefford et al., 2022).

Thermal performance curves can be used to have insights about some expected impacts of night-warming on population growth. Since night-warming increases the daily mean temperature and decreases temperature variance, it should be generally beneficial to ectotherms that are in environments with current mean temperatures below their optimum (Speights et al., 2018). This prediction aligns with Jensen's inequality, and is the expected impact even if night-warming has no particular physiological effects on organisms. Thus, it is important that night-warming studies separate effects of seasonal, statistical, and temporal factors of temperature variations, which may influence plant population growth.

To address the lack of studies on night-warming and aquatic plants, we investigate the population growth rate and biomass production of common duckweeds under current and projected changes to diel temperature cycles. We selected common duckweeds (*Lemna minor*) as our model organism because these aquatic floating plants have rapid growth rates, important impacts on the community structure of some freshwater systems (Shao et al., 2018), and play an economic role in protein production and water treatment (Ziegler, Appenroth, & Sree, 2023).

We based our hypotheses on physiological explanations from the literature regarding night-warming and tested them to determine if the timing of warming matters by controlling other factors

which we know affect plant population growth rates: changes to mean temperature and temperature variance, which are also associated with the night-warming phenomena. In the cooler spring temperatures, we predict that night-warming will benefit population growth rates because of photosynthetic overcompensation. In hot summer conditions, we predict that night-warming will reduce population growth because elevated nighttime temperatures will inhibit recovery from daytime heat stress, a phenomenon observed in other ectotherms (Dickinson et al., 2018; Ma et al., 2021).

## 3.3 Methods

To determine the impacts of climate change-induced diel temperature increases marked by warmer nights on duckweed population growth rates, we compared the number of fronds after exposure to 10-day temperature treatments based on current conditions and those with various warming scenarios and patterns of temperature fluctuations. The treatments were designed to differentiate between the potential effects of warming timing, average temperature increase, and temperature variance. In addition, we did two sets of experiments: one designed to mimic spring conditions in southern Ontario and one for hot summer conditions.

### 3.3.1 Duckweed culture origin and cultivation

Common duckweeds (*Lemna minor*) were selected as model species for this study. These aquatic plants are convenient organisms for biological studies due to their fast growth, small body size, and mostly asexual reproduction (Laird & Barks, 2018). Also, common duckweed has a simple life history, considering its preferential asexual reproductive strategy and the formation of resting fronds which do not structurally change during dormancy (Ziegler, Appenroth, & Sree, 2023). Most importantly, temperature is a major abiotic factor affecting the species growth, survival, and distribution (Rejmánková, 1975; Mcilraith, Robinson, & Shay, 1989; Echelpoel, 2020).

Two different strains were used for the two different experiments. For the spring experiment, a strain of common duckweed (*L. minor*) collected in the field on an unknown date was obtained from Carolina Biological Supplies, Burlington, North Carolina, USA in 2020 and maintained aseptically. For the summer experiment, a strain of common duckweed was collected on August 29, 2022, in a stormwater pond located in Waterloo, ON (43.47, -80.55) was sterilized according to the methodology outlined in Jewell, van Moorsel, & Bell (2022), and aseptically cultured.

Plants were kept in 20°C LED lit incubators (IPP-500) and grown on modified Hoagland's medium (Environment Canada Hoagland's E+ medium w/o organics, sterile) obtained from the Canadian Phycological Culture Centre (CPCC), University of Waterloo, Canada. This macronutrient solution is widely used to culture duckweed species (e.g., Lasfar et al., 2007; Rippner et al., 2018, Wang et al., 2020). We used a 16:8 photoperiod, with daylight intensity at 78.00-91.68 μmol $m^{-2}\,s^{-1}$, which is a moderate range for the growth of duckweed species (Paolacci, Harrison, & Jansen, 2018c; Pasos-Panqueva, Baker, & Camargo-Valero, 2024).

Plants used in experiments were even-aged individuals. Using same-aged individuals avoids impacts of differing senescence effects when assessing the performance of duckweed species during these experiments (Barks & Laird, 2015). To obtain these individuals we used birth order standardization, which is a procedure where first born fronds are successively separated across generations. This procedure reduces parental effects resulting from processes such as mutation accumulation on the performance of subsequent duckweed generations (Ankutowicz & Laird, 2018; Barks & Laird, 2015; Mejbel & Simons, 2018). To implement this standardization, about 2 weeks before experiments ~70 fronds of common duckweed were aseptically transferred to 24-well plates containing 2 mL of modified Hoagland's solution. After reaching maturity (about 4-5 days later), the first daughter fronds within each well were manually separated from their mother fronds by gently pressing a loop in between fronds. Mother fronds were discarded, and daughter fronds were considered as generation zero. This process was repeated up to the second generation, when these focal plants were then used in experiments, following a similar methodology to that described by Thwaites et al. (2024). Following the generation standardization, focal plants used in experiments were selected based on general health (size and colour) and age (about 4-5 days following germination), which corresponds to reproductive maturity.

### 3.3.2 Temperature sequence treatments

We created temperature treatments with diel fluctuations using two different methods for spring and summer conditions. For the spring experiment, we created a diel fluctuating treatment called "ambient" using mean daily temperatures observed at the weather station of the University of Waterloo from 1998 to 2020 during the dates of June 1-10 (Fig 1a). Similarly to the methodology in Higashi, Barton, & Oliver (2020), our temperature treatments incorporated the observed maximum

(20.2ºC) and minimum (12.4ºC) temperatures, and a mean of 16.4ºC. Using these temperatures, we generated a triangular thermal pattern using a simple linear regression.

For hot summer periods, we used maximum (35.0ºC), minimum (13.0ºC), and mean (24ºC) temperatures similarly to Ma et al. (2021), which are occasionally observed during hot summers in Southern Ontario (ambient treatment, Fig. 3.1b). For instance, in the Kitchener/Waterloo area, warm years such as 2016, 2020, and 2022 experienced between 3-12% of days in a given summer month with peak temperatures ranging from 33-37ºC. Additionally, 54-67% of days had minimum temperatures between 11-15ºC, and 16-55% of days had average temperatures of 22-26ºC (Environment Canada, 2023). To introduce diel fluctuations in the summer treatment, we used a sinusoidal model, a widely used approach for replicating diel temperature regimes (National Institute of Water and Atmospheric Research, 2023), where hourly temperatures are given by the equation: $T(hour) = min(T) + (max(T) - min(T)) * (0.5 + 0.5 \sin(2\pi (hour) / 24))$. Here $T(hour)$ represents the hourly temperature, *hour* is the index for each time step, and $min(T)$ and $max(T)$ are the minimum and maximum temperature values. Thermal sequences with hourly temperatures are generated by interpolating between the minimum and maximum values, ensuring smooth transitions over a 24-hour period to represent natural daily temperature cycles (Fig. 3.1b). The triangular or sinusoidal diel pattern was repeated for each treatment over a span of ten days. This repetition ensured that all treatments have strong positive day-to-day temperature autocorrelation, thereby reducing potential differences in temporal structure between treatments.

To create warming treatments that represent future conditions, we used predicted mean surface air temperature increases for Ontario, Canada in 2060-2079: 2.5°C in June for the spring experiment and 3.5°C in July for the summer experiment. These values are within average monthly median temperatures predicted by a multi-model ensemble under the SSP2-4.5 scenario (June: +2.5°C; July: +2.68°C), representing an intermediate emission pathway, and the SSP5-8.5 scenario (June: +4.01°C; July: +4.35°C), representing a business-as-usual emissions scenario (World Bank, 2021).

For each season, we created three warmed treatments with diel fluctuations as described above. The first treatment, “night-warming,” involved warming exclusively during nighttime hours, resulting in reduced temperature variance. The remaining treatments had either the same mean temperature or a similar variance, with different timings of temperature patterns so that we could distinguish between impacts caused by the statistical characteristics of the treatments, and those which might be the result

of a physiological response to night-warming. The second treatment, “ambient+,” maintained the same variance as the ambient treatment but increased hourly temperatures evenly to produce a higher mean. The final treatment, “random,” had the same variance and mean temperatures as the night-warming treatment but hourly temperatures varied randomly throughout the day. Finally, in the summer experiment, we also added two constant temperature treatments, one corresponding to current conditions (24ºC), and another to the future scenario (27.5ºC), to investigate the impacts of temperature variance (Fig. 3.1a,b).

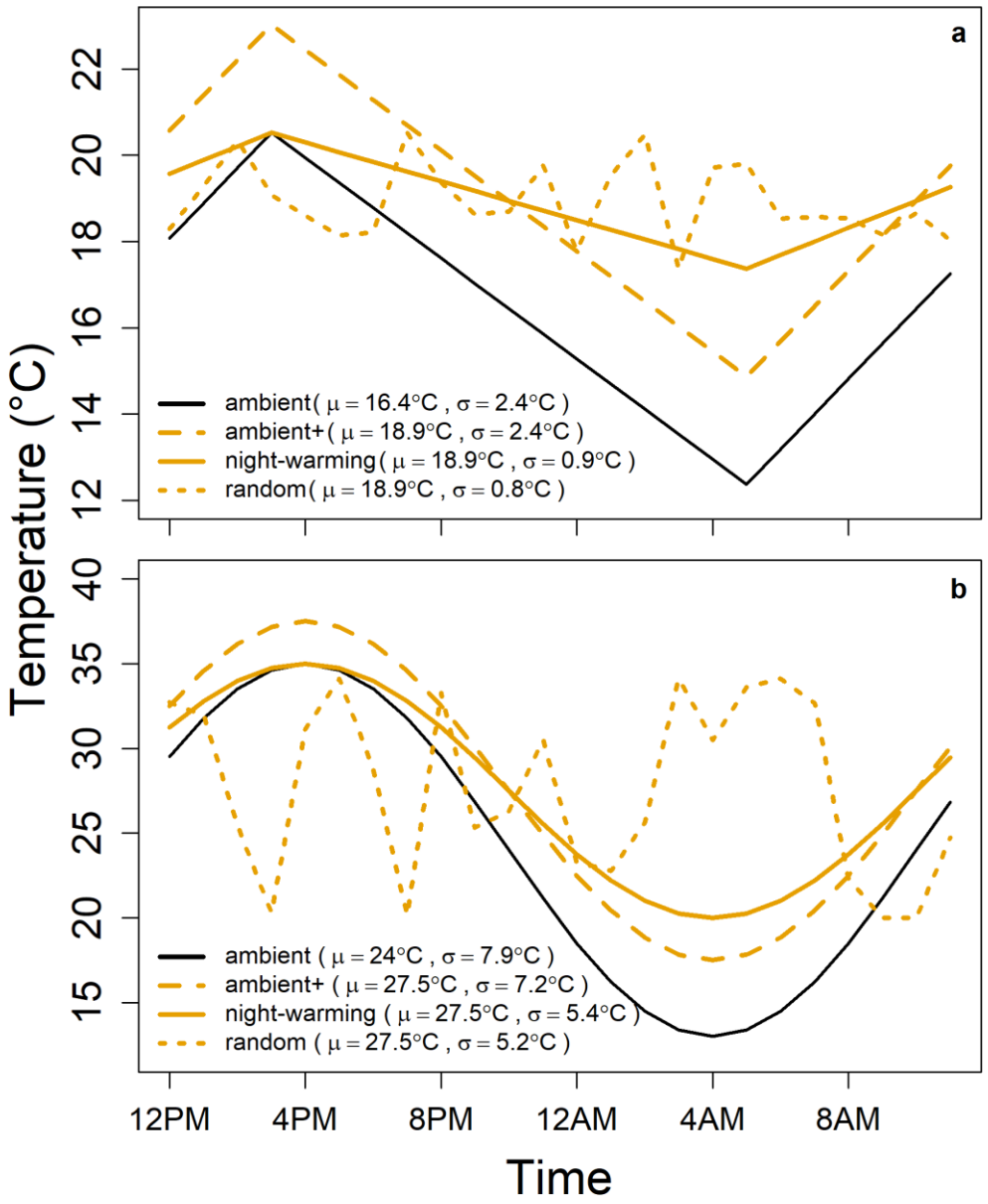



Figure 3.1 Fluctuating treatments in spring (a) and summer (b).
The graph illustrates 24-hour cycles for ease of visualization, though the experiments had a 10-day period. Ambient conditions are represented in black, while warming conditions are shown in orange. Note the difference in scales for the y-axis.

In constructing these temperature treatments, we ensured that the timing of maximum and minimum temperatures, as well as the photoperiod, correspond to historical data for each respective

period in Southern Ontario, Canada. For the spring treatments, maximum temperatures occurred at 3 PM and minimum temperatures at 5 AM, with a photoperiod of 15.5 hours of light and 8.5 hours of darkness. In the summer treatments, maximum temperatures occurred at 2 PM and minimum temperatures at 4 AM, with a photoperiod of 15 hours of light and 9 hours of darkness (National Research Council, 2022). We also note that in both the spring and the summer experiments, the range of temperatures used in all conditions (13ºC - 37ºC) were higher than those that induce dormancy in common duckweed (Landolt, 1986; Cao, Fourounjian, & Wang, 2020; Jacono & Pfingsten, 2022).

The 10-day (240 hours) temperature sequences were programmed into the incubators using the proprietary software Celsius (version 10.1.4). The incubators in general followed the input temperature sequences well, but there were deviations. We excluded replicates with departures greater than 0.7ºC from the programmed standard deviations and 1.1ºC from programmed average temperatures for each 10-day sequence comprising each treatment (data validation available here). All data manipulation and all reported analysis were completed in R (R Core Team, 2022).

### 3.3.3 Population growth and biomass measurements

For each experiment, we completed 28 replicates (7 replicate repetitions x 4 treatments). Each replicate consisted of 12 fully-grown, healthy, second generation fronds, individually placed in a single well in a 24-well plate exposed to a given temperature sequence in a single incubator. Each well contained 2.8 mL of modified Hoagland's solution, and this amount of nutrition media provided to each frond per time period closely corresponds to the volume indicated in the ISO 20227:2017 biotest protocol (Baudo et al., 2015). Replicates for individual treatments were randomly assigned to one of the 4 Memmert ICP-500 incubators.

We measured the number of live fronds after exposure to the 10-day temperature sequence by counting the total number in each replicate plate of 12 initial fronds. We considered individual fronds as alive when they displayed <90% loss of green pigmentation (Thomson & Dennis, 2013). Fronds of all sizes were included in the count, including any visible protuberance indicating a new germinating frond from the lateral meristematic pocket of a given mother frond.

We also estimated the change in biomass at the end of the experiment using dry weight. To obtain this measure, living plants were placed in between layers of filter paper, and dried at 80°C for 12 hours (Radić et al., 2010) in a Thermo Scientific Heratherm Mechanical oven. Dry weights were measured using an Optima high precision balance accurate to $10^{-5}$ grams. Change in dry weight was

estimated by using the initial biomass of 12 fronds from the same cohort of plants for each replicate. In the spring experiment, dry weight data for the random treatment were not obtained. However, this does not affect the overall results, as this predictor was found to be non-significant when comparing the three other treatments.

### 3.3.4 Statistical analysis

We evaluated the effect of treatment using either linear or generalized linear models. For count data, analysis was completed using Poisson distribution to describe errors, or when overdispersion was detected, a quasipoisson distribution. We compared candidate models using either simple linear or mixed models using maximum likelihood estimation from the R package lme4, depending on whether there was a significant random effect of grouping replicates obtained at the same time. This package estimates the best-fitting parameters while accounting for random effects and the grouping structure in the data (Bates et al., 2015; RStudio Team, 2020). Fixed factors included treatments (ambient, ambient+, night-warming, and random), and for the interaction model comparing constant and fluctuating treatments, it included temperature (current vs future) and treatment (constant vs fluctuating, the latter corresponding to ambient and ambient+). Data for the random treatment were obtained exclusively for frond count, as a preliminary analysis showed that the main effect of treatment would not be significant when using biomass. To compare treatments, we carried out post hoc tests using the package emmeans (Lenth, 2024). For linear models, degrees of freedom were approximated using the Satterthwaite method. We used the marginal and conditional $R^2$ to determine if random factors needed to be included in the statistical analysis. The marginal $R^2$ refers to the variance explained by fixed effects, which is calculated by comparing the variance of predicted values based solely on fixed effects to the total variance in the data. The conditional $R^2$, on the other hand, includes both fixed and random effects (Nakagawa & Schielzeth, 2013). Final candidate models were evaluated using the Akaike Information Criterion (AIC) estimator. Although we used different duckweed strains for the spring and summer experiments, we did not find significantly different growth rates across temperatures when comparing them at several constant temperatures in a preliminary statistical analysis.

## 3.4 Results

In our comparison of current and future climate change temperature scenarios, we found that the timing of warming had no significant effect on the population growth of duckweeds. Instead, these

factors were determined by mean temperature increases in the spring and the interaction between altered mean temperatures and variance in the summer. For all analyses, the mixed-effects models were identified as the best-fit according to the Akaike Information Criterion (AIC).

### 3.4.1 Spring temperatures

Our analysis based on live frond counts as a proxy for population growth ($\chi^2 = 26.28$, df = 3, $p < 0.001$) indicates that all warming patterns in spring enhance population growth compared to current ambient conditions. This improvement aligns with our expectations that increases in average temperature move population growth rates closer to optimal levels. Specifically, ambient+ (z-ratio = -4.72, $p < 0.001$), night-warming (z-ratio = -3.97, $p < 0.001$), and random treatments (z-ratio = -2.65, $p = 0.03$) have significantly increased population growth in comparison with ambient conditions. However, night-warming does not provide a distinct advantage compared to other warming patterns, as its effects are not significantly different from the ambient+ treatment, where warming occurs uniformly throughout the day (z-ratio = 0.77, $p = 0.870$) and from the random treatment (z-ratio = -0.86, $p = 0.825$) (Fig. 3.2a, Supplemental Table 3.1). We note that we did not observe any mortality during our experiments, thus changes to population growth are exclusively due to changes in reproductive output. Changes in total biomass follow a similar pattern, although the main effect of treatment was not significant ($F = 0.52$, df = 2, $p = 0.603$) (Fig. 3.2b, Supplemental Table 3.1).

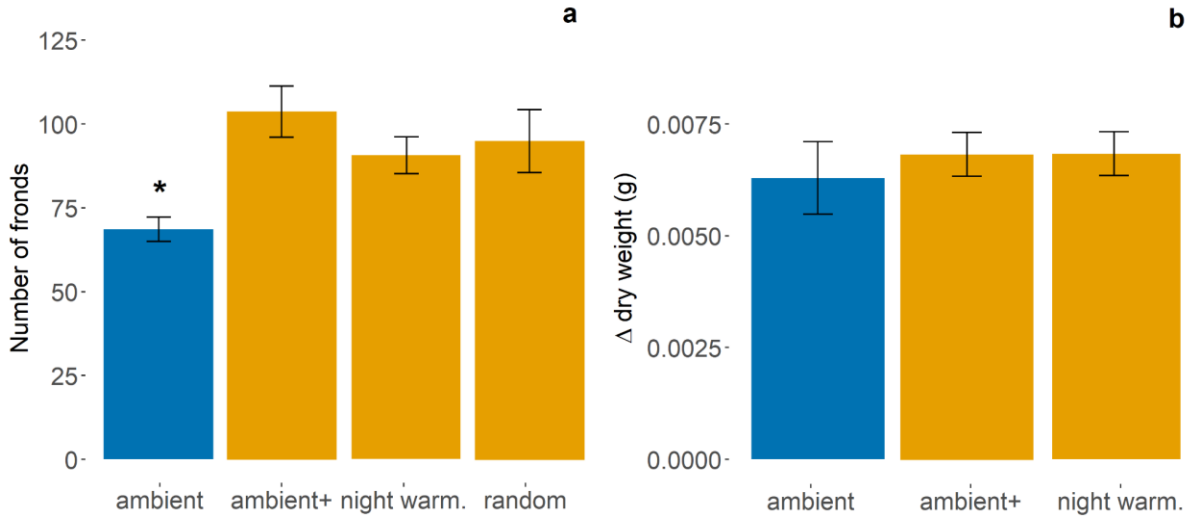



Figure 3.2 Effects of different patterns of diel temperature increase on population growth, measured as the average number of live fronds (a), and average change in biomass of common duckweeds (b) during typical temperate spring temperatures.
Thin bars are standard errors calculated using a Poisson distribution for the number of fronds and a normal distribution for biomass. The asterisk concludes that the ambient treatment has a significantly lower number of fronds than the other three treatments.

### 3.4.2 Summer temperatures

In summer conditions ($\chi^2 = 30.33$, df = 3, $p < 0.001$), the treatment with even hourly warming, ambient+, had lower population growth than all of the other treatments, both for current (ambient: z-ratio = 2.65, p = 0.041) and some future scenarios (random warming: z-ratio = -5.44, $p < 0.001$), except for night-warming (z-ratio = -2.36, p = 0.084). However, the other future scenarios did not differ significantly from current ambient conditions (night-warming: z-ratio = 0.51, p = 0.958; random warming: z-ratio = -0.56, p = 0.946). There was no significant effect of the timing of the warming as measured by the difference between the night-warming treatment and the treatment with random fluctuations (z-ratio = -0.84, p = 0.836) (Fig. 3.3a, Supplemental Table 3.1). Changes in total biomass exhibit a similar trend but the main effect of treatment is not significant (F = 0.66, df = 3, p = 0.588) (Fig. 3.3b, Supplemental Table 3.1).

A comparison of the treatments allows us to isolate any impacts of night-warming independent of changes to the mean and variance of the temperatures. Specifically, if increases only in mean temperature were responsible for any observed detrimental effects, we would expect the population growth in the ambient+ treatment to be lower than ambient, which was observed, and the same as the

night-warming and random treatments, which was not entirely observed (ambient+ = night-warming; ambient+ ≠ random). We note that ambient+ and night-warming are significantly different before adjusting for quasipoisson errors. Similarly, if increased variance were the cause of any negative impacts at increased temperatures, then the growth rates in the night-warming and random treatment should be higher than those of the ambient+ treatment, which has higher variance, which was observed. Population growth rates measured under hot constant temperatures should be even higher, which was also observed, as we will detail subsequently.

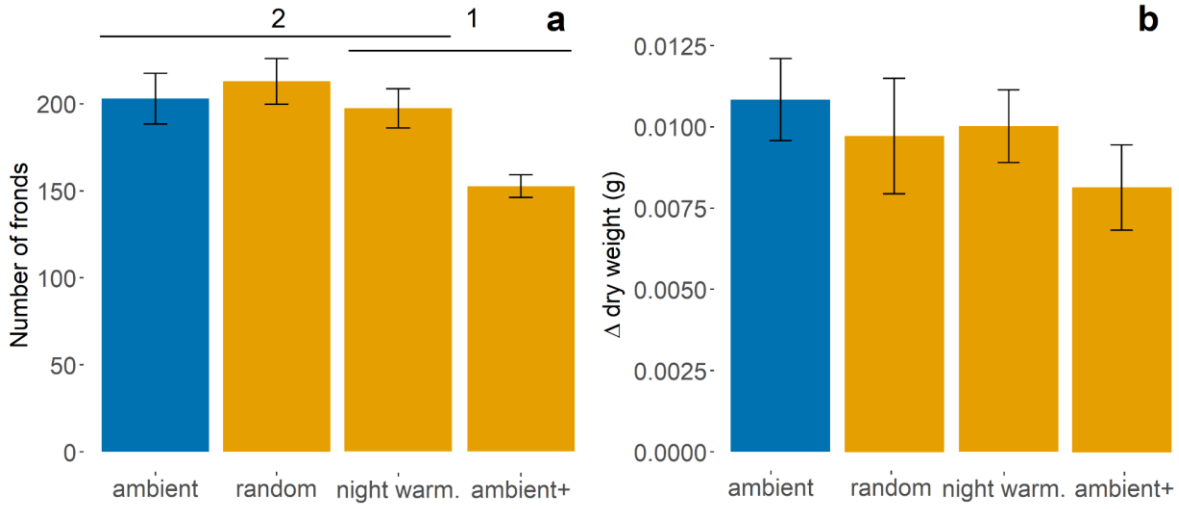



Figure 3.3 Effects of different patterns of diel temperature increase on the average number of live fronds (a), and average change in biomass (b) of common duckweeds during hot summer temperatures.
Thin bars are standard errors using a Poisson distribution for the number of fronds and a normal distribution for change in biomass. Same numbers indicate treatments not significantly different from each other under a given horizontal line. Thus, ambient+ is significantly different from ambient and random treatments, but not from night-warming.

We note that there is a significant interaction between increasing average temperatures and variance. Temperature fluctuations have a negative impact on population growth and biomass at higher mean temperature (number of fronds: $\chi^2 = 21.10$, df = 1, $p < 0.001$, Fig. 3.4a; biomass: $F = 8.75$, df = 1, $p = 0.007$, Fig. 3.4b), but not at cooler temperatures.

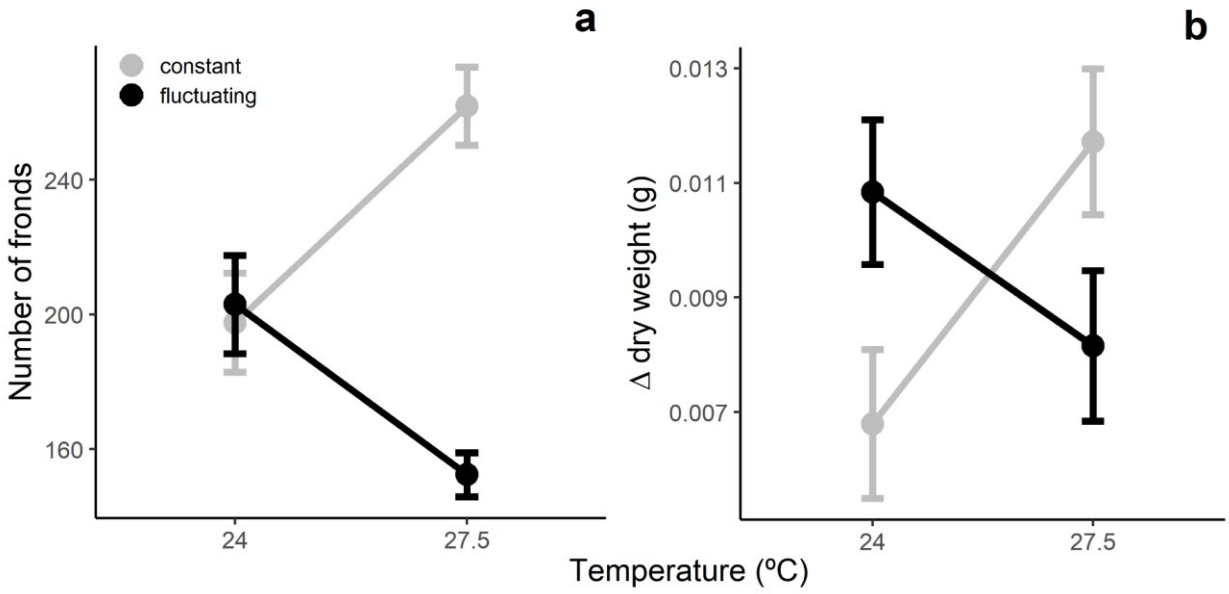



Figure 3.4 Mean number of live fronds (a) and change in dry weight (b) for two different mean temperatures and either constant (grey) or fluctuating (black) temperature treatments. Lines are standard errors using a Poisson distribution for the number or fronds and a normal distribution for biomass. Points are mean values within replicates for each treatment.

### 3.5 Discussion

Previous studies on night-warming have not differentiated between the potential effects of average temperature increases, changes in temperature variance, and their interaction, making it difficult to draw conclusions about the underlying mechanisms behind observed effects. We find that for common duckweeds, the timing of warming does not affect population growth in either summer or spring simulated conditions. Instead, all responses were driven by either changes to the mean or variance of temperatures. We highlight the need to differentiate between mean temperature increases, changes in variance, and alterations in the timing of warming when predicting the potential consequences of climate change.

Common duckweeds responded differently to temperature variability depending on the mean temperature. In cool spring conditions, population growth improved in night-warming treatments due to the overall rise in average temperature generated by this warming. The timing of warming did not appear to play a critical role, as population growth rate under night-warming was not different from other warming treatments with different timings. Moreover, the different magnitudes of temperature variance we tested in the warming scenarios did not affect duckweed population growth. Changes in

temperature variance are expected to have a beneficial effect at mild average temperatures towards the optimum (Deutsch et al., 2008; Kefford et al., 2022). However, we suggest that variance under cool conditions had little impact because of the gradual slope on the left side of the thermal performance curve of common duckweeds (Andrade-Pereira & Cuddington, 2024). Because of the shallow slope of the curve in this region, any variance would have rather small impacts.

Our findings regarding the beneficial impact of future warming under cool conditions support previous studies suggesting that species living in cooler environments relative to their optimal temperature benefit from temperature increase, provided temperatures do not surpass their optimum threshold (Deutsch et al., 2008; Kefford et al., 2022), including aquatic plants (Wu et al., 2021). A meta-analysis on plant species looked at the effects of both decreased and increased nighttime temperatures and found non-linear photosynthetic rate responses to nighttime temperatures, with very low and high night temperatures being detrimental and an optimum range (Jing et al., 2016). Although our results support this general temperature-dependent response shape, our findings indicate that, at least for duckweeds, mean temperature warming is the primary driver of performance in the spring, rather than any effects specific to the timing of warming.

The impacts of night-warming under hot summer conditions did not differ from other variable temperature regimes with similar variance. Constant hot temperature increased population growth and biomass. However, when temperatures were allowed to fluctuate, we observed decreased population growth in the treatment with the highest variance. Therefore, temperature fluctuations produced a range of effects from neutral to detrimental, depending on the magnitude of variance. As in the experiments with cool spring temperatures, the timing of warming did not appear to be a critical factor. Population growth under night-warming conditions was similar to that observed with random temperature variations.

There was no evidence suggesting that night-warming impedes heat stress recovery. This is not in accordance with literature suggesting that the combination of intense summer daytime heat and night-time warming could result in harmful effects due to insufficient recovery from daytime temperatures in other ectothermic species (Bai et al., 2019; Ma et al., 2021; Speights, Harmon, & Barton, 2017; Zhao et al., 2013). In plants, although lower yields were observed in some crops during hot summers with night-time warming (e.g., Narayanan et al., 2015), this may be due to other reasons. Instead, variance proved to be the key factor: night-warming, which reduces variance, either maintains

population growth rates similar to current temperature conditions or enhances them compared to scenarios with higher temperatures and greater variance.

We demonstrate, therefore, that high temperatures and high variance could interact to negatively affect aquatic plant population growth and biomass in the summer, supporting previous research indicating that climate change can have more negative effects when both mean temperature and temperature variability increase simultaneously (Kefford et al., 2022; Slein et al., 2023). This finding emphasizes the need for climate change experiments on plants to consider temperature variability across seasons and thermal tolerances. For instance, although some studies suggest that climate change will enhance plant performance of floating aquatic plants in general (Lind, Eckstein, & Relyea, 2022), locations facing simultaneous increases in mean temperature and variance might see different outcomes.

As a limitation of our experiments, we note that while we directly incorporated temperature variance in our treatments, the experiments lack day-to-day variability, and the temperature sequences are also identical for all replicates. However, most existing night-warming controlled experiments use constant daytime and night temperatures (Table 1.1, General Introduction), thus our fluctuating conditions introduce more realistic diel variations. Given the strong effects of variance, future research might incorporate this source of variability while also prioritizing the separation of the effects of mean temperature, variance, and warming timing. Extending experiments in duration and conducting field studies are recommended for understanding long-term effects of climate change and its interactions with other environmental changes.

Population growth in our experiments was exclusively driven by reproduction since no mortality was observed. Our results indicating decreased reproduction when temperatures fluctuated above the optimum align with previous duckweed research showing slower reproduction between <30°C and >34°C. The survival of common duckweeds during periods above their optimum also supports research suggesting tolerance to high temperatures over several days (Ziegler, Appenroth, & Sree, 2023). Additionally, since the common duckweed strains we used do not exhibit a highly asymmetrical curve (Andrade-Pereira & Cuddington, 2024), we expect mortality to occur only at very high temperatures around the curve's right end (~40 ºC), whereas the maximum temperatures we tested are below this threshold.

Our results differ from previously published works that identify either positive or negative impacts of night-warming. We suspect that many of these previously published findings are due to a failure to distinguish between the impacts of mean, variance and timing of warming. However, another possibility for why our results differ from published research on night-warming is that most studies focus on terrestrial plants. Terrestrial plants, which exhibit stomatal activity and are susceptible to drying, may be more affected by night-warming. For example, species capable of increasing stomatal density may experience positive impacts (Fan et al., 2022), by reducing negative effects of excess heat and water loss. Species without this strategy may experience negative impacts. In contrast, aquatic plants such as duckweeds have permanently opened stomata (Raven, 2002) and may be less susceptible to such negative effects of night-warming such as water loss. Moreover, while studies which identified negative night-warming effects suggest it relates to impaired reproductive organ biomass allocation (Jing et al., 2016), the plant we used in our experiments reproduces asexually and may therefore not be as adversely affected. Future studies comparing terrestrial and aquatic plants and plants with different reproductive strategies are needed to elucidate physiological responses to night-warming. We also call for future studies on night-warming to account for changes in mean temperature, variance, and timing of warming when evaluating potential impacts on plant performance.

In locations such as Southern Ontario, where diel temperature variance is decreasing due to night-warming (Cox et al., 2020), the effects on the population growth of duckweeds and other aquatic plants with a similar life history may be seasonally dependent. In spring, higher mean temperatures could boost population growth rates. Environmental managers can thus expect increasingly favourable conditions for duckweed mat formation, as predicted for floating aquatic plants resulting from climate warming (Lind, Eckstein, & Relyea, 2022; Netten et al., 2010), with negative impacts to submerged organisms in aquatic ecosystems (Ceschin, 2020; Lind, Eckstein, & Relyea, 2022).

However, during summer heat waves in these temperate regions, we show that the balance between reduced variance and increased mean temperature results in neutral effects. Therefore, under future summer conditions, environmental managers can anticipate growth patterns similar to those observed in the present. However, as the climate continues to warm, average temperatures higher than those we tested may become highly detrimental to population growth due to temperature-dependent mortality, regardless of changes in temperature variance. Our findings also indicate that areas where both daily mean temperature and variance are increasing, such as Europe (Zhong et al., 2023), may

increasingly experience decreases in plant population growth during hot summer periods as our climate continues changing. Our results are in alignment with recent research positioning Europe as a heat wave hotspot, where heat waves are getting more intense and more frequent than in other northern midlatitudes, with damage reported and predicted to ecosystems and food production systems (Brás et al., 2021; Rousi et al., 2022).

Our findings that duckweeds will benefit from a reduction in temperature variance associated with night-warming in future warmer scenarios are also relevant to food production. We can expect that duckweeds and similar aquatic plants will generally be more resilient to climate change than terrestrial crops. Temperatures tend to fluctuate less in aquatic environments due to thermal inertia (Brans et al., 2018). Additionally, the upper layer of mat-forming plants absorbs strong incoming radiation, creating a more stable temperature environment for plants underneath (Dale & Gillespie, 1977). This resilience makes duckweeds a promising alternative for outdoor protein production for both animal and human consumption, while also offering a more sustainable option compared to widely consumed terrestrial crops (Nirmal et al., 2024).

Managers tasked with controlling duckweeds as pest species should consider that climate change impacts suspected to have negative impacts on other plant species, such as night-warming and increased variance, are unlikely to similarly affect aquatic species. This is particularly important in scenarios where duckweeds dominate the water surface due to their remarkable clonal reproduction ability (Ziegler, Appenroth, & Sree, 2023), leading to ecological and economic losses (Ceschin et al., 2020). During summer in nutrient-rich waters in regions like Canada, we then expect that reduced temperature variance associated with both night-warming and the aquatic environment may mitigate extreme heat damage and allow overgrowth.

# Chapter 4

# Growth rates of duckweeds in strongly autocorrelated thermal regimes

## 4.1 Abstract

Heat waves are impacting plants globally and are predicted to increase in frequency and magnitude due to climate change. Increased temporal autocorrelation in temperatures contributes to these events. When temperatures are strongly autocorrelated there are periods of time when temperatures are either cooler or hotter than average conditions. Duckweeds, cosmopolitan aquatic plants with fast growth rates, have economic potential but also pose ecological concerns due to overgrowth and invasive spread. We conducted experiments testing the effects of strong autocorrelation in temperatures to the population growth rates and survival of common and dotted duckweeds, across several average temperatures. We find that at high mean temperatures, strong autocorrelation can lead to periods of time that are very hot resembling sudden heat waves. If these conditions occur when the population is small, they will significantly reduce the subsequent population growth of two duckweed species. This is due to increased mortality from sudden exposure to prolonged extreme conditions. We found no effects of strong autocorrelation at optimum and cold temperatures. We expect that plants with similar life histories are likely to be negatively affected by increased autocorrelation that is occurring with climate change, especially during sudden hot summer periods.

## 4.2 Introduction

As climate change intensifies, research on its biological impacts has gained attention, especially for ectotherms, whose body temperatures track ambient conditions. Studies focused on long-term average temperature projections find that tropical species would be negatively impacted by increases in mean temperature. This negative impact occurs because often these species are already in locations with temperatures near their thermal limits. In contrast, temperate species may benefit from these temperature increases since they are often in locations with means below their optimum (Estay, Lima, & Bozinovic, 2014).

Climate change is not only altering long-term average temperatures, but it is also increasing temperature variability, with heat events becoming more intense, longer, and more frequent (Meehl and Tebaldi, 2004). Changes in temperature variance can lead to performance predictions that differ from those based on constant conditions (Ruel & Ayres, 1999). When temperatures fluctuate, thermal

performance changes due to Jensen's inequality (Jensen, 1906), which means responses to fluctuating conditions differ from those to constant conditions, based on proximity to the optimum temperature (Ruel & Ayres, 1999). Temperature variance can impact biological responses more significantly than changes in average temperature (Dillon et al., 2016; Vasseur et al., 2014). The main effects include reduced performance across the thermal tolerance range and a lower optimum temperature (Denny, 2019; Vasseur et al., 2014), as shown in experiments (e.g., Paaijmans et al., 2013). When both mean and variance are considered in thermal impact analyses (Vasseur et al., 2014), conclusions change. For instance, temperate species may be at higher risk because increased temperature variance during summer can cause heat stress, leading to negative effects for ectotherms like plants (Kingsolver, Diamond, & Buckley, 2013).

In addition to changes in mean temperature and variance, climate change is causing increased positive autocorrelation of temperatures in nearly two-thirds of terrestrial areas (Di Cecco & Gouhier, 2018). Autocorrelation refers to the tendency for deviations from mean conditions to be clustered together in time. Calculating the strength of temporal temperature autocorrelation helps capture how climate change increases the frequency and duration of extreme temperature events. Strong positive autocorrelation can lead to longer sequences of unusually high or low temperatures. While air temperature is inherently positively autocorrelated (Pelletier, 1997; Vasseur & Yodzis, 2004), climate change intensifies this tendency, exposing organisms to prolonged unfavorable conditions like heat waves (Vasseur & Yodzis, 2004), which can increase extinction risk (Cuddington & Yodzis, 1999; Lawton, 1998; Robey & Vasseur, 2024, preprint). Therefore, increased autocorrelation can pose ecological and public health risks, such as massive tree die-offs in Australia (Breshears et al., 2021) and over 900 deaths during the 2021 Pacific Northwest heat wave (White et al., 2022).

Thermal performance curves can be used to provide insights into impacts of mean temperature changes, variance (Bernhardt et al., 2018; Sinclair et al., 2016), and autocorrelation on species. These are smooth functions fitted to measures of biological performance, such as metabolic, reproductive, or developmental rates, across a range of temperatures (Huey & Kingsolver, 1989). For ectotherms, like plants, these curves are typically left-skewed with the optimum temperature at the peak (Huey & Kingsolver, 1989). This asymmetry results from physiological processes, such as slower and more damaging heat shock responses compared to cold exposures (Roitberg et al., 2016).

While autocorrelation is important in determining the probability of events like heat waves, it has rarely been examined experimentally. A very limited number of studies have found impacts of strong autocorrelation. Around mild average temperatures, future warmer scenarios with strong autocorrelation increased the population density of a warm-adapted invasive zooplankton species compared to uncorrelated warmer environments, current temperature scenarios, and native species performance (Fey & Wieczynski, 2016). At high temperatures above the optimum, increased autocorrelation in diel cycles was detrimental across aphid life history traits (Ma et al., 2018).

An important characteristic of strong autocorrelation is that the temporal clustering of similar temperatures means organisms have an equal chance of being exposed to initial conditions which are either hotter or milder than the average conditions. A modeling study showed that initial beneficial conditions can increase the risk of invasive species establishment (Cuddington & Hastings, 2016). Similarly, an experimental study found that negative autocorrelation effects occurred during initial exposure to a series of hot days, which was more harmful than starting with milder days (Ma et al., 2018). This suggests that the timing of unfavourable temperature clustering in strongly autocorrelated regimes can affect performance by either enhancing reproductive and developmental processes early on or causing population declines due to early stress from detrimental conditions.

Studies of heat waves, which are related to the autocorrelation of temperatures, often reveal sub-lethal effects on photosynthesis, respiration, growth, and reproduction, as well as mass mortality in trees and shrubs (Breshears et al., 2021). Most reported effects on terrestrial plants involve negative impacts on respiration and interactions between heat waves and drought (Breshears et al., 2021). On aquatic plants, there are only a few existing studies on heat waves (Cao et al. 2015), which report species-specific responses including neutral and detrimental effects to biomass accumulation and reproductive processes (e.g., Cao et al. 2015; Li et al., 2017), and ecosystem responses including change in species composition (Li et al., 2017). We are unaware of single-species studies on the effects of heat waves on floating aquatic plants. Many aquatic plants such as duckweeds have permanently open stomata, implying that they may respond differently to prolonged heat and may be less likely to suffer from heat waves and drought in permanent water bodies (Raven, 2002). Studies on this species growth may be particularly important since they have significant economic value as a sustainable crop, water treatment solution, and in various industrial applications (Oláh, Appenroth, & Sree, 2023). However, this plant group also raises ecological concerns due to its potential for

overgrowth and spread of invasive species (Andrade-Pereira & Cuddington, 2024; Durborow, 2014). Thus, more research is needed to understand these impacts.

Strong positive autocorrelation in temperatures can shift thermal performance curves due to prolonged exposure to unfavorable but non-lethal temperature fluctuations, leading to greater performance reduction than anticipated due to non-linearity (Kingsolver & Woods, 2016). Around the optimum, we do not expect autocorrelation to change mean performance or performance distribution, similar to the effects of increased temperature variance. However, away from the optimum, populations exposed to strongly autocorrelated temperatures with the same mean and variance could initially experience clustered conditions that were either beneficial or detrimental. If it takes a long time to recover from the impact of detrimental conditions, we might expect that some populations would therefore have low growth rates, while others would have high growth rates.

We examine the impact of strongly autocorrelated temperatures on the population growth and survival of two duckweed species. We know that increased mean temperatures near common duckweed's optimum (28°C) enhances offspring number compared to cooler conditions (Paiha & Laird, 2022). Given this understanding, we formulated three hypotheses. First, we predicted that when average temperatures deviate from the optimum, population growth in strongly autocorrelated temperature treatments would exhibit a bimodal distribution arising from the timing of exposure to hotter or colder periods. We further expect that in strongly autocorrelated sequences with a high average temperature, initially cooler conditions would be beneficial, while in sequences with a lower than average mean temperature, starting warmer would be beneficial. In particular, we predict that exposure to excessive heat at the start of the sequence will diminish survival due to the acute impact of early thermal stress.

### 4.3 Methods

To assess the effects of autocorrelation on duckweed population growth rates, we first compared two treatments with distinct autocorrelation regimes, no autocorrelation and strong positive autocorrelation, across four different average temperatures. Subsequently, we divided the strong positive autocorrelation treatment into two subgroups, hot-cold and cold-hot, based on the timing of exposure to unfavorable conditions, and compared these treatments, also across the four different average temperatures. For high average temperatures, we included additional experiments with an

extended duration of 10 days to investigate if the same effects observed in a 5-day experiment still hold after a longer period of time.

### 4.3.1 Species origin and maintenance

Common and dotted duckweeds (*Lemna minor* and *Landoltia punctata*, respectively) were selected as model species for this study. Temperature is a major abiotic factor affecting their growth, survival, and distribution (Echelpoel, 2020; Mcilraith, Robinson, & Shay, 1989; Pasos-Panqueva, Baker, & Camargo-Valero, 2024; Rejmánková, 1975). The optimum temperature for population growth is around 28°C for the common and dotted duckweed strains we used (Andrade-Pereira & Cuddington, 2024). These aquatic plants are convenient model organisms for biological studies due to their fast growth, small body size, and mostly asexual reproduction (Laird & Barks, 2018). Also, both species have a simple life history, where reproduction is primarily asexual and resting fronds do not structurally change during dormancy (Ziegler, Appenroth, & Sree, 2023).

A strain of common duckweed collected in the field on an unknown date was obtained from Carolina Biological Supplies, Burlington, North Carolina, USA in 2020 and maintained aseptically. We collected another strain of common duckweed on August 29, 2022, in a stormwater pond located in Waterloo, Ontario, Canada (43.47, -80.55) and sterilized it according to the methodology outlined in Jewell, van Moorsel, & Bell (2022), and maintained it aseptically. Strains of dotted duckweed (accession number SG008) originally collected in United Water Princeton Meadows, New Jersey, Plainsboro, USA in 2015 and curated and stored at Rutgers Duckweed Stock Cooperative (RDSC) were acquired in May 2022 and maintained aseptically.

Plants were kept in 20°C LED lit incubators (IPP-500) and grown on modified Hoagland's medium (Environment Canada Hoagland's E+ medium w/o organics, sterile) obtained from the Canadian Phycological Culture Centre (CPCC), University of Waterloo, Canada. This temperature and those used during experiments (10-37°C) were higher than those that induce dormancy in common and dotted duckweed (Landolt, 1986; Cao, Fourounjian, & Wang, 2020; Jacono & Pfingsten, 2022). This macronutrient solution is widely used to culture duckweed species (e.g., Lasfar et al., 2007; Rippner et al., 2018, Zhang & Liang, 2020; Wang et al., 2016). We used a 16:8 photoperiod, and daylight intensity of 78.00-91.68 µmol $m^{-2}$ $s^{-1}$, which are moderate conditions for the growth of common duckweeds (Paolacci, Harrison, & Jansen, 2018c; Pasos-Panqueva, Baker, & Camargo-Valero, 2024).

### 4.3.2 Temperature sequences

For experimental treatments we generated 5-day temperature sequences with four different mean temperatures: 10°C, 15°C, 27°C, and 37°C, and different levels of autocorrelation (no autocorrelation, $\alpha$=0, and strong positive autocorrelation, $\alpha$=0.95, where the autocorrelation coefficient, $\alpha$, measures the correlation of temperatures in time, ranging from -1 to +1). At 37°C, we also created 10-day temperature sequences with the same properties. Over this 5-day or 10-day period the temperatures changed every hour, but all sequences had the same variance ($\sigma$ = 2.5°C). While all average temperatures are within the tolerance limits where activity for this species is observed, fluctuations in low and high temperature sequences could expose organisms to lethal conditions.

These 5-day or 10-day temperature sequences were generated using spectral mimicry (Cohen et al., 1999). The spectral mimicry approach ensures that identical temperatures are used across treatments; however, the temperatures are differentially ordered to match desired level of autocorrelation (also see Cuddington & Hastings, 2016). This procedure produces multiple temperature sequences with identical maximums, minimums, means and variance but differing autocorrelation (e.g., see R code at the Cuddington Lab Git Hub repository). Using this procedure, we created a unique set of 3 matched temperature sequences for 1 replicate of each treatment. One sequence had no autocorrelation ($\alpha$=0) and there were two sequences with strong autocorrelation ($\alpha$ at lag 1=0.95): one with temperatures that are colder than the average temperature in the first half of the sequence, and the second with warmer temperatures in the first of the sequence (see Supplemental Fig. 4.1, Appendix B). Using this procedure of matched temperature sequences with the same mean, maximum, minimum and variance, we have increased confidence that the effect of a treatment was due to its autocorrelation structure, rather than other statistical moments of the sequence.

To categorize a thermal regime as starting hot or cold, we regressed temperatures against time considering the entire thermal sequence of 120 or 240 hours. We classified positive slopes as cold-hot (temperatures going from low to high), and negative slopes as hot-cold (temperatures going from high to low). Where the linear regression was not significant, the sequence was discarded.

The five-day (120 hours) or ten-day (240 hours) temperature treatments were run in Memmert ICP-500 incubators using the proprietary software Celsius (version 10.1.4). In general, the incubators followed in the input temperature sequences well, but there were deviations. We set threshold

tolerances for deviations from the desired mean, variance and autocorrelation of the sequences, and excluded sequences that exceeded these criteria (see Supplemental Table 4.1, Appendix B).

### 4.3.3 Generation homogenization

In these experiments, we used even-aged individuals to avoid senescence effects when assessing the population growth rates of the duckweed species (Thwaites et al., 2024; Barks & Laird, 2015). Duckweed individuals are easily identified by their highly reduced, oval-shaped stem structure called a frond, which usually floats on water. As mostly asexual plants, duckweeds produce daughter fronds from lateral meristematic pockets, which eventually detach to form new colonies (Vu et al., 2020). Birth order standardization is a procedure where first born fronds are successively separated across generations in order to reduce parental effects resulting from processes such as mutation accumulation on the performance of subsequent duckweed generations (Ankutowicz & Laird, 2018; Barks & Laird, 2015; Mejbel & Simons, 2018). To implement this standardization, about 2 weeks before experiments ~70 fronds of common duckweed were aseptically transferred to 24-well plates containing 2 mL of modified Hoagland's solution. After reaching maturity (about 4-5 days later), the first daughter fronds within each well were manually separated from their mother fronds by gently pressing a loop in between fronds. Mother fronds were discarded, and daughter fronds were considered as generation zero. This process was repeated through the second generation, following a similar methodology to that described by Thwaites et al. (2024). Following the generation standardization, focal plants used in experiments were selected based on general health (size and colour) and age (about 4-5 days following germination, which corresponds to reproductive maturity).

### 4.3.4 Experimental procedure

For each temperature treatment, a replicate consisted of 12 fully-grown, healthy, third generation fronds of a given species (common or dotted duckweed), each placed in a single well containing 2 mL of modified Hoagland’s solution in a 24-well plate. For the 10-day experiment, each well contained 2.8 mL of this solution. This amount of nutrition media provided to each frond per time period closely corresponds to the volume indicated in the ISO 20227:2017 biotest protocol (Baudo et al., 2015). Each replicate was placed in an individual incubator and exposed to a designated temperature sequence for either 5 or 10 days. In most cases, replicates were exposed to matched temperature sequences (i.e., see above, sequences which only differed in the degree of autocorrelation) in different incubators at the same time. Some replicates done early in the experimental period using cold average

temperatures consisted of three small sterilized 60x15mm petri dishes, each containing 10 mL of Hoagland solution and 4 fully-grown, healthy duckweed fronds (total of 12 fronds/replicate) for each species, which were also randomly assigned to each treatment.

### 4.3.5 Growth rate measurements

We measured any impacts of strong positive autocorrelation on population growth using counts of live and dead fronds and surface area. We counted fronds for each replicate plate of 12 initial fronds after exposure to the 5-day or 10-day temperature sequence. Fronds of all sizes were considered, including any visible protuberance indicating a new germinating frond from the lateral meristematic pocket of a given mother frond. We considered individual fronds as alive when they displayed <90% loss of green pigmentation (Thomson & Dennis, 2013).

To measure surface area, we took an image of each replicate using a 50 MP Octa PD Quad Bayer wide camera at the beginning and end of the 5-day or 10-day sequence. The duckweed surface area in these images was obtained using the colour threshold routine from ImageJ (Rasband, 1997-2018). This function calculates live frond areas by identifying occupied pixels and using a known scale included in the photographs to generate the total surface area in $cm^2$. The analysis focused exclusively on live fronds, which were categorized using the same methodology described for obtaining the number of live fronds. We calculated the change in surface area of all live fronds within each replicate from the beginning to the end of the experiment and analyzed these data.

For treatments with mean temperature of 37°C, we observed some mortality, which was negligible in other average temperatures. For these treatments, we counted the number of dead fronds at the end of the experiment for a subsequent survival analysis.

### 4.3.6 Statistical analysis

For all statistical analyses, we excluded failed replicates with thermal sequences that fell outside the inclusion criteria (Supplemental Table 4.1, Appendix B), and all sets of replicates which did not contain the complete set of 3 treatments (Supplemental Table 4.3, Appendix B). To avoid overly complex models and loss of statistical power, we analyzed common and dotted duckweed performance separately, which aligns with our aim to investigate climatic responses rather than interspecific differences. We also note that throughout the experiment, we changed some sample preparation procedures to optimize the frond selection process, e.g. by replacing petri dishes with well

plates. We conducted statistical tests and found no significant difference in growth rates as a result of these procedural changes; thus, our final dataset contains all replicates prepared using different techniques. For cold temperatures only, replicates exposed to 10°C and 15°C of the two different common duckweed strains were grouped together for statistical analyses, as there are no significant differences in their growth rates. For cold temperatures, we also included a few replicates which were not generated using the generation homogenization procedure. This inclusion occurred to increase the sample size, and was justified because there were no significant differences in growth rates with or without this procedure in colder temperatures. We evaluated the effect of autocorrelation treatments using either simple linear, or mixed linear models, depending on whether there was a significant random effect of matched sequence replicates. We initially compared population growth under conditions of strong and no autocorrelation using the number of live fronds and surface area at the end of the 5-day or 10-day experiment. However, we also compared treatments where the initial sequence of autocorrelated temperatures was either below or above the mean, incorporating the same mean temperature levels and interactions as fixed factors. For high average temperatures (37°C) we also analyzed differences in the number of dead fronds across treatments. For frond count data, the analyses were either completed using a Poisson distribution to describe errors, or when overdispersion was detected, a quasipoisson distribution. Post-hoc comparisons were conducted using the package emmeans (Lenth, 2024). For linear models, degrees of freedom were approximated using the Satterthwaite method. For all analyses, we fitted candidate models using maximum likelihood estimation from the R package lme4. This package estimates the best-fitting parameters while accounting for random effects and the grouping structure in the data (Bates et al., 2015; RStudio Team, 2020). For generalized linear models, we selected the nloptwrap optimizer in package lme4, as all models converged when using this optimizer. Fixed factors included autocorrelation type, mean temperatures (10-15°C, 27°C, and 37°C), and their interactions. Replicate time was included as a random factor, matching replicates with different treatments that used the same set of temperatures (as for each replicate we created new temperature sequences). We used the marginal and conditional $R^2$ to determine if this random factor needed to be included in the statistical analysis. The marginal $R^2$ refers to the variance explained by fixed effects, which is calculated by comparing the variance of predicted values based solely on fixed effects to the total variance in the data. The conditional $R^2$, on the other hand, includes both fixed and random effects (Nakagawa & Schielzeth, 2013). Final candidate models were evaluated using the Akaike Information Criterion (AIC) estimator.

To visualize the frequency distribution of population growth rates, we created histograms of the number of live fronds of common duckweed across mean temperature and autocorrelation treatments from the 5-day experiment. Only common duckweed was used due to its higher number of replicates compared to the other species. For improved visualization, we included the full dataset, incorporating replicates that did not have matched temperature sequences in other treatments (Supplemental Table 4.2, Appendix B).

### 4.3.7 Thermal performance curves for autocorrelated treatments

We used the number of live fronds on the 5-day experiments for common duckweeds to calculate relative growth rates across several fluctuating temperature regimes. Daily relative growth rates (RGR) were calculated from the number of live fronds using the formula RGR = (log(Nf) - log(Ni)) / t, where Nf represents the final number of fronds, Ni is the initial number, and t is the time in days. We then fitted thermal performance curves to data for each treatment using the R package nls.multstart (Padfield & Matheson, 2023). We compared several candidate models as reviewed by Bernhardt et al., 2018 and Shi & Ge, 2010 based on the Akaike criterion corrected for small sample sizes (AICc).

## 4.4 Results

We found no significant effects of autocorrelation on population growth of duckweeds except at hot temperatures. At mean temperatures of 37ºC, we observed significantly lower population growth rates and higher mortality in strong autocorrelation treatments starting hot, compared to both strong autocorrelation treatments starting cold and random temperature variation controls, in both 5-day and 10-day experiments. In most analyses, the best-fit model according to the Akaike criterion was a mixed-effects model. This type of model was subsequently used across all analyses to maintain consistency, although we indicate in the text when it was not the best fit-model.

### 4.4.1 Effects of strong positive autocorrelation on the mean and distribution of population growth

There were no significant differences in the number of live fronds between treatments with no autocorrelation ($\alpha=0$) or strong positive autocorrelation ($\alpha=0.95$) for either duckweed species (common duckweed: $\chi^2 = 0.54$, df = 1, $p = 0.464$; dotted duckweed: $\chi^2 = 0.30$, df = 1, $p = 0.585$). Mean temperature alone explained the majority of the variance ($p < 0.001$ for both species). Growth

rates were significantly higher closer to optimal temperatures (27ºC) in comparison to growth at cold (10-15ºC) and hot temperatures (37ºC). We did not find an interaction between mean temperature and autocorrelation treatment (common duckweed: $\chi^2 = 2.98$, df = 2, p = 0.225; dotted duckweed: $\chi^2 = 0.46$, df = 2, p = 0.795).

A change from a unimodal to a bimodal frequency distribution of population growth was expected in temperatures away from the optimum as a result of exposure to thermal regimes starting either hotter or colder than the average. While this pattern is not seen in low temperatures (10-15ºC), it is apparent in high average temperatures (Fig. 1). At high average temperatures (37 °C), the treatment with no autocorrelation had a unimodal distribution resembling a normal curve (Fig. 1c). In contrast, in the strong autocorrelation treatment, replicates exposed to initial hot conditions tended to have a lower number of live fronds than those exposed to initial cold conditions (Fig. 4.1f). For instance, 83% of hot-cold replicates had frond counts below the mean (21.52 fronds) compared to only 9% of cold-hot replicates in the strong autocorrelation treatment (Fig. 4.1f). At low and optimum temperatures, no distinct distribution pattern emerged in any treatment, and hot-cold and cold-hot replicates are more evenly spread across population growth measurements (Fig. 4.1a-e).

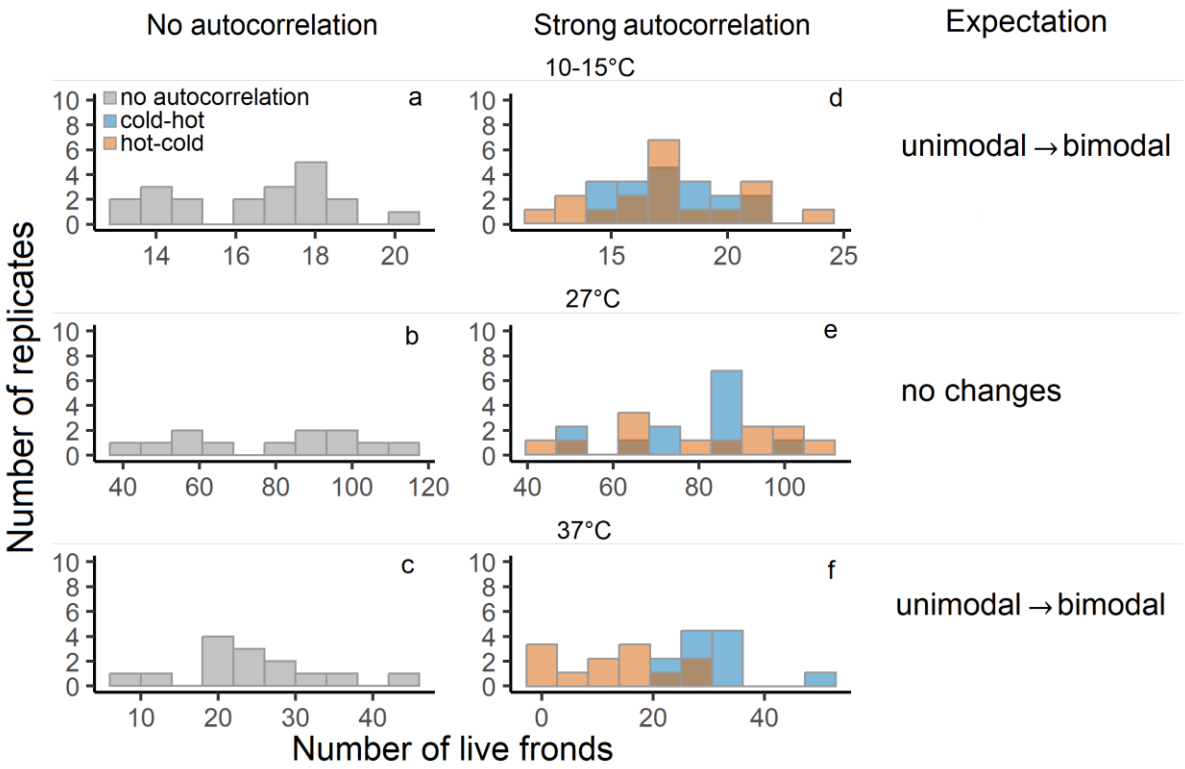



Figure 4.1 Histograms of common duckweed population growth following exposure to 5-day temperature regimes characterized by no autocorrelation (a-c, grey bars) versus strong positive autocorrelation (d-f) in sequences with 3 different average temperatures.
Data for strong autocorrelation treatments is superimposed and is shown separately depending on whether the temperatures are either initially hot (orange bars) or cold (blue bars).

### 4.4.2 Effect of initial conditions in strongly autocorrelated temperature sequences

We completed a new analysis of the same data using three treatments (i.e., no autocorrelation, strong autocorrelation starting cold, and strong autocorrelation starting hot) instead of two (no autocorrelation vs. strong autocorrelation), to account for this influence of initial conditions on population growth. There was a significant interaction between treatments and mean temperatures for the 5-day experiment on the number of live fronds for common duckweed ($\chi^2 = 35.95$, df = 4, $p < 0.001$), while this interaction was not significant for dotted duckweed ($\chi^2 = 4.43$, df = 4, $p = 0.351$). As with the previous analysis, frond counts fluctuated with average temperatures as expected considering the thermal performance curve for the species (Andrade-Pereira & Cuddington, 2024). Our post-hoc analysis showed that exclusively at 37ºC, there was a significantly lower number of live fronds in the hot-cold treatment in comparison with both the control group (no autocorrelation) (z.ratio = 4.77, $p < 0.001$) and the cold-hot treatment (z.ratio = 6.20, $p < 0.001$) (Fig. 2a). There were

no other significant differences between treatments (Supplemental Table 4.4, Appendix B). Surface area of common duckweeds was significantly affected by the interaction between temperature and autocorrelation in a mixed linear model ($\chi^2 = 11.92$, df = 4, p = 0.018), which was selected over a simple model because both were very similar based on the model assessment (AIC without random effect = 65.45, AIC with random effect = 73.14). Our post-hoc analysis revealed that at 37ºC, the surface area in the hot-cold treatment was significantly lower compared to the cold-hot treatment (t-ratio = 3.93; p-value = 0.001) (Fig. 2b). There were no other significant differences between treatments (Supplemental Table 4.4, Appendix B). For dotted duckweeds, neither the simple nor the random effect model has a significant effect of autocorrelation treatment (mixed effect model: $\chi^2 = 1.30$, df = 4, p = 0.861).

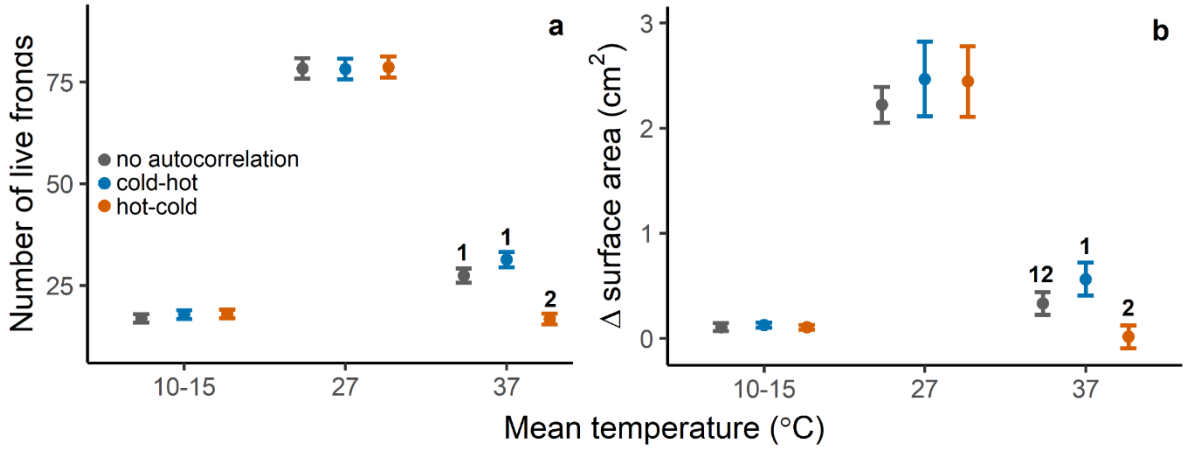



Figure 4.2 The number of live fronds (a) or surface area (b) of common duckweed following a 5-day exposure to temperature regimes with no autocorrelation (grey) or strong positive autocorrelation in temperatures.
Strong autocorrelation sequences have either initially colder than average temperatures (blue) or initially warmer than average temperatures (orange). Points are mean live fronds for replicates within each treatment; thin bars are standard errors based on a Poisson (a) or normal (b) distribution. Different numbers indicate significantly different treatments from others within a given mean temperature.

We observed the same trends in autocorrelation effects during 10-day experiments conducted at an average temperature of 37ºC. For common duckweeds, there was a significant effect of autocorrelation on the number of live fronds at 37°C at the end of the 10-day period ($\chi^2 = 11.42$, df = 2, p = 0.003). The post-hoc analysis showed that there were significantly fewer live fronds in the hot-cold treatment compared to the no autocorrelation treatment (z.ratio = 3.38, p = 0.002). The number of live fronds in the hot-cold treatment was also significantly lower than in the cold-hot treatment for

both species (z.ratio = 2.58, p = 0.027, Fig. 3). Also, as in the 5-day experiments, there were no significant differences between the no autocorrelation and the cold-hot treatments (Supplemental Table 4.4, Appendix B).

Unlike the 5-day experiment, the 10-day experiment revealed a significant autocorrelation effect on the number of live fronds in dotted duckweeds at 37°C ($\chi^2 = 21.45$, df = 2, $p < 0.001$). For this species, the post-hoc analysis showed the same results as in common duckweeds. The hot-cold treatment resulted in significantly fewer live fronds compared to the no autocorrelation treatment (z.ratio = 4.59, $p < 0.001$; Fig. 3). Additionally, the number of live fronds in the hot-cold treatment was markedly lower than in the cold-hot treatment (z.ratio = 4.49, $p < 0.001$). As in previous analyses, there were no significant differences in the number of live fronds between the no autocorrelation and cold-hot treatments (Supplemental Table 4.4, Appendix B).

In these 10-day follow-up experiments conducted at 37°C, the surface area analysis in common duckweeds also followed the same general trends as previous analyses. There was a significant effect of autocorrelation treatment in a mixed model ($\chi^2 = 10.21$, df = 2, p = 0.006), which was selected over a simple model because both were very similar based on the model assessment (AIC without random effect = 34.12, AIC with random effect = 38.40). Change in live frond surface area was significantly lower in the hot-cold treatment compared to the no autocorrelation treatment (t = 3.18, p = 0.005), but not when comparing it to the cold-hot treatment (Supplemental Table 4.4, Appendix B). As in previous analyses, there was no significant difference between the cold-hot vs. no autocorrelation (Supplemental Table 4.4, Appendix B).

For dotted duckweeds, there was also a significant effect of autocorrelation treatment in a mixed model ($\chi^2 = 11.56$, df = 2, p = 0.003), which was also selected due to similarity and to keep consistency of using mixed effect models (AIC without random effect = 9.49, AIC with random effect = 18.69). The change in live frond surface area was significantly lower in the hot-cold treatment compared to the cold-hot treatment (t = 3.10, p = 0.03), but not from the no autocorrelation treatment (t.ratio = 2.77, p = 0.057). Again, there was no significant difference between the cold-hot vs. no autocorrelation (Supplemental Table 4.4, Appendix B).

Autocorrelation strength was a significant predictor of the number of dead fronds for common duckweed in 5-day experiments ($\chi^2 = 32.01$, df = 2, $p < 0.001$). Post-hoc analyses revealed that mortality was significantly higher in the hot-cold treatment compared to both the no autocorrelation

treatment (z.ratio = -4.38, $p < 0.001$) and the cold-hot treatment (z.ratio = -3.85, $p = <0.001$). Unlike previous metrics where these two groups showed no differences, mortality was significantly lower in the cold-hot treatment compared to the no autocorrelation treatment (z.ratio = 2.48, $p = 0.035$). In the 10-day experiment (Fig. 3), we observed results for common duckweeds that were mostly similar to those in the 5-day experiment, with autocorrelation strength being a significant predictor ($\chi^2 = 12.88$, df = 2, $p = 0.001$) and post-hoc analyses showing higher mortality the hot-cold treatment compared to both no autocorrelation (z.ratio = -2.59, $p = 0.026$) and cold-hot treatments (z.ratio = -2.69, $p = 0.020$). However, in this extended experiment, the reduction in the 5-day experiment mortality observed in the cold-hot treatment compared to the no autocorrelation treatment was no longer present (Supplemental Table 4.4, Appendix B).

For dotted duckweeds, autocorrelation strength was not a significant predictor ($\chi^2 = 5.55$, df = 2, $p = 0.062$) for mortality in the 5-day experiment, while it was significant in the 10-day experiment ($\chi^2 = 19.31$, df = 2, $p < 0.001$), similar to the number of live fronds and change in surface area analyses. For this species, post-hoc comparisons also showed several similar trends as common duckweeds. Mortality was significantly higher in the hot-cold treatment compared to the no autocorrelation (z.ratio = -4.24, $p = <0.001$), but not for the cold-hot treatment (z.ratio = -2.04, $p = 0.102$). However, mortality was significantly lower in the cold-hot treatment in comparison with the no autocorrelation treatment (z.ratio = -2.89, $p = 0.011$) (Fig. 3).

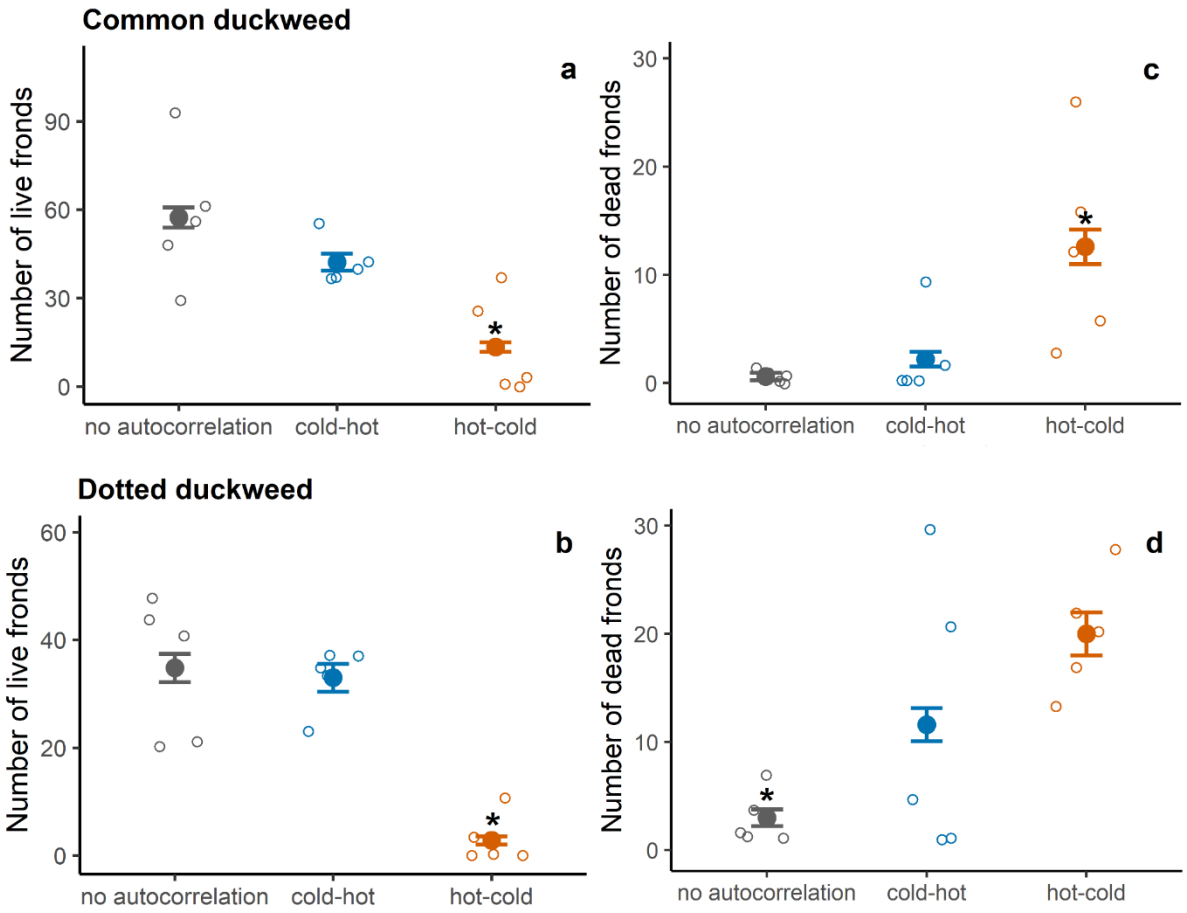



Figure 4.3 The number of live and dead fronds of common (a&c) and dotted duckweed (b&d) following a 10-day exposure to temperature regimes with mean of 37ºC and zero (grey) or strong positive autocorrelation in temperatures.
Strong autocorrelation sequences have either initially colder than average temperatures (blue) or initially warmer than average temperatures (orange) Open circles are individual replicates, solid circles are treatment means, and vertical bars are standard errors based on a Poisson distribution. Asterisks indicate significantly different treatments.

### 4.4.3 Strong autocorrelation and thermal performance curves

The Eppley and Brière1 thermal performance models (in bold and italics in Table 4.1) were selected because they were among the best fits according to the AICc assessment while also providing the most biologically meaningful estimates of optimum and threshold temperatures during a visual inspection of fitted curves. All three curves for each treatment follow the general asymmetric shape of ectotherm performance curves, and they do not differ much from each other around cool and optimal temperatures. However, at high temperatures above the optimum, the hot-cold curve has a sharper

drop in relative growth rates in relation to the two other curves, in alignment with the other described results from number of fronds, surface area, and survival. As with previously described results, the strong autocorrelation curve starting with milder temperatures is very similar to the no autocorrelation curve (Figure 4.4).

The selected models have the following equations and description of coefficients: Eppley model: $r(T)=ae^{(bT)}(1-(T-z/(w/2))2)$. T: temperature, a: scaling factor, b: exponential increase with temperature, z: central temperature where the curve is symmetric, w: curve width. Brière1 model: $r(T)=aT(T-Tmin)\sqrt{(Tmax-T)}$. a: scaling factor, Tmin: minimum temperature for positive growth, Tmax: maximum temperature for positive growth.

Table 4.1 Model assessment based on AICc (Akaike Information Criterion corrected for small sample sizes) for the thermal performance of common duckweed based on the number of live fronds following exposure to different autocorrelation strengths.

| | AICc | | |
|---|---|---|---|
| Thermal performance model | no autocorrelation | cold-hot | hot-cold |
| ***Brière1*** | ***-113.49*** | ***-121.03*** | 85.50 |
| ***Eppley*** | -117.17 | -140.14 | ***83.00*** |
| Hinshelwood | -57.66 | -65.65 | 85.2 |
| Logistic1 | -64.86 | -68.25 | 93.89 |
| Quadratic | -88.2 | -87.83 | 82.02 |
| Sharpe Schoolfield | -99.32 | -108.89 | 90.81 |
| Weibull | -39.04 | -39.23 | 94.24 |

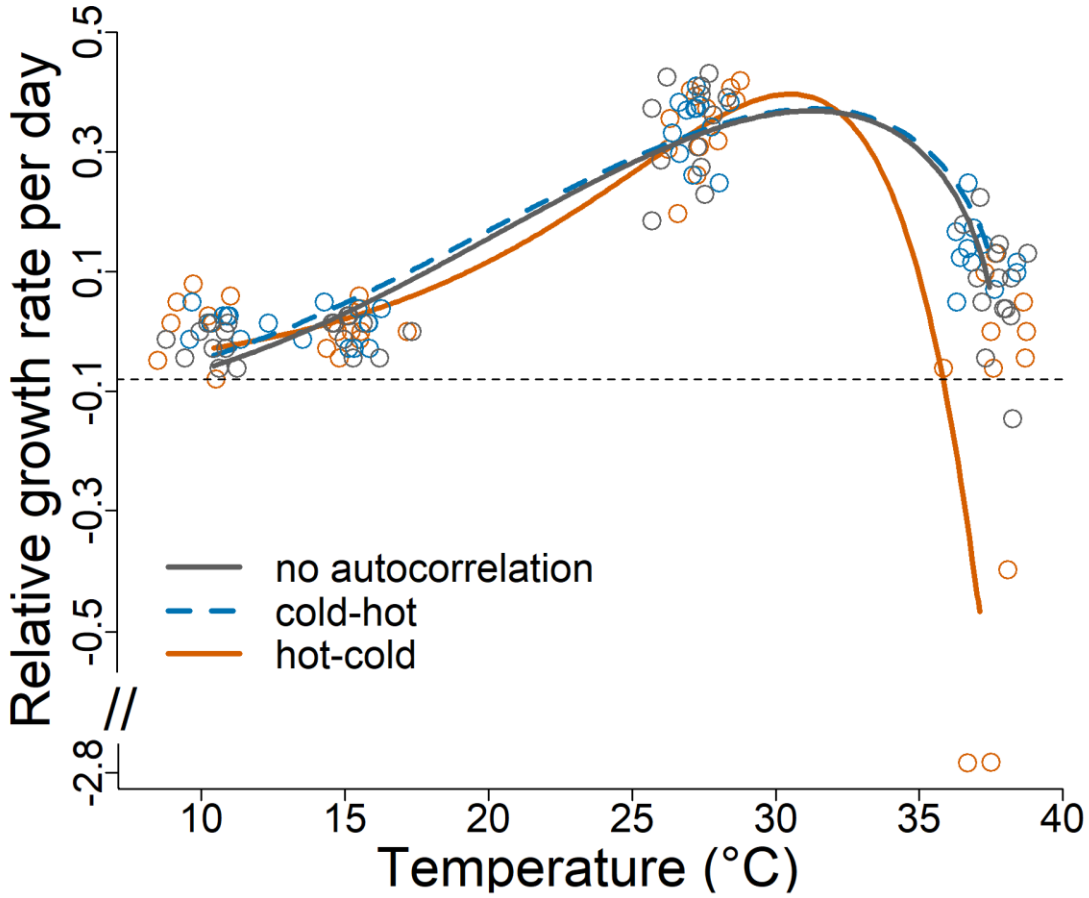



Figure 4.4 Thermal performance curves fitted to the relative growth rate of common duckweeds. Relative growth rates obtained following a 5-day exposure to temperature regimes characterized by zero (grey) versus strong autocorrelation with either initial temperatures which are colder (blue) or hotter than average (orange). Open circles are relative growth rates of individual replicates, curves are model fits. The best fitting curve for the hot-cold treatment is Eppley, with parameters a = -0.03, b = 0.19, z = 289.85, and w = 508.94, while curves for no autocorrelation and cold-hot treatments are Brière1 (no autocorrelation: a = 0.00, tmin = 8.28 , tmax = 37.33; cold-hot: a = 0.00, tmin = 6.74 tmax = 37.66). Note that jitter was applied to points along the x-axis to improve visibility.

### 4.5 Discussion

Climate change is increasing temporal autocorrelation of temperatures globally. Our controlled experiment is the first to measure its impact on plant population growth. We found few effects of strong positive autocorrelation on floating aquatic plants' growth. Autocorrelation in temperatures only affected duckweed growth at very high temperatures, driven by significant mortality in sequences where initial temperatures were above average, similar to heat waves in real systems. As

climate change continues, increased mean temperatures and autocorrelation can raise the frequency of harmful heatwave events for aquatic plants. However, autocorrelation itself is unlikely to have a larger impact.

Other studies which have not measured temperature autocorrelation provide insights into its impacts, such as those focused on phenology, early warming conditions, and heat wave effects on ectotherms (e.g., Ma, 2018). For example, a modeling study showed climate change is extending the growing season in temperate regions, increasing annual fitness in some ectotherms, but also raising the frequency of heat stress, decreasing fitness for others, leading to varied responses based on species-specific thermal tolerances (Kingsolver et al., 2013). In the context of heat stress, an experiment on seagrass species demonstrated that heatwaves significantly increased mortality in this species (Sawall, Ito, & Pansch, 2021). By isolating autocorrelation, we identified one of its potential negative impacts on population growth rates related to survival in high temperatures.

We predicted that strong autocorrelation would lead to bimodal distributions in the population growth rates due to the clustering of very low versus high performances, driven by periods that start either too hot or milder, respectively, which are characteristic of strong autocorrelation sequences. We did not observe this distribution at cold temperatures, but at high temperatures, growth rates were clustered at very low and high values, as expected. The lack of bimodality for cold temperatures resulted from a failure of initially warmer temperatures to provide a benefit at cool average temperatures in strongly autocorrelated sequences. Conversely, starting too hot in sequences with hot average temperatures had a detrimental impact, explaining the clustering around very low growth rates seen in the bimodal distribution. These low growth rates were due to increased mortality in these hot sequences. Overall, while we did not observe a general effect of strong autocorrelation, we do find that in high temperatures and strong autocorrelation, average performance is not a reliable indicator of plant performance. At these temperatures, the distribution of outcomes changes from unimodal to bimodal due to increased mortality.

Our findings regarding hot temperatures and strong autocorrelation aligns with ectotherm experiments showing sudden and prolonged heat is detrimental (Ma et al., 2018). Elevated heat-shock protein production after exposure to temperature extremes imposes costs on population performance, especially when gradual acclimation is not feasible (Sinclair et al., 2016; Sobek et al., 2011). However, when hot periods start gradually, mortality may not occur because organisms have time to

adjust their thermal tolerance levels (Woodin et al., 2013). This explanation is supported by thermotolerance studies on other aquatic plants (Amano, Iida, & Kosuge, 2012). Together, our results align with studies suggesting beneficial acclimation may mitigate thermal stress, but its effects are limited if individuals cannot survive the initial stress or if cumulative damage leads to mass mortality (Siegle, Taylor, & O'Connor, 2018; Williams et al., 2016). Our results also support modelling research suggesting that autocorrelation increases extinction risk (Cuddington & Yodzis, 1999; Lawton, 1998; Robey & Vasseur, 2024, preprint).

The lack of an impact of strong autocorrelation at colder temperatures was also found by Robey & Vasseur (2024, preprint). In their modelling study, they show that strong temperature autocorrelation across a thermal performance curve increases extinction risk more than random temperatures by quickly reducing populations at high temperatures, but has no significant effect on ectotherm growth rates at low temperatures (Robey & Vasseur, 2024, preprint). In retrospect, we note that the typical asymmetrical shape of thermal performance curve would suggest that ectotherms typically respond to cold temperature changes more seamlessly than they do when faced with hotter temperatures exceeding their optimum range (Roitberg et al., 2016).

We note that thermal performance curves are a useful first step for understanding climate change impacts. Our previously published thermal performance curve for common duckweed (Andrade-Pereira & Cuddington, 2024) predicts that relative growth rates are positive close to 0°C and so we would not have seen large negative impacts of cold mean temperatures in this experiment. Although this curve based on constant temperatures does not sharply decline on the right side (Andrade-Pereira & Cuddington, 2024), we still observed notable impacts following simulated sudden heat waves. In the presence of hot temperatures, the shape of the curve shifted due to strong autocorrelation when periods start hot (Fig. 4.4). This finding implies species or strains with steeper curves than the ones we used, such as strains of *Wolffia columbiana* and *Wolffia brasiliensis* found in the US (Docauer, 1983), would experience even more severe negative effects from sudden heat events when autocorrelation strength increases and environments are close to their thermal optimum. Our findings align with the understanding that thermal performance curves shift based on temporal history (Kingsolver & Woods, 2016; Sinclair et al., 2016), which would require future models to incorporate threshold-crossing events and frequency and duration of unfavourable temperature exposure (Sinclair et al., 2016).

We acknowledge several limitations in our study. Some factors relate to the difficulty of simulating realistic temperature conditions in a short term experiment in the lab. Our experiments focused on extreme scenarios, while typical autocorrelation values for air temperature are more intermediate (Colombo, Etkin, & Karney, 1999). However, strong autocorrelation values may correspond well to aquatic plant environments, as the high heat capacity (Letcher et al., 2016) and thermal inertia (Coats et al., 2006) of water can amplify temperature autocorrelation. It is also possible that the duration of the experiment was too short to observe effects of strong autocorrelation in cold conditions, as physiological processes are slowed down (Roitberg et al., 2016). Another limitation is that we used a constant variance in our treatments, which may have been too small to impact population growth at lower temperatures.

There are also some limitations related to the selection and treatment of the model organisms. We also controlled for variability due to factors such as maternal effects, age, nutrition, light, and contamination, but natural environments could introduce greater heterogeneity in responses. We used a lab strain of dotted duckweeds, which has probably been exposed to constant lab conditions for several years. In contrast, for the experiments with common duckweeds, we collected a strain from the field and used it for most replicates. This may explain why common duckweeds responded to our temperature fluctuation treatments faster than dotted duckweeds, as we observed most results within the 5-day experiments with common duckweeds, whereas responses in dotted duckweeds were only evident in the 10-day experiment.

Our results may apply well to plants with simple life histories like duckweeds. However, in plants with more complex developmental stages, strong autocorrelation may affect other vital rates, as different life stages may have distinct thermal performance curves (Sinclair et al., 2016). For example, Gascoigne et al. (2025) found autocorrelation altered the population structure of perennial plants and disrupted stable states, as favorable periods may accelerate developmental rates at specific stages. Although we are not aware of other studies on effects on plants, an insect study found increased compensatory reproduction and pest outbreaks due to strong autocorrelation (Chaves et al., 2014). Therefore, we call for more research on the effects of autocorrelation on plant populations with different life histories to examine impacts on vital rates other than survival, which may also affect population structure.

We show that duckweeds, with significant economic applications and ecological impact, are affected by climate change-induced autocorrelation at high temperatures. Outdoor applications such as production for animal nutrition, protein for human consumption, and biofuel production (Appenroth et al., 2015; Liu et al., 2021) would be especially impacted, where production would be negatively affected by sudden heat waves due to mortality. Alternatively, nuisance duckweed overgrowth would be harder to control in years when seasons start milder, exacerbating issues related to mat formation and consequent oxygen and light depletion, harming ecosystems (Ceschin et al., 2020) and recreational areas.

Extended periods of abnormally hot or cold conditions are expected with climate change, with phenomena like heat waves becoming more frequent (Di Cecco & Gouhier, 2018; Vasseur & Yodzis, 2004). Few experiments evaluate the effects of this increased autocorrelation on ectotherms. We present the first experiment testing strong temporal autocorrelation in plants, showing that at high temperatures, population growth rates exhibit a bimodal distribution despite being exposed to treatments with the same mean and variance. This distribution is due to high mortality when periods start hot, while periods starting milder do not have the same effect. Our results support findings that heat wave impacts natural and crop terrestrial plant survival (Breshears et al., 2021; Chen, 2022; Ledesma & Nawabata, 2016) and submerged aquatic plants due to heat shock and increased mortality (Amano, Iida, & Kosuge, 2012; Cao et al., 2015). This information can be used to predict responses to climate change in regions affected by increased autocorrelation, such as Canada and Russia (Duffy, Gouhier & Ganguly, 2022; Dunn et al., 2019).

# Chapter 5
# General conclusion

In this thesis, I determined the current thermal performance and predicted the future population growth rates and distribution of duckweed species, aquatic plants of economic interest and ecological concern, considering temperature fluctuation patterns driven by climate change. This is the first study to provide a risk map for the invasive dotted duckweed, to conduct climate change-induced night-warming experiments with plants that separate temperature and temporal components, and to measure effects of temperature autocorrelation to plants. Temperature variability can drive changes in the demographic rates of plant populations, highlighting the need to incorporate this factor into ecological research (Gascoine et al., 2025), especially as our climate continues to change.

In the second chapter, I provided current and future risk maps of dotted duckweed distribution, an invasive, herbicide-resistant species spreading in North America and Europe. Unlike most available research, I customized maximum entropy distribution models (Maxent) in an attempt to obtain more realistic predictions according to what we know about temperature-dependence in duckweed species. First, I incorporated water temperature variables, and selected the ones most relevant to the life history of this species. I also constrained the shape of model functions to match thermal lab-obtained performance curves, which may effectively represent species-level responses, as population-level curves have been shown to reflect species-level niche breadth reasonably well in plants (Wooliver et al., 2022). Similar consistency across genotypes has also been observed in duckweeds (Usui & Angert, 2024, Fig. S5). This niche-modelling shows that there are several suitable habitats for this species in Canada and Europe, especially as our climate warms, which can contribute to preventative or early detection and management measures.

In the third chapter, I conducted climate change experiments to compare current and future population growth rates of common duckweeds in Ontario in spring and summer periods. Differently from previous research, I inserted controls for mean temperature warming and temperature variance. I did not find an effect of the warming timing in night-warming; instead, mean temperature and variance determine population growth rates. I demonstrate that duckweed species will see favourable temperature conditions which may drive overgrowth and mat formation in future spring and summer scenarios, consistent with studies showing increased temperature variance affects duckweed thermal performance evolution and reduces their range expansion (Usui & Angert, 2024), and such information can inform both environmental managers and industries focused on outdoor applications.

In the fourth chapter, I conducted experiments testing the effects of strong autocorrelation in temperatures to population growth rates of duckweed species. Differently from previous research, my experiments measured and isolated effects of autocorrelation from other statistical aspects known to affect ectotherms. At high temperatures, I show that periods starting hot cause high mortality due to prolonged extreme heat too early in the period. My observations support predictions of increased extinction risk and establishment of invasive ectotherm populations. My research also supports the notion that predicting mean population growth rates is becoming increasingly difficult due to strong autocorrelation-induced bimodal distributions of growth rates. Lastly, as alternating outcomes due to strong autocorrelation become more frequent as our climate changes, ecological management can use this information to plan control based on periods starting to hot leading duckweeds susceptible to die-offs while milder beginnings could facilitate overgrowth and mat formation.

In temperate ponds where duckweeds are usually found, mean daily surface water temperatures are highly correlated to the surrounding air in temperate areas (Martinsen, Andersen, & Sand-Jensen, 2019), making air-based experimental setups such as mine a reasonable proxy for natural environments. However, surface water temperatures tend to fluctuate less than air temperatures, especially with increased water depth (e.g. Martinsen, Andersen, & Sand-Jensen, 2019, Fig. 1 and 2), and to have greater autocorrelation due to water's high heat capacity (Letcher et al., 2016) and thermal inertia (Brans et al., 2018; Coats et al., 2006). These properties may help explain why surface lake water temperatures were better predictors of duckweed distribution in relation to air temperatures. Moreover, these thermal characteristics of pond surface water were reflected in the temperature patterns I tested. These patterns, where reduced diel variance supported growth and strong autocorrelation corresponded to alternating periods of mortality and normal growth, may reflect future summer conditions in temperate ponds in regions like Canada.

Together, my findings can be used to make predictions of when climate change fluctuating patterns will have a greater significance across the thermal performance curve of duckweeds. For example, at low average temperatures, neither autocorrelation nor variance affected population growth. Likewise, autocorrelation was not detrimental when levels of variance were moderate at optimum temperatures. However, at high temperatures, an increase in both factors led to reduced growth compared to controls. Thus, both increased variance and autocorrelation may be most detrimental to plants at high average temperatures, which is concerning given that all three factors are rising in some global regions due to climate change. Moreover, while changes in the timing of warming in diel cycles do

not affect fluctuating aquatic plants like duckweeds, the timing of warming across multiple days is relevant, as periods starting very hot due to strong autocorrelation result in mortality.

As climate change progresses, freshwater ecosystem managers in Canada should expect increasingly favourable conditions for the spread and overgrowth of invasive and nuisance duckweed species, except during sudden heat waves that may favour submerged plants. Rising average temperatures are likely to promote rapid duckweed expansion, while low temperature variance during hot summer periods may help sustain dense surface mats. These conditions can lead to ecological and recreational challenges, including reduced oxygen and light availability, loss of biodiversity, particularly among submerged plants, and impaired water quality. Additionally, stronger temperature autocorrelation associated with heat waves may lead to alternating dominance, where floating plant mortality allows submerged nuisance species to thrive, as they may be more buffered from extreme surface temperatures in deep waters (Amano, Iida, & Kosuge, 2012).

Overall, my research indicates potential limits to climate change adaptation, consistent with previous findings on constraints in the evolution of thermal performance in plants (Wooliver, Tittes, & Sheth, 2020). Since increased temperature variance has been shown to result in the evolution of reduced thresholds of tolerance and broader optimum ranges in duckweeds (Usui & Angert, 2024), the trend of reduced diel variance in regions like Canada may cause thermal responses to evolve to resemble those observed under constant conditions. However, strong autocorrelation complicates making predictions based on thermal performance curves, as I showed that they become less reliable when periods begin with extreme heat, leading to high mortality which effectively shrinks the curves. Such effects may be linked to the limitations that autocorrelation imposes on plasticity; while maintenance of population growth under gradual-onset heat waves illustrate acclimation as a buffering strategy, high mortality during abrupt-onset events reveals its limits when individuals cannot survive initial exposures or cumulative stress (Siegle, Taylor, & O'Connor, 2018; Williams et al., 2016).

To continue advancing our understanding of aquatic plant responses to climate change, future research could focus on several areas. Species distribution models focused on aquatic plants would benefit from future climate predictions for water temperatures, which may result in more realistic species distributions across climate change emission scenarios. In terms of experiments, given the significance of temperature variance, incorporating more variability across days in night-warming

experiments could offer more realistic temperature sequences to simulate climate change impacts. Also, implementing acclimation periods, including temperature fluctuations and varying average temperatures, may also serve as a progression for future research. Moreover, increasing variance in autocorrelation experiments will help elucidate the combined effects of strong autocorrelation and high variance. Finally, research including moderate autocorrelation levels can provide insights into plant responses in diverse environments.

My research is a step towards a more realistic and biologically relevant understanding of aquatic plant responses to climate change. I identified which aspects of temperature matter most to duckweeds and under what conditions: mean temperature consistently plays a key role; variance and autocorrelation become critical as temperatures exceed the optimal range; diel timing has little effect; and multi-day timing becomes important during heat events. My findings can be incorporated in population dynamics models attempting to make more realistic predictions of climate change responses. Additionally, my research supports developing strategies to manage or prevent ecological impacts, such as the risk map I created for an invasive species approaching Canada, and sustainable economic uses of aquatic plants in a warming world.

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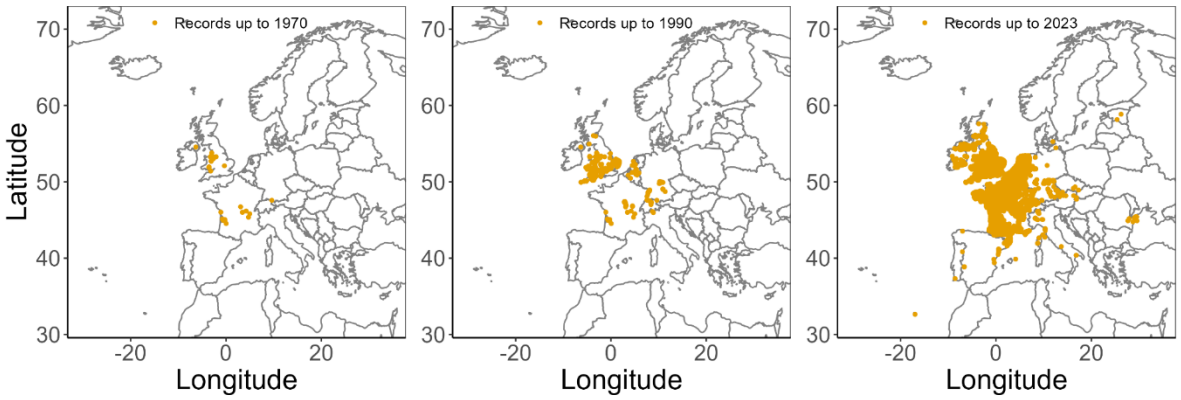
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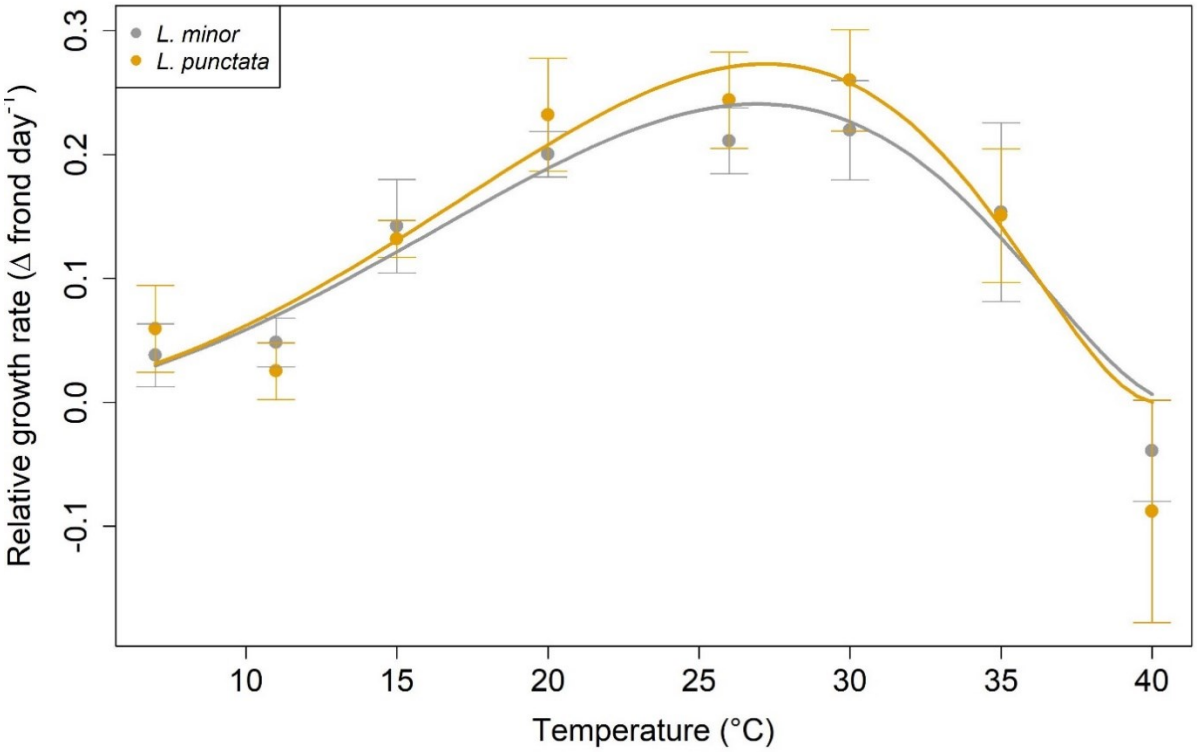
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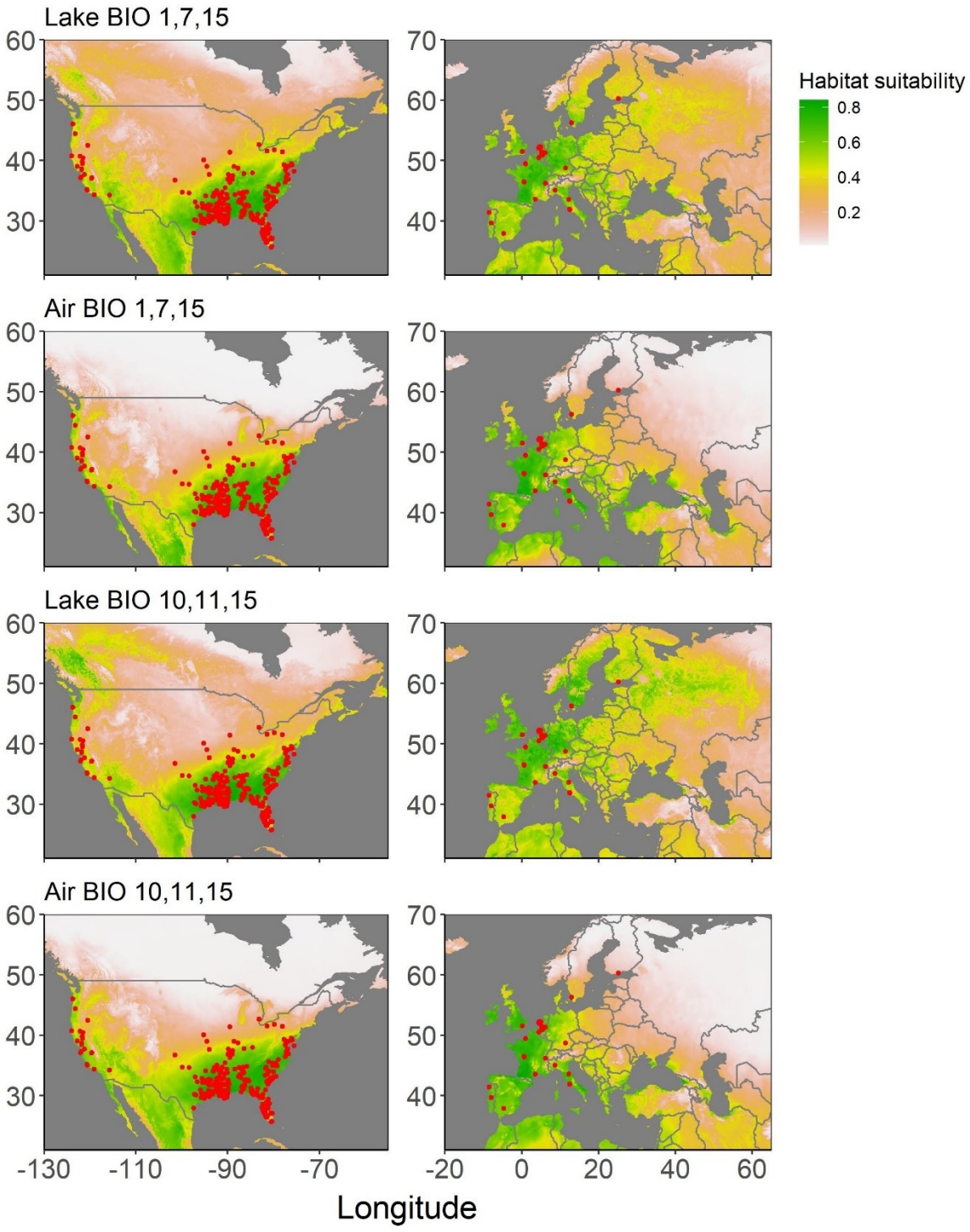
## Appendix A



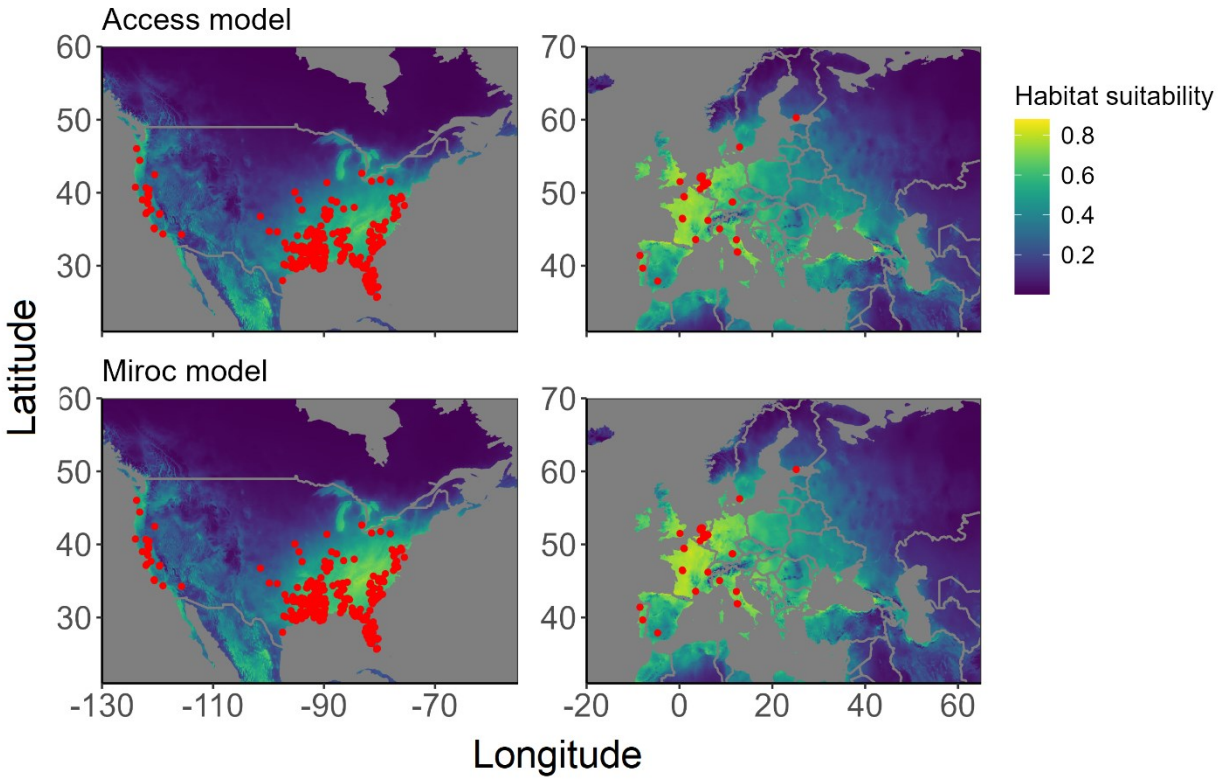

Supplemental Figure 2.1: Recent range expansion of least duckweed in Europe. Occurrence maps are based on unique records retrieved from GBIF.org on 2023-10-02. (https://doi.org/10.15468/dl.g5m5h8)




Supplemental Figure 2.2: Thermal performance curve for a native and an invasive strain in the US. Thermal performance curve for a native strain in the US (common duckweed, *L. minor*) and an invasive strain (dotted duckweed, *L. punctata*). Relative growth rates were calculated based on individual frond counts after a 5-day experimental period. Points indicate mean performance and bars are standard errors. Curves were calculated using the Ratkowsky function (Ratkowskly, 1983). Linear mixed models, relative growth rate ~ species * mean temperature, were used to compare growth rates of each species across mean temperatures, and included experiment run ID as a random factor (model including ascending portion of curve < 31 ºC = Marginal $R^2$ / Conditional $R^2$ = 0.776 / 0.892; model including descending portion of curve > 31 ºC = Marginal $R^2$ / Conditional $R^2$ = 0.772 / 0.985). The models revealed no significant differences between the 2 species within any of the temperatures tested. The number of replicates in each temperature, for *L. minor* and *L. punctata*, respectively, are: 7 °C (5,6), 11 °C (3,3), 15 °C (4 ,4), 20 °C (4,6), 26 °C (3,5), 30 °C (5,5), 35 °C (5,7), 40 °C (3,4).




Supplemental Figure 2.3: Prediction maps from all models included in the study, based on different predictor groups and predictor types (lake and air temperature).
Red dots are dotted duckweed occurrence records.




Supplemental Figure 2.4: Future predictions based on different global circulation models, made using the air temperature model with the highest predictive performance (BIO 1, 7, and 15).
Red dots are dotted duckweed occurrence records.

Supplemental Table 2.1 Additional sources of *L. punctata* observation records.

| Location | Lon | Lat | Year | Reference |
|---|---|---|---|---|
| Amstelveen, Netherlands | 4°51"E | 52°18"N | 2007 | van Valkenburg & Pot, 2008 |
| Belfeld, Netherlands | 6°12"E | 51°31"N | 2007 | van Valkenburg & Pot, 2008 |
| Bleiswijk, Netherlands | 4°31"E | 52°00"N | 2007 | van Valkenburg & Pot, 2008 |
| Helsinki, Finland | 25°15"E | 60°27"N | 1942 | Finnish Biodiversity Information Facility, 2024 |
| North Brabant, Netherlands | 5°23"E | 51°48"N | 2007 | van Valkenburg & Pot, 2008 |
| Pijnacker, Netherlands | 4°25"E | 52°00"N | 2007 | van Valkenburg & Pot, 2008 |
| San Marino, Italy | 12°27"E | 43°56"N | NA | EU, 2023 |
| Skåne, Sweden | 12°97"E | 56°25"N | 2000 | NOBANIS, 2011 |
| Unreported, Belgium* | 4°47"E | 50°50"N | 2020 | Neucker & Scheers, 2023 |
| Unreported, England* | 0°11"E | 51°50"N | 2006 | EPPO, 2015 |
| Unreported, Italy* | 12°57"E | 41°87"N | NA | EPPO, 2015 |
| Unreported, Spain* | 12°57"E | 41°87"N | NA | EPPO, 2015 |
| Unreported, Switzerland* | 6°14"E | 46°20"N | NA | EPPO, 2015 |
| Vatican City, Italy | 12°45"E | 41°90"N | NA | EU, 2023 |
| Vinkeveense Plassen, Netherlands | 4°95"E | 52°23"N | 2007 | van Valkenburg & Pot, 2008 |

* For unreported locations, the country capital coordinates were used to geolocate the occurrences

Supplemental Table 2.2 Candidate model selection: models using lake water temperature-based BIO 1, 7, and 15 predictors.

| Features | Regularization multiplier | Training AUC | Mean testing AUC | AICc | Delta AICc | Parameters |
|---|---|---|---|---|---|---|
| L | 0.5 | 0.73 | 0.7 | 18,419.14 | 616.72 | 3 |
| **LQ** | **0.5** | **0.81** | **0.75** | **17,802.42** | **0** | **6** |
| L | 1 | 0.73 | 0.7 | 18,424.04 | 621.62 | 3 |
| LQ | 1 | 0.81 | 0.76 | 17,860.95 | 58.53 | 6 |
| L | 1.5 | 0.73 | 0.7 | 18,429.06 | 626.65 | 3 |
| LQ | 1.5 | 0.8 | 0.76 | 17,953.27 | 150.86 | 6 |
| L | 2 | 0.73 | 0.7 | 18,434.19 | 631.78 | 3 |
| LQ | 2 | 0.8 | 0.76 | 18,037.61 | 235.19 | 5 |
| L | 2.5 | 0.73 | 0.7 | 18,439.47 | 637.05 | 3 |
| LQ | 2.5 | 0.79 | 0.75 | 18,122.97 | 320.55 | 5 |
| L | 3 | 0.73 | 0.7 | 18,440.85 | 638.43 | 2 |
| LQ | 3 | 0.78 | 0.75 | 18,203.27 | 400.86 | 5 |
| L | 3.5 | 0.73 | 0.7 | 18,443.4 | 640.98 | 2 |
| LQ | 3.5 | 0.77 | 0.74 | 18,288.97 | 486.56 | 5 |
| L | 4 | 0.73 | 0.71 | 18,445.99 | 643.57 | 2 |
| LQ | 4 | 0.75 | 0.74 | 18,387.38 | 584.96 | 5 |

Supplemental Table 2.3. Candidate model selection: Models using air temperature-based BIO 1, 7, and 15 predictors.

| Features | Regularization multiplier | Training AUC | Mean testing AUC | AICc | Delta AICc | Parameters |
|---|---|---|---|---|---|---|
| L | 0.5 | 0.74 | 0.73 | 18,331.2 | 755.59 | 3 |
| **LQ** | **0.5** | **0.83** | **0.77** | **17,575.61** | **0** | **6** |
| L | 1 | 0.74 | 0.73 | 18,336.95 | 761.34 | 3 |
| LQ | 1 | 0.82 | 0.77 | 17,642.03 | 66.42 | 6 |
| L | 1.5 | 0.74 | 0.74 | 18,342.87 | 767.26 | 3 |
| LQ | 1.5 | 0.82 | 0.77 | 17,724.33 | 148.72 | 6 |
| L | 2 | 0.74 | 0.74 | 18,348.97 | 773.36 | 3 |
| LQ | 2 | 0.82 | 0.77 | 17,789.08 | 213.47 | 5 |
| L | 2.5 | 0.74 | 0.74 | 18,352.18 | 776.57 | 2 |
| LQ | 2.5 | 0.81 | 0.78 | 17,872.84 | 297.23 | 5 |
| L | 3 | 0.74 | 0.74 | 18,355.72 | 780.11 | 2 |
| LQ | 3 | 0.8 | 0.77 | 17,949.27 | 373.66 | 5 |
| L | 3.5 | 0.74 | 0.74 | 18,359.31 | 783.7 | 2 |
| LQ | 3.5 | 0.79 | 0.77 | 18,035.28 | 459.67 | 5 |
| L | 4 | 0.74 | 0.74 | 18,362.96 | 787.35 | 2 |
| LQ | 4 | 0.78 | 0.77 | 18,118.11 | 542.5 | 5 |

Supplemental Table 2.4 Candidate model selection: Models using lake water temperature-based BIO 10, 11, and 15 predictors.

| Features | Regularization multiplier | Training AUC | Mean testing AUC | AICc | Delta AICc | Parameters |
|---|---|---|---|---|---|---|
| L | 0.5 | 0.73 | 0.7 | 18,429.15 | 680.64 | 3 |
| **LQ** | **0.5** | **0.8** | **0.75** | **17,748.51** | **0** | **5** |
| L | 1 | 0.73 | 0.7 | 18,430.34 | 681.83 | 3 |
| LQ | 1 | 0.8 | 0.76 | 17,774.54 | 26.03 | 5 |
| L | 1.5 | 0.73 | 0.7 | 18,431.64 | 683.12 | 3 |
| LQ | 1.5 | 0.8 | 0.76 | 17,801.64 | 53.13 | 5 |
| L | 2 | 0.73 | 0.7 | 18,433.05 | 684.54 | 3 |
| LQ | 2 | 0.8 | 0.77 | 17,832.54 | 84.02 | 4 |
| L | 2.5 | 0.73 | 0.7 | 18,434.57 | 686.06 | 3 |
| LQ | 2.5 | 0.8 | 0.77 | 17,867.5 | 118.99 | 4 |
| L | 3 | 0.73 | 0.7 | 18,436.2 | 687.69 | 3 |
| LQ | 3 | 0.8 | 0.76 | 17,903 | 154.48 | 4 |
| L | 3.5 | 0.73 | 0.7 | 18,437.95 | 689.44 | 3 |
| LQ | 3.5 | 0.8 | 0.76 | 17,937.78 | 189.27 | 4 |
| L | 4 | 0.72 | 0.7 | 18,439.8 | 691.29 | 3 |
| LQ | 4 | 0.8 | 0.76 | 17,973.28 | 224.77 | 4 |

Supplemental Table 2.5 Candidate model selection: Models using air temperature-based BIO 10, 11, and 15 predictors.

| Features | Regularization multiplier | Training AUC | Mean testing AUC | AICc | Delta AICc | Parameters |
|---|---|---|---|---|---|---|
| L | 0.5 | 0.74 | 0.72 | 18,331.75 | 758.73 | 3 |
| **LQ** | **0.5** | **0.81** | **0.75** | **17,573.02** | **0** | **6** |
| L | 1 | 0.74 | 0.72 | 18,335.51 | 762.49 | 3 |
| LQ | 1 | 0.81 | 0.76 | 17,626.14 | 53.12 | 5 |
| L | 1.5 | 0.74 | 0.72 | 18,339.44 | 766.42 | 3 |
| LQ | 1.5 | 0.81 | 0.76 | 17,644.19 | 71.16 | 5 |
| L | 2 | 0.74 | 0.72 | 18,343.55 | 770.53 | 3 |
| LQ | 2 | 0.81 | 0.76 | 17,666.33 | 93.3 | 4 |
| L | 2.5 | 0.74 | 0.72 | 18,347.83 | 774.81 | 3 |
| LQ | 2.5 | 0.81 | 0.76 | 17,693.73 | 120.7 | 4 |
| L | 3 | 0.74 | 0.72 | 18,352.29 | 779.26 | 3 |
| LQ | 3 | 0.81 | 0.77 | 17,721.36 | 148.33 | 4 |
| L | 3.5 | 0.74 | 0.72 | 18,356.92 | 783.89 | 3 |
| LQ | 3.5 | 0.81 | 0.77 | 17,749.2 | 176.18 | 4 |
| L | 0.5 | 0.74 | 0.72 | 18,331.75 | 758.73 | 3 |
| LQ | 0.5 | 0.81 | 0.75 | 17,573.02 | 0 | 6 |

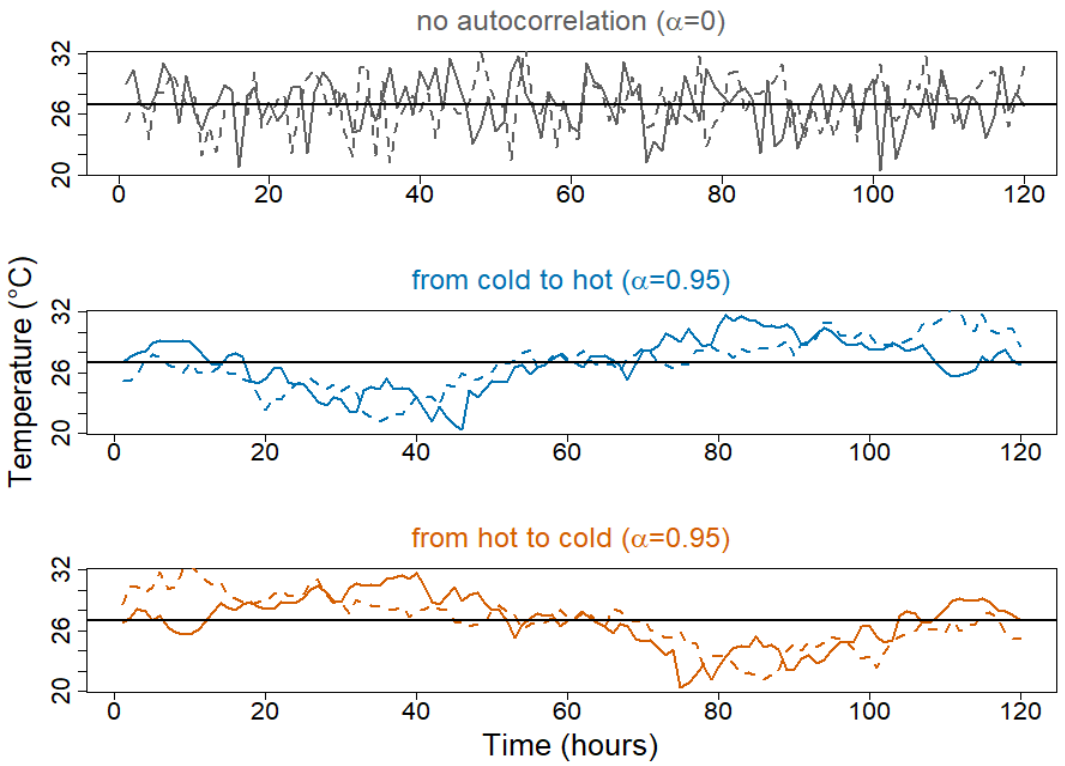
## Appendix B

Supplemental Table 3.1 Post hoc least-squares treatment comparisons for the night-warming experiment.
Linear models: degrees of freedom approximations used Satterthwaite method, pairwise comparisons used Tukey method; Generalized linear: Wald z-tests, Tukey; Significant results in bold. SE: standard errors; df: degrees of freedom.

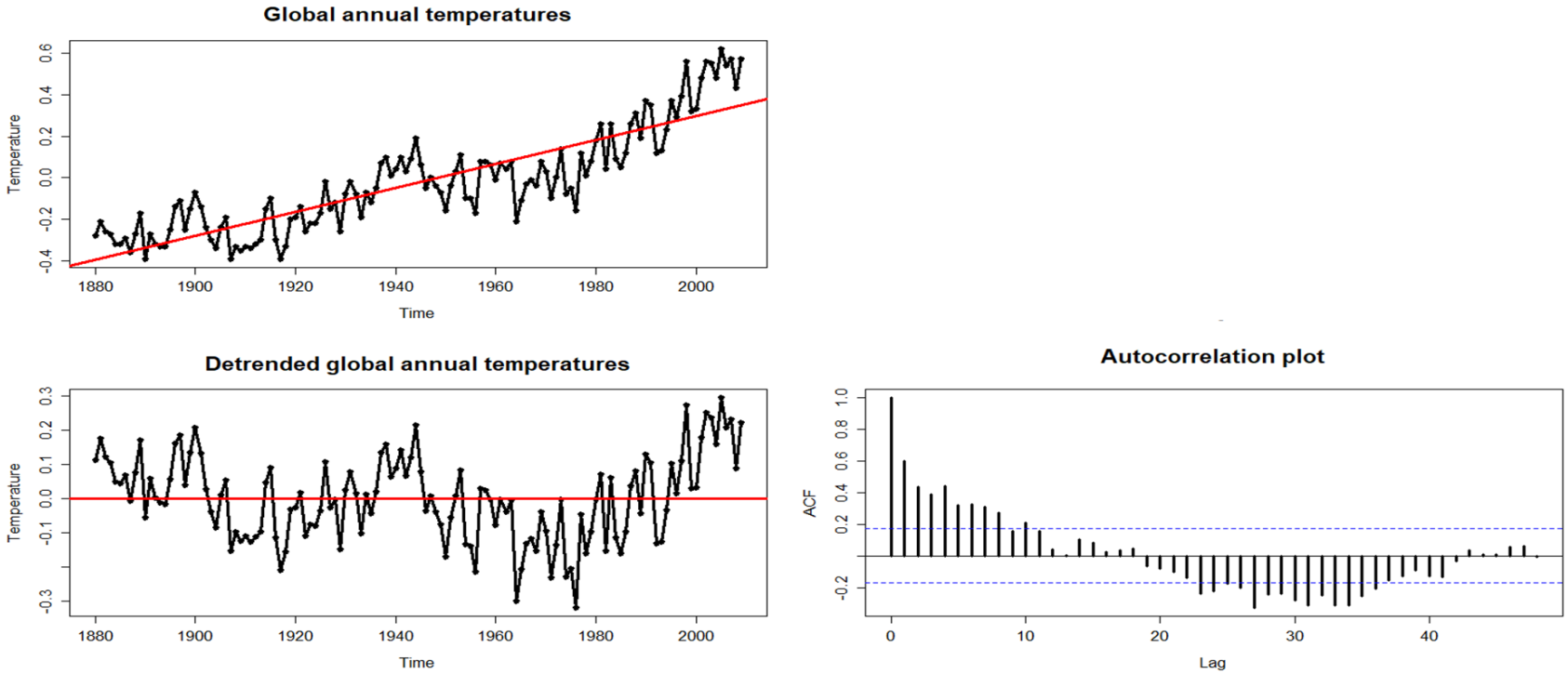
**Number of live fronds**

| | Spring | | | | Summer | | | |
|---|---|---|---|---|---|---|---|---|
| **Treatment** | **ratio** | **SE** | **z.ratio** | **p.value** | **estimate** | **SE** | **z.ratio** | **p.value** |
| **ambient vs ambient+** | **0.72** | **0.05** | **-4.72** | **<0.001** | **1.32** | **0.14** | **2.65** | **0.041** |
| **ambient vs night-warming** | **0.76** | **0.05** | **-3.97** | **<0.001** | 1.03 | 0.06 | 0.51 | 0.958 |
| **ambient vs random** | **0.67** | **0.10** | **-2.65** | **0.039** | 0.95 | 0.10 | -0.56 | 0.945 |
| ambient+ vs night-warming | 1.05 | 0.07 | 0.77 | 0.870 | 0.78 | 0.08 | -2.36 | 0.084 |
| **ambient+ vs random** | 0.92 | 0.14 | -0.54 | 0.950 | **0.72** | **0.04** | **-5.44** | **<0.001** |
| night warming vs random | 0.88 | 0.13 | -0.86 | 0.826 | 0.92 | 0.09 | -0.84 | 0.835 |

## Appendix C




Supplemental Figure 4.1 Autocorrelation experiment: Temperature regimes.
Solid and dashed lines illustrate 2 different thermal regimes, as we generated a new sequence at each replicate run. Each sequence is composed of different hourly temperatures, but have the same statistical properties. All sequences have the same mean temperature (μ=27°C) and the same variance (σ=2.5°C). However, the same temperatures are ordered differently across treatments: while they vary randomly in the first treatment (no autocorrelation), the second sequence has longer runs of warmer temperatures at first and later colder temperatures. Meanwhile, the third sequence is the exact opposite of the second, starting with low temperatures. Such longer sequences of unfavorable temperatures can amplify potential negative impacts observed in a randomly fluctuating environment.




Supplemental Figure 4.2 Calculating autocorrelation after detrending time series of temperatures.
The plot to the bottom left represents detrended temperatures seen in the top plot, which is done by using the residues from a linear regression. The autocorrelation plot (bottom right) shows autocorrelation coefficients for these detrended temperatures. For several lags, temperatures are strongly autocorrelated, which is indicated by vertical spikes rising above the dashed line representing statistical significance. Global mean land-ocean temperature deviations were obtained using R package astsa (Stoffer & Poison, 2024). Autocorrelation is calculated by correlating a given time series with a lagged version of this same time series, for any desired time interval, using the equation: $\alpha_k = [\sum_{t=k+1}^{T}(r_t - \bar{r})(r_{t-k} - \bar{r})] / [\sum_{t=1}^{T}(r_t - \bar{r})^2]$ where $r_t$: time series data, $r_{t-k}$ = time series data shifted by k lags, $\bar{r}$ = average of time series data The presence of autocorrelation in temperatures is detected even after detrending temperature sequences, such as global annual temperatures

Supplemental Table 4.1 Exclusion criteria for output temperature regimes.

| Parameter | Set value | Output exclusion criteria |
|---|---|---|
| Standard deviation | 2.5°C | +- 0.4°C |
| Mean temperature | 10, 15, 27, 37°C | +- 0.2°C |
| Autocorrelation coefficient | 0 or 0.95 | +- 0.3 |

Supplemental Table 4.2 Total number of replicates obtained in each treatment of the autocorrelation experiment, for the number of live fronds, after removing failed replicates. This dataset was used for the histograms.

| 5-day experiments | | | | | | |
|---|---|---|---|---|---|---|
| | Common duckweed | | | Dotted duckweed | | |
| Average temperature | no autocorrelation | cold-hot | hot-cold | no autocorrelation | cold-hot | hot-cold |
| 10-15°C | 19 | 17 | 18 | 6 | 5 | 7 |
| 27°C | 12 | 12 | 12 | 5 | 12 | 12 |
| 37°C | 14 | 11 | 12 | 7 | 11 | 12 |
| Total for each treatment | 45 | 40 | 42 | 18 | 11 | 12 |
| Grand total | 127 | | | 91 | | |

Supplemental Table 4.3 Numbers of replicates obtained in each treatment (no autocorrelation, cold-hot, and hot-cold) of the autocorrelation experiment.
Numbers are for each measurement (number of live or dead fronds or change in live frond surface area) after removing failed replicates and subsetting to obtain replicates containing the full set of three treatments. This dataset was used for all statistical models.

| 5-day experiments | | | | | 10-day experiments | | | |
|---|---|---|---|---|---|---|---|---|
| Species | Common duckweed | | Dotted duckweed | | Common duckweed | | Dotted duckweed | |
| Average temperature | number of fronds | change in surface area | number of fronds | change in surface area | number of fronds | change in surface area | number of fronds | change in surface area |
| 10-15°C | 16 | 9 | 5 | 5 | | | | |
| 27°C | 12 | 5 | 12 | 5 | | | | |
| 37°C | 9 | 7 | 9 | 6 | 5 | 5 | 5 | 5 |
| Total for each treatment | 37 | 21 | 26 | 16 | 5 | 5 | 5 | 5 |
| Grand total (Total x 3) | 111 | 63 | 78 | 48 | 15 | 15 | 15 | 15 |

Supplemental Table 4.4 Post hoc least-squares treatment comparisons for the autocorrelation experiment.
Linear models: degrees of freedom approximations used Satterthwaite method, pairwise comparisons used Tukey method; Generalized linear: Wald z-tests, Tukey; Significant results in bold. SE: standard errors; df: degrees of freedom.

| 5-day experiments using common duckweeds | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Temperature** | **Treatment** | **Number of live fronds** | | | | **Change in surface area** | | | | | **Number of dead fronds** | | | |
| | | **ratio** | **SE** | **z.ratio** | **p.value** | **estimate** | **SE** | **df** | **t.ratio** | **p.value** | **ratio** | **SE** | **z.ratio** | **p.value** |
| **10-15ºC** | no vs cold | 0.95 | 0.08 | -0.64 | 0.799 | -0.02 | 0.12 | 11.60 | -0.16 | 0.986 | | | | |
| | no vs hot | 0.94 | 0.08 | -0.72 | 0.752 | 0.00 | 0.12 | 11.60 | 0.02 | 1.000 | | | | |
| | cold vs hot | 0.99 | 0.08 | 0.08 | 0.996 | 0.02 | 0.12 | 11.60 | 0.18 | 0.982 | | | | |
| **27ºC** | no vs cold | 1.00 | 0.04 | 0.04 | 0.999 | -0.25 | 0.17 | 37.50 | -1.50 | 0.303 | | | | |
| | no vs hot | 1.00 | 0.05 | -0.10 | 0.995 | -0.22 | 0.17 | 37.50 | -1.36 | 0.372 | | | | |
| | cold vs hot | 0.99 | 0.04 | -0.14 | 0.989 | 0.02 | 0.17 | 37.50 | 0.14 | 0.989 | | | | |
| **37ºC** | no vs cold | 0.88 | 0.08 | -1.52 | 0.282 | -0.23 | 0.14 | 27.60 | -1.66 | 0.240 | 14.00 | 14.90 | 2.48 | **0.035** |
| | no vs hot | 1.64 | 0.17 | 4.77 | **<0.001** | 0.32 | 0.14 | 27.60 | 2.28 | 0.076 | 0.26 | 0.08 | -4.38 | **<0.001** |
| | cold vs hot | 1.87 | 0.19 | 6.20 | **<0.001** | 0.55 | 0.14 | 27.60 | 3.93 | **0.001** | 0.02 | 0.02 | -3.85 | **<0.001** |
| 10-day experiments using common duckweeds | | | | | | | | | | | | | | |
| **37ºC** | no vs cold | 1.36 | 0.39 | 1.07 | 0.534 | 0.51 | 0.40 | 491.05 | 1.27 | 0.412 | 0.27 | 0.35 | -1.00 | 0.575 |
| | no vs hot | 4.28 | 1.85 | 3.38 | **0.002** | 1.28 | 0.40 | 491.05 | 3.17 | **0.005** | 0.05 | 0.06 | -2.59 | **0.026** |
| | cold vs hot | 3.15 | 1.40 | 2.58 | **0.027** | 0.77 | 0.40 | 491.05 | 1.90 | 0.139 | 0.17 | 0.11 | -2.69 | **0.020** |
| 10-day experiments using dotted duckweeds | | | | | | | | | | | | | | |
| **37ºC** | no vs cold | 1.05 | 0.23 | 0.25 | 0.967 | -0.06 | 0.18 | 8.00 | -0.33 | 0.943 | 0.259 | 0.12 | -2.89 | **0.011** |
| | no vs hot | 12.43 | 6.83 | 4.59 | **<0.001** | 0.49 | 0.18 | 8.00 | 2.77 | 0.057 | 0.15 | 0.07 | -4.24 | **<0.001** |
| | cold vs hot | 11.79 | 6.49 | 4.48 | **<0.001** | 0.55 | 0.18 | 8.00 | 3.10 | **0.035** | 0.58 | 0.15 | -2.04 | 0.102 |