THE LANSING EFFECT IN *LEMNA TURIONIFERA* (LEMNOIDEAE) AND POTENTIAL CONTRIBUTING FACTORS

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A thesis submitted
in partial fulfilment of the requirements for the degree of

MASTER OF SCIENCE

in

BIOLOGICAL SCIENCES

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University of Lethbridge
LETHBRIDGE, ALBERTA, CANADA

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Date of Defense: December 9, 2022

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DEDICATION

Dedicated to my parents, Sunil & Promila Dutt, without whom I could not have seen this dream come to fruition. Love you mama and papa.

ABSTRACT

The Lansing effect is a specific type of parental age effect whereby older parents have shorter-lived offspring than younger parents. The phenomenon is important because it implies the presence of non-genetic forms of inheritance relating to parental age, such as epigenetics or physiological effects. Further, the existence of the Lansing effect informs our understanding of the evolution of life histories because it shows that senescence – traditionally defined in terms of decreases in survival and reproduction with age – also involves a decrease in offspring quality. The Lansing effect has been observed in a wide variety of taxa, including plants. Here, I investigated the Lansing effect in the subfamily Lemnoideae (duckweeds). My objectives were two-fold: (1) testing for the Lansing effect, and (2) if the Lansing effect is present, determining whether shortened lifespans of offspring of older parents are due to a higher mortality rate at all ages (i.e. a difference in baseline mortality), or a faster-accelerating mortality with age. I recorded lifespan, reproduction, and other metrics of fitness of 392 individuals; half were their parent's first clonal offspring (offspring of younger parents), and half were fifth clonal offspring (offspring of older parents). Offspring of older parents had shorter lifespans (i.e., the Lansing effect occurred) and produced fewer offspring themselves compared to offspring of younger parents. Further, a model-selection approach indicated that offspring of older parents had a greater initial mortality rate at birth that then persisted through life compared to offspring of younger parents. Thus, greater baseline mortality was responsible for the Lansing effect for the plants in this experiment. My work emphasizes that senescence can manifest in offspring as a result of parental age effects, specifically the Lansing effect, in addition to the more well described phenomena of decreasing survival and reproduction.

ACKNOWLEDGEMENTS

I would like to thank my supervisor, Dr. Robert Laird, for all his guidance in completing my master's degree. I would also like to thank my committee members, Dr. Jenny McCune and Dr. Julie Lee-Yaw, for their thoughts and feedback. The mentorship I received from these three individuals is very much appreciated.

My sincerest of gratitude goes to my lab members, most of whom have begun their careers since I have started, including Suzanne Chmilar, Austin Paiha, and Kanishka Senevirathna. Thank you for remaining supportive as I navigated graduate school. I would also like to extend this gratitude to our new members, Amanda Luzardo, Abbe Pawluk, Vicky Thwaites, and Jemma Todoschuk, who have provided their support, friendship, and many laughs.

I would like to extend my love and appreciation specifically to Suzanne Chmilar and Maegan Trottier for their kindness, support, and friendship. In both professional and personal contexts, I am forever grateful to you both. Further, I would like to thank my family, Sunil Dutt; Promila Dutt; Raghuwar Dutt; Eeshwar Dutt, for their motivation and for ensuring that I felt their warmth every step of the way. My thanks also extend to my partner, Zachary Wanner, for his unwavering support and cheerleading. Thank you all, my friends and family, for standing by my side through my many rollercoasters.

I would like to give a special thank you to my friend and colleague, Dr. Marc Higgins, for his many warm extensions of motivation in both my exploration of the sciences as well as my dedication to education.

Finally, I would like to gratefully acknowledge the funding provided by the Alberta Conservation Association (ACA) Grants in Biodiversity as well as the Natural Sciences and Engineering Council of Canada (NSERC) Discovery Grant provided to Dr. Robert Laird.

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1. GENERAL INTRODUCTION

1.1 THE EVOLUTION OF SENESCENCE

Senescence, otherwise known as aging, occurs at both the individual level and the population level. At the individual level, senescence is the phenomenon in which there is an age-related decrease in viability of organisms with a simultaneous increase in vulnerability or physiological deterioration (Barks & Laird, 2015; Comfort, 1954; Sherratt & Wilkinson, 2009). At the population level, this leads to increased risk of death and lower fecundity in older age classes compared to younger ones (Barks & Laird, 2020; Sherratt & Wilkinson, 2009; Sherratt et al., 2011; Watkinson, 1992). While the cellular and physiological mechanisms associated with individual-level senescence are important and interesting – e.g., accumulation of oxidative damage to nucleic acids, lipids, and proteins (Bokov et al., 2004; Hamilton et al., 2001; Sherratt & Wilkinson, 2009) – this thesis is primarily concerned with population-level senescence, also known as demographic senescence.

Senescence seems maladaptive, and therefore requires an evolutionary explanation. Senescence has previously been explained as being programmed into cells to aid in the limiting of a population's size or growth, thereby aiding in the population's adaptation to change in the environment, though senescence does not contribute to natural mortality (Kirkwood & Austad, 2000; Muñoz-Espín et al., 2013). However, like most group-selectionist arguments, this line of thinking has largely been superseded by individual-selectionist arguments (but see Mitteldorf, 2004) such as kin selection (weighing the benefits of care at the maternal nest against reproductive costs) or altruism (individual behaviours that pose risks but benefit another such as in eusocial insect colonies) (Kay et al., 2019). There are three predominant, related individual-selection theories that attempt to explain the evolution of senescence: The Mutation Accumulation, Antagonistic Pleiotropy, and Disposable Soma theories.

Sir Peter Medawar's concept, known as Mutation Accumulation theory states that alleles with deleterious effects in late life may accumulate in the genome over multiple generations due to a

lessening of selection in older age classes (Kirkwood & Austad, 2000; Medawar, 1952). Because comparatively few individuals remain alive in old age-classes, even in the absence of senescence, (due to predation, accidents, infectious pathogens, and other senescence-independent processes) (Hughes & Reynolds, 2005), natural selection is relatively ineffective in removing late-acting mutations from a population (Sherratt & Wilkinson, 2009; Williams, 1957). Thus, in the Mutation Accumulation theory, senescence is a consequence of mutation-selection balance, coupled with the observation that the force of selection is likely to wane with age (Hamilton, 1966).

George C. Williams' theory of Antagonistic Pleiotropy is an elaboration of Mutation Accumulation theory. It focuses on temporally pleiotropic genes that have beneficial effects early in life and negative effects at later ages, thus being favoured by natural selection at early ages (Kirkwood & Austad, 2000; Williams, 1957). This in turn results in senescence being the consequence of early-life survival and reproduction being decisive for overall fitness (Sherratt & Wilkinson, 2009; Williams et al., 2006). In a few words, beneficial effects that occur early in life have outsized effects on a population's capacity for growth, and can therefore be selected for, even if they trade-off with deleterious effects in late life (Williams et al., 2006).

Finally, Thomas Kirkwood's Disposable Soma Theory suggests that one way that early-/late-life trade-offs can occur is if resources are optimally allocated to lifespan (somatic maintenance, repair, growth) versus reproduction (Drenos & Kirkwood, 2005; Kirkwood, 1977; Kirkwood, 2017; Kirkwood & Austad, 2000; Kirkwood & Holliday, 1979; Sherratt & Wilkinson, 2009). This means that if there are benefits to using resources for reproduction early in life despite costs to somatic components in later life, natural selection will favour that exchange of resources. In this scenario, the maintenance of somatic components is limited by the investment in reproduction at the expense of the soma, with senescence the inevitable result (Kirkwood, 2017; Sherratt & Wilkinson, 2009).

The theory of Antagonistic Pleiotropy is an extension of the Mutation Accumulation theory with trade-offs as a consideration, while the Disposable Soma theory focuses on resource allocation as a specific trade-off that organisms must navigate. Due to the interconnectivity of these three theories, the literature has coalesced around the notion, common to the theories, that the force of natural selection declining with age is the ultimate cause of senescence, a paradigm most closely associated with William Hamilton's classic 1966 paper (Hamilton, 1966).

1.2 GENERAL CHARACTERIZATION OF DEMOGRAPHIC SENESCENCE AND SURVIVORSHIP CURVES

Senescence can be characterized in part by how survival changes with age; i.e., by the shape of the survivorship curve. If the probability of mortality is independent of age, which is to say that it is a result of changes within the environment, diseases, or other external factors, it is referred to as extrinsic mortality (Hughes & Reynolds, 2005). However, when mortality is a non-constant function of age, the logarithm of the resulting survivorship curve is indicative of the nature of the senescence trajectory experienced by the species or population in question (Demetrius, 1978; Jones et al., 2014). Survivorship curves are often categorized into three broad types (Fig. 1.1): Type I, II, and III survivorship curves.

A Type I survivorship curve shows a population in which most individuals attain an age close to maximum expected lifespan, thus making the average lifespan of that species similar to the maximum (Demetrius, 1978). Type I curves apply to species with increasing death rates as they age (i.e., those that experience demographic senescence) and are depicted as a convex relationship between the proportion surviving and the age of the population (Jones et al., 2014). This curve is most often associated with human senescence, although many other species, including duckweeds, a clade of aquatic plants to be discussed at length herein, exhibit this curve as well.

Type II survivorship curves apply to populations in which mortality is independent of age, which is to say that no age class experiences a higher mortality than any others (Demetrius, 1978). The

resultant relationship between proportion surviving and age is therefore linear when viewed on a log scale (Jones et al., 2014). Type II survivorship curves, indicating negligible senescence, are associated with species in which extrinsic mortality dominates the causes of death.

The third and final type, the Type III survivorship curve, sometimes called 'negative senescence', depicts a high mortality in early life, but mortality decreases thereafter, and life expectancy increases with the age of the individual (Demetrius, 1978). Type III relationships are depicted as concave curves (Jones et al., 2014) and are often seen in species with high juvenile mortality relative to adult mortality; e.g., in plant species, because seedlings or young sprouts are highly susceptible to extrinsic factors of mortality with few individuals surviving the juvenile phase, but those that do survive may be comparatively invulnerable. In all three survivorship-curve types, while the general shape of the survivorship curve is a species-specific trait, the degree of concavity or convexity of the curve is highly sensitive to external or genetic conditions (Demetrius, 1978).

1.3 TAXONOMIC VARIATION IN PATTERNS OF SENESCENCE

Given that it is a nearly universal feature of multicellular organisms to varying degrees (Gaillard & Lemaître, 2020; Hughes & Reynolds, 2005), senescence needs to be studied across multiple species to gain a thorough understanding of its evolution (Jones et al., 2014). From a phylogenetic viewpoint, senescence has been observed in varying patterns and degrees in a multitude of species, including in plants, birds, mammals, invertebrates, and even yeasts and bacteria (Ackermann et al., 2007; Ackermann et al., 2003; Barks & Laird, 2020; Monaghan et al., 2020; Sherratt & Wilkinson, 2009; Sherratt et al., 2011). For example, in humans, a study on the ways in which senescence interacts with cancer found that cellular senescence helped prevent or slowed the development of cancer, ultimately preventing the proliferation of mutated or damaged cells (Campisi et al., 2001). Senescence has also been studied in other animals, including in the model species, *Drosophila melanogaster*, in which it was found that there was a significant number of parallels between humans and *D. melanogaster* regarding functional senescence (Grotewiel et al., 2005). For instance, memory function declines with age in both *D. melanogaster* and humans, as

do odor response, circadian rhythmicity, and reproductive ability (Grotewiel et al., 2005). While studying plants, Leopold (1961) found that changes that coincide with senescence typically manifest as decreases in growth rate or increases in susceptibility to external factors such as disease or disturbance. Senescence has also been identified in duckweeds (e.g., Ashby & Wangermann, 1949), with details to be provided in the subsequent section.

Single-celled species might seem likely to be immune to senescence, due to their reliance on binary fission for reproduction – without a clear parent-offspring dichotomy, senescence appears unlikely at the outset. However, in bacteria, such as *Caulobacter crescentus* and *Escherichia coli*, or in yeast species, such as *Saccharomyces cerevisiae*, the rate of fission tends to decline over time and asymmetric allocation of damaged components is actually what distinguishes aging parent cells from their comparatively undamaged daughters (Ackermann et al., 2007; Sherratt & Wilkinson, 2009). Because these single-celled organisms also show signs of senescence, it can be deduced that senescence has an extremely long evolutionary history (Ackermann et al., 2007; Sherratt & Wilkinson, 2009).

It is important to note that in some species, senescence is not observed at all or is often negligible. This lack of senescence has been identified in several species including naked mole rats (Buffenstein, 2008), ocean quahog (Abele et al., 2008), rockfish (Guerin, 2004), and Greenland sharks (Stenvinkel & Shiels, 2019). In *Heterocephalus glaber*, naked mole rats, there is no correlation between age and risk of death, which describes a survivorship curve in which there is no change in survivorship as a result of mortality, indicative of the aforementioned lack of senescence (Stenvinkel & Shiels, 2019). Interestingly, this species does not have typical repair mechanisms that other species use to protect their cell components against oxidative damage (Stenvinkel & Shiels, 2019). Additionally, naked mole rats exhibit a tolerance to age-related disease, such as neurodegeneration and cancer (Stenvinkel & Shiels, 2019). It is possible, then, that this particular species has evolved to have metabolic adaptations in concert with protein regulation to regulate factors of senescence, in contrast to more conventionally senescing species (Stenvinkel

& Shiels, 2019). Overall, this leads to this species having relatively constant mortality across age classes, the hallmark of negligible senescence.

1.4 THE LANSING EFFECT

In addition to the prototypical aspects of population-level senescence – i.e., the age-related decreases in survival and reproduction – there is a much less well-known third component of senescence known as the Lansing effect. The Lansing effect is the phenomenon in which older parents have offspring with shortened lifespan compared to the offspring of younger parents (Jennings & Lynch, 1928; Priest et al., 2002). The Lansing effect arises from non-genetic factors such as phenotypic plasticity (Monaghan et al., 2020; Plaistow et al., 2015; Uller, 2008), the ability for an organism to respond readily to changes in the environment without changing the genotype by altering the amount of mRNA produced as a result of exposure to environmental variance (Hale, 2018). For example, a recent study on plant plasticity found that leaf morphology, physiology, and size were positive plastic traits in response to mean annual temperatures (Stotz et al., 2021). Another nongenetic possibility is epigenetics (Monaghan et al., 2020; Xie et al., 2018), modifications to DNA (not including sequence differences) resulting in changes in gene expression. Other potential factors contributing to the Lansing effect include physiological changes such as a deterioration of gametes with age (Monaghan et al., 2020) and individual changes such as a decrease in parental care provisioned to the offspring (Monaghan et al., 2020).

The Lansing effect was first observed by Alexander Graham Bell when he analyzed the genealogy of human settler populations using archived data which consisted of birth and death records (Bell, 1918; Priest et al., 2002). This archived data was from the 1600s and in relation to William Hyde, a settler of Norwich, Connecticut of the early American colonies, and his descendants (Bell, 1918). Bell observed a decrease in the life expectancy of settler children with older mothers as compared to those of younger mothers, while younger fathers seemed to also increase children's lifespan (Bell, 1918; Priest et al., 2002). Bell's study was succeeded by Albert Lansing's work on rotifers in which he observed multiple orthoclones, clonal offspring derived from the same parent (Lansing, 1948), of varying ages and found a decrease not only in the number of generations the lineage

survived, but also a decrease in mean lifespan with increasing age (Lansing, 1947; Lansing, 1954; Monaghan et al., 2020; Priest et al., 2002).

In more recent research, *Daphnia pulex*, the common water flea, showed evidence of the Lansing effect during embryogenesis of offspring as a result of maternal age (Plaistow et al., 2015). Older maternal age influenced offspring in several ways, including by elevating the biological age of the offspring, determined from the increased age-independent mortality rate in tandem with lower reproductive potential and growth rates, which could be observed as lower growth rates and higher intrinsic mortality (Plaistow et al., 2015). Also in *D. pulex*, maternal age changed the way in which offspring responded to caloric restriction and showed a general effect on fecundity (Bock et al., 2019). Specifically, there was an effect of maternal age on the offspring lifespan-reproduction trade off (Bock et al., 2019), where offspring of older mothers had increased rates of reproduction, but with shortened lifespan.

While the Lansing effect has been studied in multiple taxa extensively, how the effect is generated or how it persists in a species' population is not well understood (Monaghan et al., 2020). It is important to better understand the extent to which parental age can have a negative effect on offspring and with which population-level patterns (Monaghan et al., 2020). Furthermore, research done on both an inter- and multi- generational scale should be considered in studying the Lansing effect as lifespan studies have been largely restricted to the first offspring generation (Monaghan et al., 2020). The Lansing effect and the patterns of senescence that may generate it have often been ignored in demographic research due to the general assumption that offspring fitness is independent of parental age (Monaghan et al., 2020; Wolf & Wade, 2001).

To begin understanding the Lansing effect, we must also understand that there are, at the very least, two demographic processes that may contribute to it, either simultaneously or independently. The first is a faster increase in the rate of mortality experienced by the offspring of older parents (Monaghan et al., 2020) (Figure 2a). The second is an increase in baseline

mortality (the level of vulnerability offspring begin with and subsequently experience throughout their lives) of offspring of older parents in comparison to those of younger parents (Monaghan et al., 2020) (Figure 2b). Though my study focuses on population-level traits, we must also eventually seek to understand the molecular mechanisms at play in concert with the effects of senescence, such as those contributing to changes on a molecular level (Monaghan et al., 2020).

In studying these patterns associated with the Lansing effect, we may begin to understand the trade-offs of senescence. One possible trade-off is that reproductive scheduling (the time in which an organism is reproducing rather than maintaining, repairing, or growing) varies with individual lifespan, which may result in a higher reproductive output during a shorter lifespan or lower reproductive output during a longer lifespan but ultimately reaching similar reproductive output maximums (Monaghan et al., 2020). In one study on *Daphnia pulex*, it was found that increased maternal age resulted in larger offspring that had an increased growth rate and fecundity in early life (Plaistow et al., 2015). In terms of reproductive scheduling, however, these offspring reached the peak of their reproductive output earlier and were senescing at faster rates (Plaistow et al., 2015). In a few words, these offspring were shorter-lived and produced fewer eggs over the entirety of their lifespans as a possible response to the parental age effects from the older mothers (Plaistow et al., 2015).

1.5 LEMNA SPECIES, SENESCENCE & PARENTAL AGE EFFECTS

As alluded to above, observations of senescence have also been made in Lemnoideae, otherwise known as the duckweeds, which are among the simplest flowering plants (Hillman, 1961). Duckweeds are free-floating plants typically found on slow-moving bodies of water (Hillman, 1961). The fronds, or the leaves of the plants, can range from 0.5 mm to slightly less than two centimetres with a simple root system, with most having two meristematic pockets from which offspring grow and detach (Ashby & Wangermann, 1949; Hillman, 1961). With these characteristics of the duckweeds, their short lifespans, and their simple structure, they have great potential as experimental model organisms for further demographic studies (Hillman, 1961; Laird & Barks, 2018; Wangermann & Ashby, 1950). Duckweeds are also predominantly asexually reproducing

plants, aiding in the elimination of genetic variability between individuals in laboratory practice (Hillman, 1961), which helps reduce noise in the data, as all experimental subjects derived from a common clone are genetically identical. Further, genetic similarity and simplistic species allow for variations in specimens to be abundantly clear with little connection drawn to a genetic component, a particularly helpful quality when assessing the Lansing effect, a nongenetic phenomenon.

Studies on senescence in *Lemna*, a prominent genus in Lemniodeae, have been performed on various species. In *Lemna minor* L., for example, survival, reproduction, and offspring quality all decrease with age (Barks & Laird, 2015). The age-related declines in offspring quality are also known as parental age effects (Barks & Laird, 2015, 2016) and can be seen in various traits relating to fitness including offspring size, lifespan, and reproductive output (Ashby & Wangermann, 1951; Barks & Laird, 2015). Research pertaining to *Lemna minor* has also reported a decrease in cell number (and thus, offspring size) associated with successive daughter fronds because of senescence in parental fronds (Ashby & Wangermann, 1951). Parental age effects in *L. minor*, in combination with the determinate growth patterns of the plant, and high rates of extrinsic mortality (herbivory, disease, environmental disruption), are consistent with evolutionary theories of senescence pertaining to survivorship-age relationships reflected by Type I survivorship curves in laboratory conditions (Barks & Laird, 2015). Recall that Type I survivorship curves show a species with high survival until old age which coincides with determinate growth patterns of *L. minor* and the elimination of factors of mortality.

Senescence has been reported in other *Lemna* species as well. In *Lemna turionifera* Landolt, offspring size and fitness declined with increasing immediate birth order (the order in which offspring are born from a single parent, where increasing parental age is akin to increasing birth order), which is to say that a significant decrease in offspring fitness was seen in response to increasing parental age (Barks & Laird, 2016). These changes in fitness and the resulting death rate also make *L. turionifera* a species with a Type I survivorship curve. *Lemna turionifera* will serve as the study species in the research reported in this thesis.

1.6 STUDY OVERVIEW

In this study, I ask the following questions in *Lemna turionifera:*

(1) Does the Lansing effect exist?

(2) And, if so, do offspring of older parents undergo a more rapidly increasing mortality rate than offspring of younger parents, and/or do offspring of older parents experience increased baseline morality at every age compared to offspring of younger parents?

The null hypothesis is that there is no difference between older and younger parents in the effect of age on offspring mortality, that is parental ages at the time of a reproduction event is independent of offspring mortality trajectories.

To answer my study questions, I observed the lifetime of 392 specimens of *Lemna turionifera*, each referred to as a focal frond, half of which were from older parents and half of which were from younger parents, with birth order of the individual being a proxy for parental age. While observing these individuals, I recorded the number of offspring produced by each focal frond and also recorded the size of each focal frond at time of death. This is followed with analysis of lifespan data, offspring reproduction data, as well as frond size analysis. In completing these observations and analyzing the data collected, I conclude that the Lansing effect does exist in *Lemna turionifera* and is associated with a change in baseline mortality of offspring with an increase in parental age.

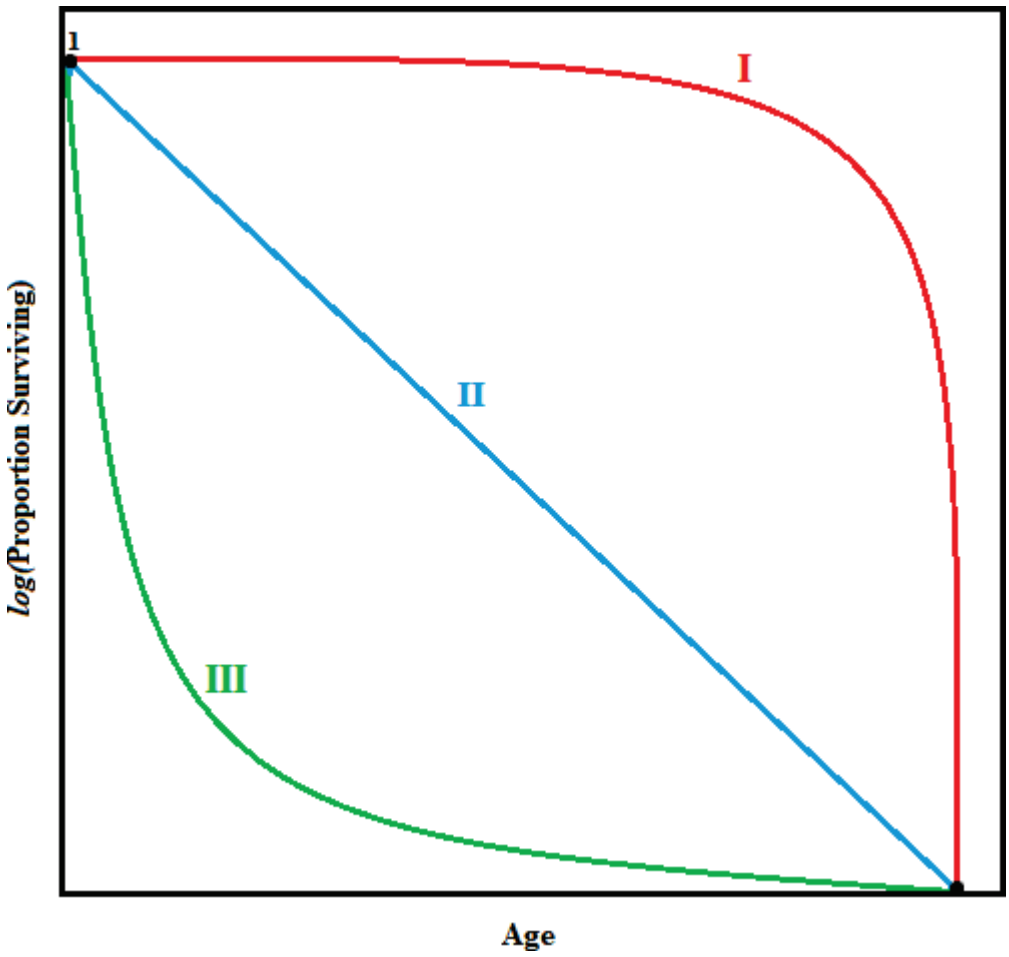

Figure 1.1: Survivorship curves where the logarithm of the proportion surviving is a function of age (Modified from Demetrius, 1978). Type I depicts high survival until old age (e.g., humans, most mammals, duckweeds under lab conditions); type II depicts steady decline in survival with age indicating constant mortality (e.g., birds); type III depicts low survival into old age (e.g., most plants).

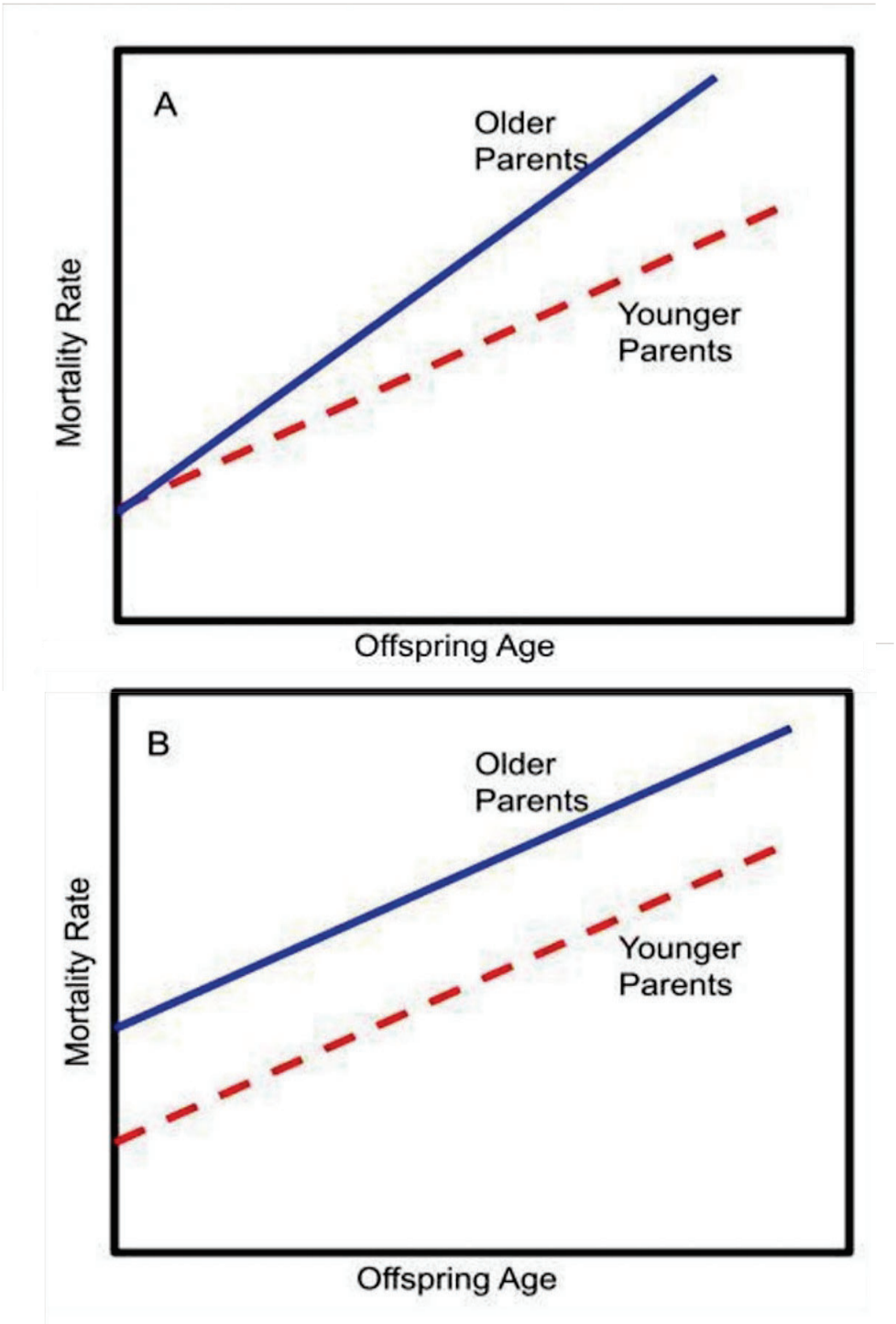

Figure 1.2: Proposed demographic patterns underlying the Lansing effect. Panels are hypothetical mortality rates as a function of offspring age and trendlines show offspring of respective parental ages. A reflects a difference in rate of increase of mortality and B portrays a change in baseline mortality (Modified from Monaghan et al., 2020).

2. THE LANSING EFFECT IN *LEMNA TURIONIFERA* (LEMNOIDEAE) AND POTENTIAL CONTRIBUTING FACTORS

2.1 INTRODUCTION

2.1.1 PREAMBLE

The Lansing effect is the phenomenon of senescence whereby older parents have shorter-lived offspring than younger parents (Jennings & Lynch, 1928; Lansing, 1954; Monaghan et al., 2020). While the age of the parents at the time of a reproductive event affects offspring lifespan, other parental age effects may also be observed such as decreased number of grand-offspring produced. The Lansing effect has been observed in a variety of animal taxa including insects, birds, and mammals such as humans (Bell, 1918; Monaghan et al., 2020; Plaistow et al., 2015). The Lansing effect has also been observed in plants (Ashby & Wangermann, 1949; Barks & Laird, 2016). Here, I investigate the Lansing effect in the plant subfamily Lemnoideae (duckweeds) - tiny, aquatic plants found on the surfaces of slow-moving bodies of water (Hillman, 1961).

2.1.2 SENESCENCE & THE LANSING EFFECT

Senescence is a seemingly maladaptive process which is characterized at the individual level as physiological deterioration with age. We recognize these changes as age-related decreases in vigor, survival, reproduction, and growth rates, and increases in susceptibility to extrinsic factors, such as environmental changes, disease, or exposure to toxins (Ashby & Wangermann, 1949; Barks & Laird, 2015; Comfort, 1954). Senescence can be associated with parental age effects, wherein an offspring's fitness depends on the age of its parent(s) when that offspring was produced. Parental age effects can result in decreases in offspring lifespan or changes to measures of fitness including, but not limited to, number of offspring produced and offspring size (Barks & Laird, 2015, 2020). When a decrease in offspring lifespan is observed with increasing parental age, this is known as the Lansing effect (Lansing, 1947; 1954; Monaghan et al., 2020).

2.1.3 OBJECTIVES

My goal is to analyze the relationship between parental age and offspring lifespan in the duckweed *Lemna turionifera* Landolt. My specific objectives are as follows:

(1) Test for the presence of the Lansing effect;

(2) Determine whether the Lansing effect is generated due to a difference in rate of increase of mortality or an increase in baseline mortality associated with increasing parental age.

Aside from these specific goals pertaining to the Lansing effect, I chose to analyze other aspects of fitness as they relate to parental age effects. These aspects of fitness included size of the focal frond, number of offspring produced, as well as the intrinsic rate of increase as a proxy for fitness at the individual level. In obtaining these data, I will be able to further deduce the effects of parental age at birth on offspring fitness prospects.

2.2 METHODS

2.2.1 STUDY SPECIES

For this experiment, the study species consist of member of the subfamily Lemnoideae (family Araceae). These are known as duckweeds, a group of small, floating, aquatic plants typically found on the surfaces of slow-moving bodies of water (Landolt, 1986). *Lemna* reproduces predominantly asexually and individual ramets (i.e., single-leaf fronds, a colony of which is known as a genet) typically begin to reproduce shortly after detaching from the mother frond or, in some cases, while still attached, and often produce no more than 10-15 daughters during their lifetime (Landolt, 1986; Lemon & Posluszny, 2000). Because of this asexual reproduction, ramets within any given genet are genetically identical (excepting rare mutants) which is beneficial when studying the Lansing effect, a non-genetic parental age effect (Hillman, 1961; Landolt, 1986). *Lemna* also exhibits a determinate growth pattern, where a maximum size is reached and thus growing ceases, effectively aiding in the visibility of senescence following maximal growth. In addition, *Lemna* individuals exhibit an average lifespan that is close to the species' maximum lifespan, wherein most individuals will not survive past 30 days even in ideal conditions. This short lifespan makes this an excellent study species, in conjunction with the simplicity of the plant and the lack of genetic variability.

Lemna turionifera L. is one of three species of *Lemna* found in Alberta (Senevirathna et al., 2021).

This species is distributed widely in the province (Alberta Biodiversity Monitoring Institute, 2020). An individual *L. turionifera* plant typically consists of a frond (a leaf and stem hybrid) and a root (Lemon & Posluszny, 2000). Fronds have two meristematic pockets from which the offspring develop and detach (Hillman, 1961). To maximize the observability of the Lansing effect in this study, *Lemna turionifera* was used because the effect had previously been observed in this species (Barks & Laird, 2016).

2.2.2 EXPERIMENTAL METHODS

Methods were adapted from Barks and Laird (2016) in which the species used was *Lemna turionifera* and a Lansing effect was observed as part of their analysis for parental age effects. I chose to use *Lemna turionifera* and similar conditions to maximize the chances of observing the Lansing effect. I also used first and fifth clonal offspring as test groups due to the results seen in the 2016 study in which the first and fifth offspring showed differences in lifespan and reproduction.

Lemna turionifera were obtained from a stock culture of a single-genotype strain originally collected from a small wetland outside the Alberta Water and Environmental Sciences Building at the University of Lethbridge (strain Wat A, *L. turionifera*; GenBank accession number: MG000496) (Barks & Laird, 2016; Laird & Barks, 2018). Working cultures grew in flasks containing 36 mL of quarter-strength Schenk and Hildebrandt (0.8 g/L) growth medium (S6765, Sigma-Adlrich, St. Louis, MO, USA) supplemented with tryptone (0.333 g/L), yeast extract (0.067 g/L), and sucrose (6.667 g/L), which act as metabolites and nutrients for cell growth in bacterial cultures, to help detect microbial contamination by increasing the growth rate of microbes present in the growth media, thus making them more visible to the naked eye. These working cultures were kept in natural light, 2.85 meters from a South-facing window at 19 °C for one month for proliferation.

Once working cultures produced an adequate number of fronds (n = 392), fronds were placed individually into sterile petri dishes. Each dish contained 10.5 mL of half-strength growth medium (1.6 g/L of Schenk and Hildebrandt; all other additive concentrations were consistent). Each of

these ‘progenitor fronds’ was marked with diluted, autoclaved India ink to make it possible to distinguish mother and daughter fronds. Each progenitor was randomly assigned to a position on one of 14 wire trays, spread across two growth chambers (Conviron E15; Controlled Environments Limited, Winnipeg, MB) at 24 °C with a photoperiod of 15:9 light:dark, with the 15 hours of light at a photon flux density of 269.46 and 259.37 μmol m^{-2} s^{-1}, as measured at the beginning of the experiment.

Throughout the experiment, weekly growth medium transfers were done in which each frond was placed in a new, sterilized petri dish with fresh growth medium to mitigate the effects of depleted nutrients or water in the media. The fourteen wire tray positions were also re-randomized daily to mitigate any variation in conditions within and between growth chambers.

2.2.3 OBTAINING DATA

Each progenitor frond and its immediate descendants underwent a series of clonal reproductions to establish ‘focal fronds’ that were used in the experimental component of the study. This was done to reduce residual age and parental age effects prior to the observational period of the study (Barks & Laird, 2015). First daughters of progenitor (P) fronds were taken until the fifth clonal generation. The second generation that was taken was called unknown (U) as the ancestral order cannot be sufficiently deduced at this stage given that the progenitors may be of any generational order from the working culture. Following this stage, first daughters were obtained with confirmation of generational order, and this was repeated for an additional three times (fifth generation). The daughters of the fifth-generation fronds comprised the focal fronds in the experiment and 196 of these focal fronds were randomly assigned to each of the two treatments. Thus, half of the sixth-generation fronds were first daughters (first-born; P-U-1-1-1-1), and the other half were fifth daughters (fifth-born; P-U-1-1-1-5). These two groups were created to establish ‘young’ and ‘old’ parent groups, referred to as B1 and B5, respectively. B1 and B5 reflect birth order, which is used as a proxy for parental age. One hundred ninety-six focal fronds were established for each of B1 and B5, for a total sample size of n = 392 fronds (Figure 2.1). A small number of samples were removed from the experiment due to microbial contamination or frond

damage. The final sample size was n = 383 (B1 = 191; B5 = 192). Further sample loss occurred due to damage or deterioration of samples during microscopic imaging for size analysis, which reduced the sample sizes relative to the other tests (sample size for size and fitness analysis: B1 = 175; B5 = 171).

Focal fronds (first daughters (B1) and fifth daughters (B5)) were observed daily from birth, defined as the day a focal frond detached from its mother frond, until death, defined as the day of a focal frond's last daughter's detachment (determined retroactively following a seven-day buffer period to ensure no further daughters were produced). Thus, death is equivalent to the cessation of reproduction, which is an evolutionarily relevant definition in species with no parental care or other intergenerational transfers (there are no other evolutionarily beneficial reasons for the parental frond to live following the final offspring's detachment in this species). Moreover, there are few characteristics that can be used to accurately pinpoint physiological death, making this "cessation of reproduction" definition of death a more consistent and quantifiable approach. Number of offspring and lifespan were recorded during these daily observations and data were analyzed for intrinsic rate of increase at the level of the individual, and size differences. Most pertinently for my objectives, I also assessed the presence of the Lansing effect, and the nature of the demographic patterns (rate of mortality or baseline mortality) that may generate the Lansing effect.

2.2.4 DATA ANALYSIS

I checked the distribution of lifespan data for normality using normal quantile-quantile plots and the Shapiro-Wilk Normality test. The quantile-quantile plots indicated that the data showed a skewed distribution, and the Shapiro-Wilk test determined that the data were non-normal ([B1] Shapiro-Wilk test, $W = 0.89$, $P = 1.98 \times 10^{-10}$, $n = 191$; [B5] Shapiro-Wilk test, $W = 0.91$, $P = 2.76 \times 10^{-9}$, $n = 192$). Due to the non-normal data, I used a Wilcoxon Rank-Sum test to compare the median lifespans of individuals in the B1 and B5 groups. I then used boxplots to visualize the difference in lifespans in the two groups.

I repeated the above steps for the number of offspring, which also showed a non-normal

distribution ([B1] Shapiro-Wilk test, W = 0.90, P = 1.0 x 10^{-8}, n = 191; [B5] Shapiro-Wilk test, W = 0.90, P = 1.0 x 10^{-9}, n = 192). I then used a Wilcoxon Rank-Sum test as well, to compare the median number of offspring of individuals in the B1 and B5 groups.

I created life tables to calculate the probability of survival and reproduction for each age class, for both B1 and B5 groups separately. I fit the survivorship data with logistic, exponential, Gompertz, and Weibull distributions using a log-likelihood approach (Table 2.1) (Barks & Laird, 2015; Pletcher et al., 2000; Sherratt et al., 2011). The four candidate survivorship curves are commonly used for analyses of demographic senescence and have previously been used in studies of duckweed senescence. A logistic curve depicts the three types of survivorship curves addressed previously (Type I, II, and III; Figure 1.1). An exponential curve, due to its arithmetic scale, often shows a more linear curve that does not accurately account for the changes in mortality at different age-time points in a species population. Both the Gompertz and Weibull distributions are defined by two parameters (Pletcher et al., 2000). The Gompertz distribution is defined by rate of mortality and *initial mortality* while the Weibull distribution is defined by rate of mortality and *baseline mortality* (Pletcher et al., 2000). It is possible that data may have fit any number of these curves; for example, if the data were to fit the Gompertz distribution, this would mean that baseline mortality is negligible in this species. For model selection, I compared the four models using Akaike's Information Criterion, corrected for small sample sizes (AIC_C), with the best-fit model being the one with the lowest AIC_C (Hu, 2007)

As the logistic model fit best for the survivorship curves of both the B1 and B5 groups taken *separately* (see *Results*), I subsequently re-analyzed the survivorship curves of B1 and B5 *simultaneously* in a single model, again using the logistic model, but this time where each of the logistic model's three parameters (Table 2.1), namely baseline mortality (A), mortality rate (B), and deceleration of mortality (C), could either be common between B1 and B5 or distinct . As a result of there being two possibilities for each parameter (common or distinct; A, B, C), there were 2^3 = eight possible candidate models to evaluate. As with before, I evaluated the eight candidate

models using AIC_C values, where uppercase versions of the three parameters (A, B, C) denote distinct values between B1 and B5, and lowercase versions of the three parameters (a, b, c) denote common values between the two test groups (Table 2.1). This means that, for example, for a resultant Abc candidate model, the A parameter is distinct between the offspring of older and younger parents while the B and C parameters are common between parental age groups. Of particular interest to resolving Objective 2 are parameters A and B, which are interpreted as baseline morality and mortality rate, respectively. Thus, analyzing the ways in which these parameters differ or coincide between offspring of older and younger parents will contribute to the understanding of the demographic pattern that is generating the Lansing effect in *Lemna turionifera*.

I modelled the probability of reproduction as a function of age using generalized estimating equations (GEEs), specifically using first-order autoregressive (AR1) correlation structure to model separately all reproductive events and reproductive events involving multiple reproductions (i.e., cases in which more than one daughter frond is produced on the same day) (Chmilar & Laird, 2019). It was necessary to separate the two types of reproductions because reproductions leading to multiple offspring are rare in this species (i.e., with rare exception, reproduction is a binary variable with fronds either producing a daughter frond – or not – on any given day). This GEE approach was ideal for my analysis due to the within-individual temporal autocorrelation in which the probability of an individual reproducing the immediate next day following a reproductive event, decreases (Barks & Laird, 2015). I plotted the probability of reproduction with fitted GEE curves.

I measured frond size of each focal frond by first taking digital images via microscope then running said images through code written in MATLAB (version R2016a, The MathWorks Inc., Natick, USA, 2016; Ankutowicz & Laird, 2018) to analyze for surface area (mm^2). The code converts the image of the frond into a binary image (0 = background, 1 = frond), then detects the frond's edge, and generates a set of ordered xy coordinates for the edge (Ankutowicz & Laird, 2018). The area of

the resultant polygon can then be found by using the shoelace algorithm – named for the repetitive cross-multiplication of coordinates - which is a standard mathematical algorithm used to determine the area of a polygon whose vertices have been determined by coordinates on a plane (Ankutowicz & Laird, 2018).

I also analyzed the intrinsic rate of increase (*r*) at the individual level by constructing a Leslie matrix for each individual and then calculating the leading eigenvalue. The intrinsic rate of increase could be calculated as the natural log of this eigenvalue (McGraw & Caswell, 1996). The intrinsic rate of increase is an appropriate proxy for fitness in this analysis (Williams et al., 2006); as with other comparisons between B1 and B5, the differences in the intrinsic rate of increase was evaluated using boxplots and Wilcoxon Rank Sum tests.

2.3 RESULTS

I observed demographic senescence for offspring of both younger and older parents, whose log-transformed survivorship exhibited a downward arc (Figure 2.2). In addition, I observed a decrease in reproduction in both groups of offspring and for single and multiple reproductive events with age (Figure 2.2). This is also in accordance with previous studies (Barks & Laird, 2016).

Model fitting and model selection showed that the logistic model (Figure 2.3) was the best fit for survival for both birth orders (Table 2.2; Figure 2.4). Once established, the logistic model was re-fit for both groups simultaneously to assess for parameter-fit in accordance with Pletcher et al.'s (2009) parameters for logistic models (Table 2.3). This analysis indicated that the best-fitting of the eight candidate models was the one denoted as 'Abc'; i.e., the model with distinct baseline mortalities for offspring of younger and older individuals, with offspring of older individuals experiencing a higher baseline mortality (parameter A). However, this model also predicted that the other two parameters, mortality rate and deceleration of mortality (parameters B and C, respectively), hold constant between the two parental age groups (Table 2.3).

Boxplots and associated statistical tests indicate that lifespans were different between the offspring of young and old parents, where offspring of younger parents had significantly greater lifespans (Figure 2.5) (Wilcoxon Rank-Sum Test, W = 27473, $P < 2.2 \times 10^{-16}$, $n_{[B1]} = 191$, $n_{[B5]} = 192$; mean lifespans: B1 = 25.94 days, B5 = 21.97 days). Thus, the Lansing Effect was observed, and the relationship between lifespan in the two groups (where B1 > B5) corresponded with the relationship between the baseline mortality parameter for the simultaneous logistic fit (i.e., parameter A, where again B1 > B5).

Likewise, boxplots and associated statistical analyses also showed that the number of offspring differed between the two groups where offspring of older individuals produced fewer offspring than those of younger parents (Figure 2.6) (Wilcoxon Rank-Sum Test, W = 24540, $P < 7.0 \times 10^{-9}$, $n_{[B1]} = 191$, $n_{[B5]} = 192$; mean number of offspring: B1= 11.17, B5 = 9.93). Contrarily, offspring of older individuals were larger and had a greater intrinsic rate of increase than those of younger parents (Figure 2.7, 2.8) ([Area] Wilcoxon Rank-Sum Test, W = 5664, $P < 2.2 \times 10^{-16}$, $n_{[B1]} = 175$, $n_{[B5]} = 171$; mean area: B1 = 5.82 mm^2, B5 = 6.89 mm^2; [*r*] Wilcoxon Rank-Sum Test, W = 14103, $P = 9.0 \times 10^{-5}$, $n_{[B1]} = 175$, $n_{[B5]} = 171$; [B1], mean r: B1 = 0.277, B5 = 0.307; mean log(r): B1 = -1.33, B5 = -1.22).

2.4 DISCUSSION

The data support the hypothesis that the Lansing effect exists in *Lemna turionifera* as there is a statistically significant difference in lifespan in the test groups, with those in the B5 group (offspring of older parents) having a shorter average lifespan than those in the B1 group (offspring of younger parents).

The logistic model was the best-fit distribution for the data obtained, which was determined by analysis of AIC_C values. No senescence would present as a straight line on a log-survivorship by age graph, indicating constant instantaneous mortality. This is in accordance with previous studies done on *Lemna* in which senescence was observed (Barks & Laird, 2016). I then overlaid both resultant survivorship curves which suggests that the difference in lifespan is due to greater

baseline mortality - initial vulnerability of the offspring - in the offspring of older parents, as opposed to a more rapid increase in mortality. My analysis of the survivorship curves in this study depicted parallel but distinct curves of the log survivorship, indicative of different values of logistic parameter A (Pletcher et al., 2000). This implies that the offspring of older parents begin life with and retain an increased mortality throughout their lifespan (Monaghan et al., 2020).

However, there is an indication of other possibilities which may be generating the Lansing effect in models with different combinations of common or distinct parameters. For example, model ABc indicated differences in both baseline mortality and rate of increase of mortality ($\Delta AIC_C = 2.080$, Table 2.3). This was ultimately determined not to be the demographic pattern in the case of *L. turionifera* based on ΔAIC_C values, which are considered most parsimonious when falling below 2.0. Based on these analyses, it can be said that the most relevant contribution to the Lansing effect in *L. turionifera* is a change in baseline mortality and, thus, increased frailty at all ages for individuals with parents of an older age class.

In addition, it was found that offspring of older individuals produced fewer offspring themselves, which has previously been documented as a parental age effect in *Lemna turionifera* and in other species of duckweed (Barks & Laird, 2015; 2016; Chmilar & Laird, 2019; Monaghan et al., 2020). However, offspring of older parents were initially faster to reproduce than offspring of younger parents which resulted in a higher peak of reproduction earlier in life in comparison to offspring of younger parents. The change in reproductive rates and the timing of said change may coincide with the higher fitness associated with the older parents (measured by the intrinsic rate of increase at the individual level, as a proxy for fitness). This may also imply a change in parental investment with age (Barks & Laird, 2016), leading to a different outcome in the trade-off between parental investment in offspring versus parental somatic maintenance. In this manner, increased parental investment in offspring could result in decreased parental lifespan (as a result of decreased investment in parental somatic maintenance) but increased offspring fitness (Hansen & Price, 1995; Plaistow et al., 2015; Poizat et al., 1999).

Future directions for studying the Lansing effect in *Lemna turionifera* include performing a multigenerational study to evaluate the persistence, extent, and possible cumulative nature of the effect. Previous studies have embarked on multigenerational analysis in which several generations were observed (Barks & Laird, 2016); however placing focus on the Lansing effect, specifically in regard to demographic patterns such as those analyzed here, or in regard to generating factors such as somatic deterioration or parental investment strategies, would be a logical next-step. It may also be beneficial to consider a nutritional uptake study to evaluate whether larger fronds are better equipped for the physiological mechanisms of maintenance, such as resource allocation for growth, maintenance, and repair.

Table 2.1: Parameters and equations for each model used to ascertain demographic survival patterns in the birth order groups. Each model is associated with its own set of parameters, which were assigned new names (A, B, and C) for the purposes of statistical analysis. The best-fit model was assessed using AIC_C values for the birth order groups taken separately, indicating a preference for the logistic model (Table 2.2), shown in bold. Models with different combinations of common versus distinct logistic parameters were then compared for the two birth groups simultaneously to assess for similarity or differences between the survivorship curves of offspring of young and old parents (Table 2.3) (following Pletcher et al., 2000).

Model	**Parameter Definitions***	**Survivorship Equation***	**Parameter Substitution**
Logistic	λ = baseline mortality; A γ = mortality rate; B s = decreasing rate; C	$[1 + s\frac{\lambda}{\gamma}(e^{\gamma t} - 1)]^{-1/s}$	$[1 + C\frac{A}{B}(e^{Bt} - 1)^{-1/C}$
Weibull	λ = baseline mortality; A β = rate of increase in mortality, B	$\exp[-(\lambda t)^{\beta}]$	$\exp[-(At)^{B}]$
Gompertz	λ = baseline mortality; A γ = mortality rate; B	$\exp[-\frac{\lambda}{\gamma}(e^{\gamma t} - 1)]$	$\exp[-\frac{A}{B}(e^{Bt} - 1)$
Exponential	λ = baseline mortality; A	$\exp(-\lambda t)$	$\exp(-At)$

Table 2.2: Model fitting for each birth group, taken separately. Table A and B reflect B1 and B5, respectively. ΔAIC_C values were used to determine the best-fit model. Tables are in ascending order of ΔAIC_C values. In both B1 and B5, the logistic model (given in bold) was deemed the best fit.

(A)	Model	meters (k)	Deviance	AIC_C	ΔAIC_C	AIC_C weight
	Logistic	***3***	***1139.702***	***1145.830***	***0.000***	***2.813×10^{0}***
	Weibull	2	1221.654	1225.718	79.888	4.493×10^{-18}
	Gompertz	2	1290.463	1294.526	148.696	5.141×10^{-33}
	Exponential	1	1625.163	1627.690	481.860	2.320×10^{-105}

(B)	Model	meters (k)	Deviance	AIC_C	ΔAIC_C	AIC_C weight
	Logistic	***3***	***1140.186***	***1146.315***	***0.000***	***2.813×10^{0}***
	Weibull	2	1207.667	1211.730	65.416	6.239×10^{-15}
	Gompertz	2	1288.355	1292.418	146.104	1.879×10^{-32}
	Exponential	1	1570.505	1572.527	426.212	2.813×10^{-93}

Table 2.3: Model parameters (Par.) and AIC_C values as a result of the best-fitting logistic model, for both birth order groups taken simultaneously (Figure 2.2). Given that there are three parameters associated with the logistic model and two possible relations between each parameter (distinct or common parameter values for B1 vs B5), there are a total of eight possibilities regarding distinct and common parameters, shown here. The best fit model was *Abc,* shown in bold, which indicates distinct parameter A values, but common parameter B and C values. Table is in ascending order of ΔAIC_C values.

Model	**Par. A**	**Par. B**	**Par. C**	**Deviance**	**AIC_C**	**ΔAIC_C**	**AIC_C weight**
Abc	***Distinct***	***Common***	***Common***	***2280.183***	***2288.183***	***0.000***	***4.689×10^{-01}***
ABc	Distinct	Distinct	Common	2280.154	2290.154	2.080	1.657×10^{-01}
AbC	Distinct	Common	Distinct	2280.302	2290.302	2.228	1.539×10^{-01}
aBC	Common	Distinct	Distinct	2280.867	2290.867	2.793	1.160×10^{-01}
ABC	Distinct	Distinct	Distinct	2279.888	2291.345	3.944	6.519×10^{-02}
aBc	Common	Distinct	Common	2285.664	2293.664	5.481	3.026×10^{-02}
abC	Common	Common	Distinct	2349.913	2357.913	69.729	3.385×10^{-16}
abc	Common	Common	Common	2360.180	2366.180	77.909	5.665×10^{-18}

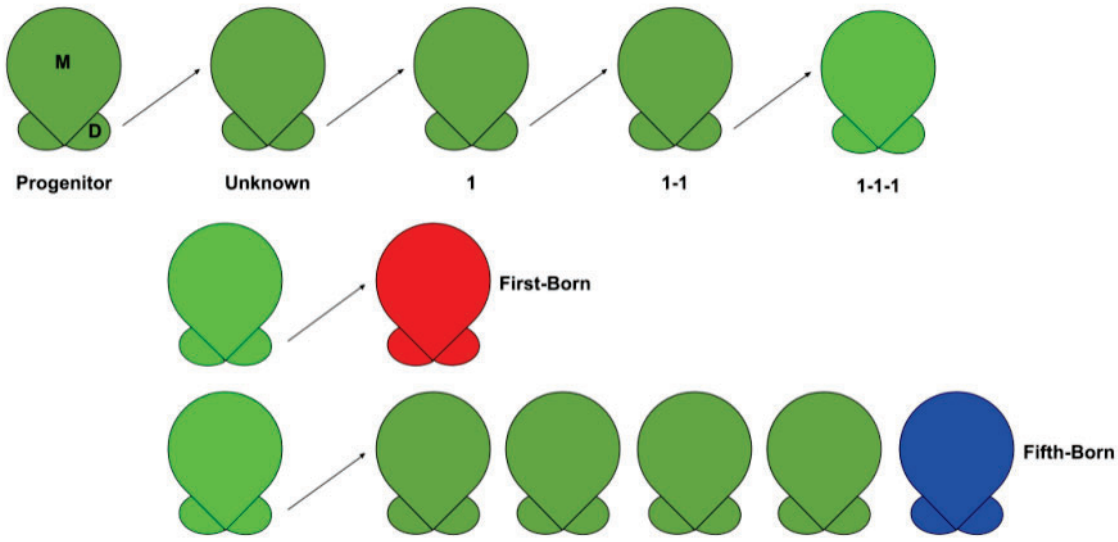

Figure 2.1: Experimental method used to acquire first- and fifth-born offspring. Progenitor fronds (P) were obtained from working cultures and deemed mother fronds (M). First daughters (D) were obtained over five generations in order to mitigate generational parental age effects, resulting in P-U-1-1-1 fronds (otherwise denoted as frond 1-1-1 in the figure). Fronds were randomly assigned to either treatment (first- or fifth-born) which required taking P-U-1-1-1 fronds and obtaining either the first- or fifth-born offspring from that frond.

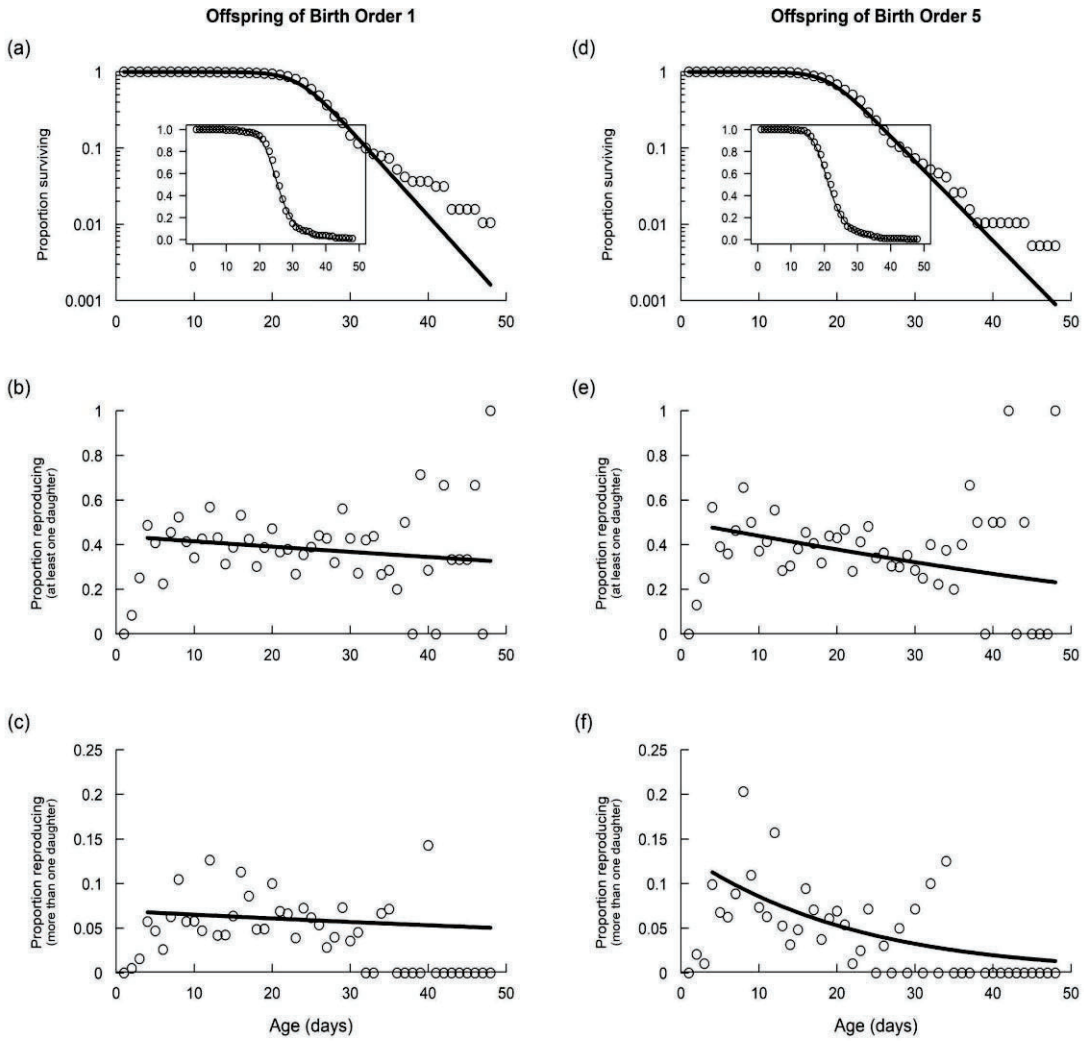

Figure 2.2: Demographic senescence and probability of reproduction for first clonal offspring (B1) compared to fifth clonal offspring (B5) (B1: panels a, b, c; B5: panels d, e, f). Panels a and d indicate a senescing population with downward-arcing log-survivorship versus age relationships. Panels b and e demonstrate a decrease in the probability to reproduce one offspring with older age classes where B5 having a slightly higher proportion reproducing at earlier ages. Panels c and f suggest a decrease in the probability to reproduce more than one offspring with older age classes. Panels a and d have logarithmic survivorship axes while insets have arithmetic survivorship axes, portraying the same data.

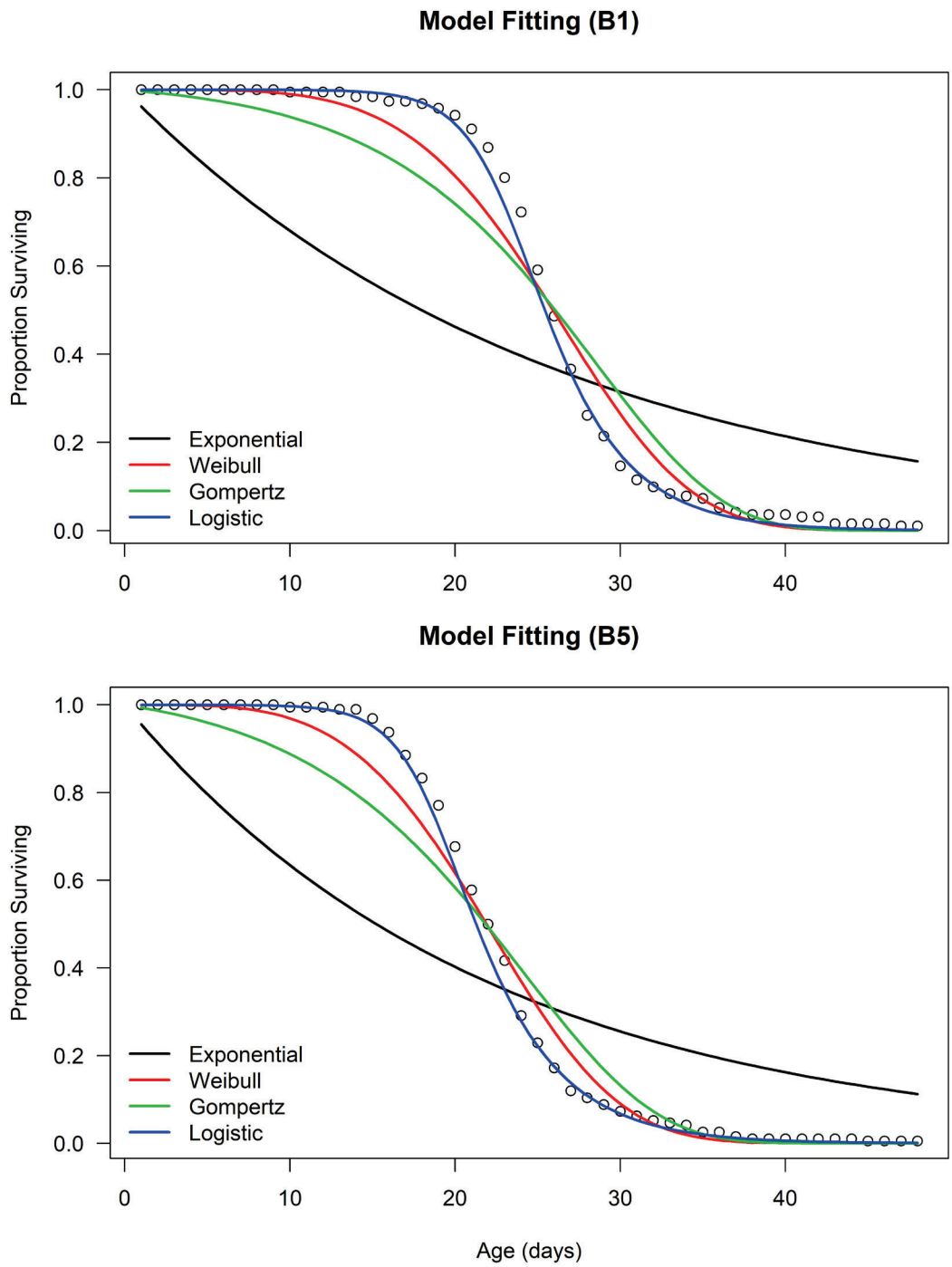

Figure 2.3: Results of demographic model fitting suggest that the logistic model is the best fit for both test groups (see also Table 2.2). Top panel depicts B1 (first clonal offspring), and bottom panel depicts B5 (fifth clonal offspring).

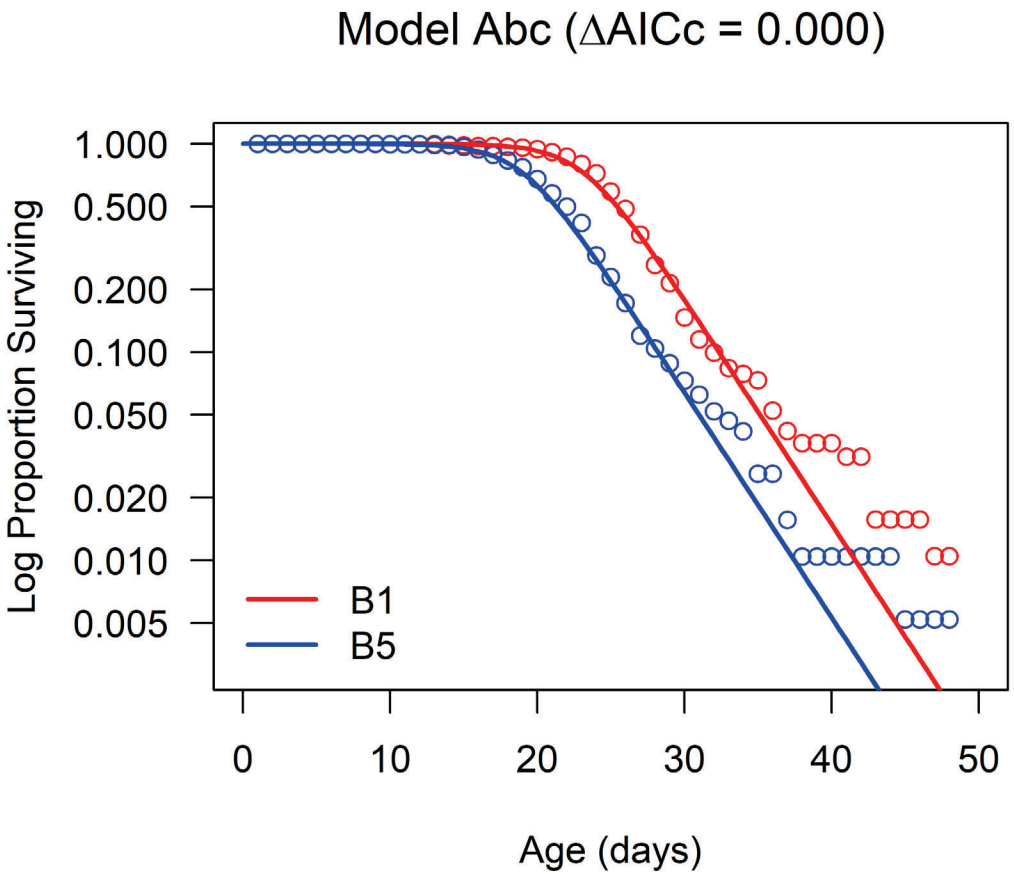

Figure 2.4: Best-fit comparative logistic models for the test groups based on the results portrayed in Table 2.3 associated with model Abc in which the proportion surviving is on a log scale. Red depicts offspring of younger parents in comparison to blue, which are offspring of older parents.

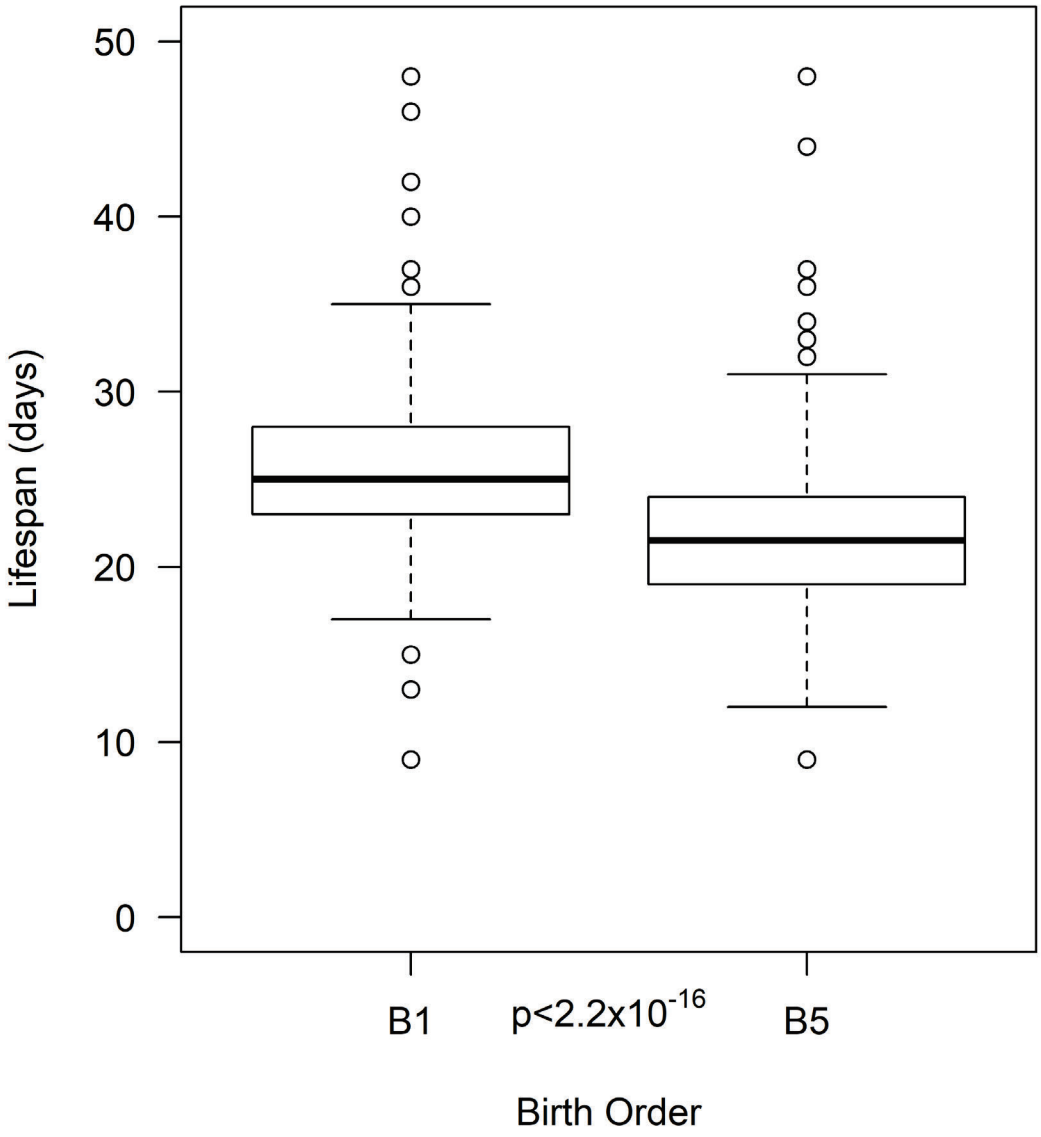

Figure 2.5: Boxplot of lifespan differences between test groups ($p < 2.2x10^{-16}$). Boxes show the median (thick black line) as well as first and third quartiles (upper and lower sections). Whiskers show the minimum and maximum non-extreme points, defined as those within 1.5 times the interquartile range from the bottom or top of the boxes. Symbols are extreme points that did not fall within this range.

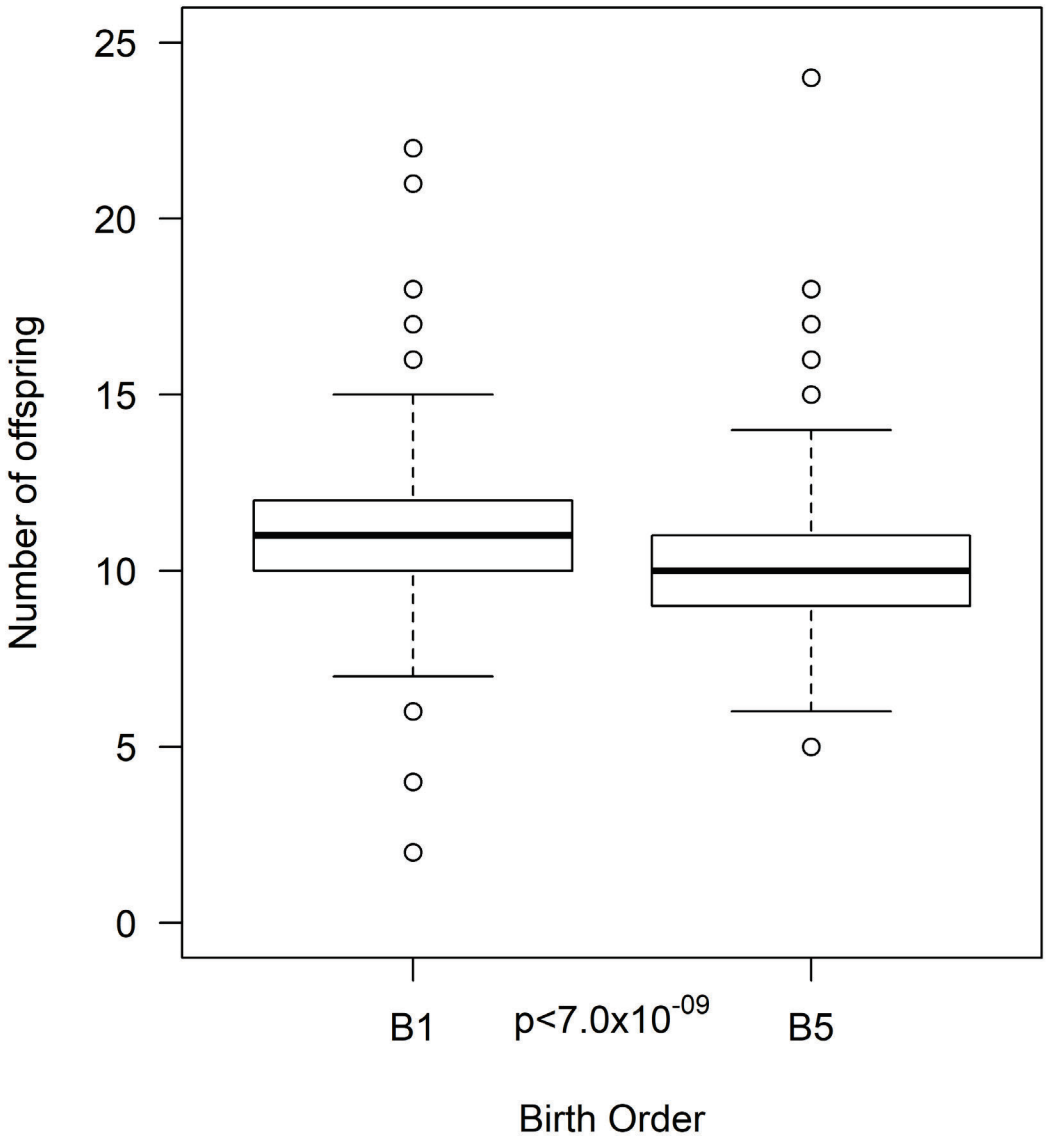

Figure 2.6: Boxplot of differences in offspring numbers ($p < 7.0x10^{-09}$). Boxes show the median (thick black line) as well as first and third quartiles (upper and lower sections show the minimum and maximum non-extreme points, defined as those within 1.5 times the interquartile range from the bottom or top of the boxes. Symbols are extreme points that did not fall within this range.

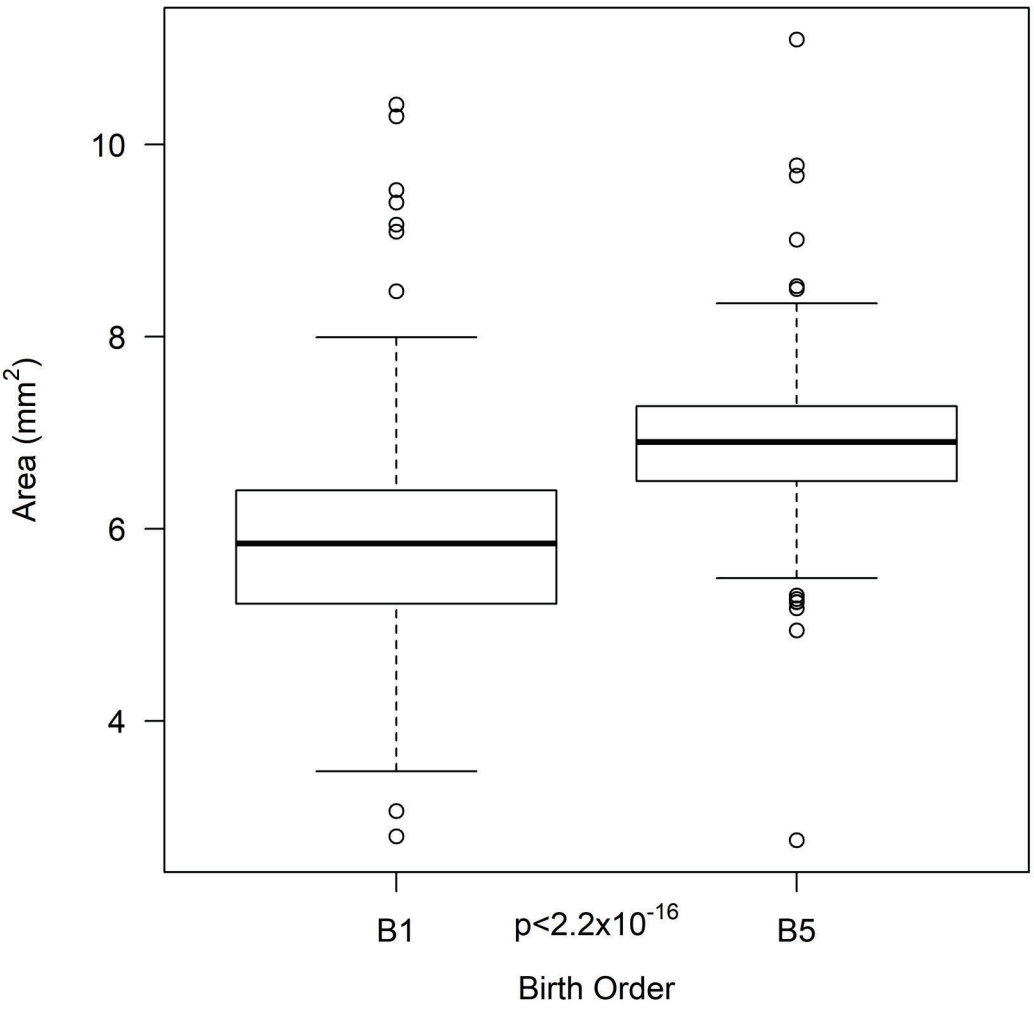

Figure 2.7: Boxplot of size differences between test groups ($p < 2.2x10^{-16}$). Boxes show the median (thick black line) as well as first and third quartiles (upper and lower sections). Whiskers show the minimum and maximum non-extreme points, defined as those within 1.5 times the interquartile range from the bottom or top of the boxes. Symbols are extreme points that did not fall within this range.

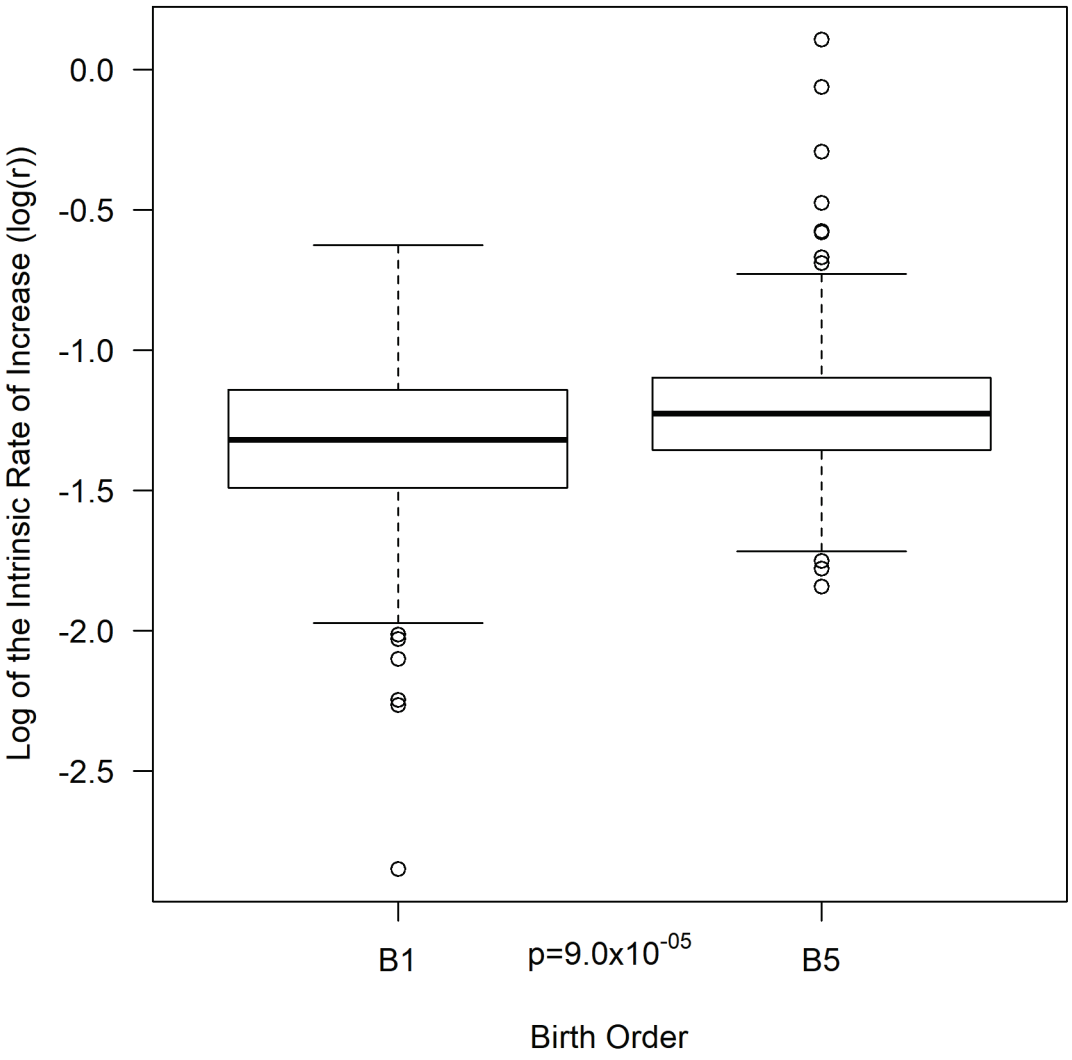

Figure 2.8: Boxplot of the log of intrinsic rate of increase (log(*r*)) between test groups ($p = 9.0x10^{-05}$). Boxes show the median (thick black line) as well as first and third quartiles (upper and lower sections). Whiskers show the minimum and maximum non-extreme points, defined as those within 1.5 times the interquartile range from the bottom or top of the boxes. Symbols are extreme points that did not fall within this range.

3. GENERAL DISCUSSION

3.1 SUMMARY OF SENESCENCE IN THIS STUDY

In this study, I found that *Lemna turionifera* exhibited positive senescence, as demonstrated by the convex relationships between the log-proportion surviving and age. Additionally, the probability of reproduction deceased with age. These trends in survival and reproduction occurred in both the offspring of young parents and the offspring of old parents and are consistent with other studies on *Lemna* species (Barks & Laird, 2015; Barks & Laird, 2016; Chmilar & Laird, 2019).

3.2 SUMMARY OF THE LANSING EFFECT IN THIS STUDY

With this study, I intended to observe the Lansing effect in *Lemna turionifera* (Objective 1) as well as analyze demographic patterns that may be generating the effect (Objective 2). In response to Objective 1, the Lansing effect was indeed present in this species, seen as a decrease in lifespan with increasing parental age. Specifically, offspring of older parents, B5, had shorter lifespans on average than offspring of younger parents, B1. In addition to the differences in lifespan, other parental age effects were observed in the study. This included a decrease in the number of offspring produced by older parents, increased size of offspring of older parents, and a change in reproductive performance. The change in reproductive performance was exemplified in the increased rate of reproduction in older parents, which altered the ratio of reproductive rate to parental age, thus resulting in a higher fitness.

Regarding Objective 2, there are two possible, non-mutually exclusive demographic patterns that may generate the Lansing effect (Monaghan et al., 2020). The first potential pattern is greater mortality at every age (i.e., greater baseline mortality) in offspring of older parents (Monaghan et al., 2020); I observed this in *Lemna turionifera*. The second is an accelerated rate of mortality in offspring of older parents but similar baseline mortalities for offspring of all ages (Monaghan et al., 2020); I found some evidence of this latter pattern being exhibited simultaneously with a change in baseline mortality. However, the difference in baseline mortality is shared between my two best—fitting models and, as such, I can conclude with confidence that the Lansing effect in *Lemna turionifera* is the result of an increase in baseline mortality in offspring of older parents.

The observed changes in parental lifespan can be associated with the parameters of the logistic model, which are typically used for populations that undergo demographic senescence, and which can be analyzed to determine which demographic pattern the population is displaying (Pletcher et al., 2000). Here, these parameters were A, B, and C, which represent baseline mortality, mortality rate, and decelerating mortality, respectively (Pletcher et al., 2000). Thus, in *L. turionifera*, analysis of parameters A, B, and C using the best-fit logistic model identified the underlying demographic pattern, which was an increase in baseline mortality associated with offspring of older individuals. Specifically, the best-fit model had distinct baseline mortalities between the B1 and B5 birth orders, but common mortality rates and rates of mortality deceleration.

3.3 SPECULATION OF MECHANISMS

While demographic patterns of mortality help explain the Lansing effect, there are also underlying mechanisms at play. Though my study did not involve the investigation of specific mechanisms, some speculation is warranted. For example, in sexually reproducing species, the quality of germ cells, specifically oocytes remaining after a breeding event, may change with increasing parental age, thus creating a Lansing effect in subsequent reproductive events and among offspring (Monaghan et al., 2020). Specifically, germ cell deterioration with age can alter the survivability and thus lifespan of offspring due to poorer quality gametes and zygotes. For species that reproduce clonally, like duckweeds, other factors such as parental investment or reproductive performance changes in life should be considered as factors that may contribute to the Lansing effect. Parental investment may change with parental age in several ways, including a decrease in parental investment with age due to increasing parental frailty (Monaghan et al., 2020). Alternatively, an increase in parental investment with age due to increased experience with reproduction and rearing offspring (Monaghan et al., 2020), though this is less relevant in this plant species that does not exhibit parental care, may also be observed.

In this study, it was observed that *L. turionifera* showed an increase in fitness associated with offspring of older individuals. One possible explanation for this change in fitness is the change in allocation of resources because of a terminal reproduction strategy, whereby an individual's

reproductive effort will increase as its residual reproductive value (the time-discounted expectation of future reproduction) decreases (Charlesworth & Leon, 1976; Creighton et al., 2009; Williams, 1957). In other words, as an organism ages, resources are spent primarily on the effort of reproduction rather than cell maintenance and growth. When an organism devotes most, if not all, of its resources available to reproduction prior to death, this is known as a terminal reproduction as there are not enough resources available for further, successful reproductive events. This is indicative of a trade-off between current and future reproduction where the benefit in reproducing in the current point in time outweighs reproduction at a later point in time, when probability of survival decreases. This balancing act becomes favourable in natural conditions where life expectancy decreases with age and, therefore, current reproduction is favoured over future reproduction (Heimpel & Rosenheim, 1995). This study, however, did not include analysis of these mechanisms and therefore, cannot ascertain whether these patterns are observed in *Lemna turionifera*.

Additionally, in *L. turionifera*, it was observed that offspring of older individuals were, on average, larger than offspring of younger individuals. It is possible that a larger individual may be able to produce larger offspring with lowered reproductive costs due to a decreased energy expenditure per unit size (Clutton-Brock, 1984) which is to say that larger individuals are simply better equipped to themselves produce larger offspring with lower resource investment. In the case of *Lemna turionifera*, offspring of older individuals were larger with a higher individual fitness, which may then be associated with a lower cost of reproduction, where smaller individuals may need greater energy investments per unit size. Simply put, smaller individuals may need to expend more energy in comparison to larger individuals to achieve the same rates of reproduction or survival.

A change in reproductive effort or output could be associated with the natural physiological deterioration - senescence - that accompanies the aging of an organism (Creighton et al., 2009). In the case of *L. turionifera*, a change in reproductive performance may result in the changes in size, offspring number, and lifespan that were observed in this study. Alternatively, a decrease in

performance can be associated with the constraints of somatic deterioration and overall senescence with increased age (Reid et al., 2003).

A change in investment may also be the result of higher-quality individuals being better suited to survive to advanced ages (Creighton et al., 2009). In asexual populations, advanced-aged individuals may simply be more fit individuals surviving to old age and thus, having more fit offspring (Bock et al., 2019). *Lemna* populations are typically genetically uniform, so this may be due to epigenetic inheritance rather than genetic transmission, in which extrinsic factors alter phenotypes, resulting in phenotypic plasticity in clonal populations (Wong et al., 2005). This is further supported by the uniform environmental conditions provided by the experimental set-up. Because the environment is uniform and the individuals are clonal, the variation in vital rates such as survival and reproduction, as well as offspring quality, must arise from a nongenetic factor such as plasticity or epigenetic inheritance.

Epigenetics is the study of heritable changes in gene function or expression without changes to the DNA sequence itself (Diez et al., 2014). These changes can be brought on by DNA methylation (the addition of a methyl group to DNA (Diez et al., 2014)), or histone modification (changes made to histone proteins that may be a consequence of DNA methylation (Burks & Wesley, 2020; Xu et al., 2021)) among other mechanisms. These changes to DNA, though indirect, may be driving modified gene expression in clonal populations, which may then result in parental age effects being a consequence of epigenetics in senescing species. One such consequence is the shortened lifespan of offspring with age (Pal & Tyler, 2016). Assessing the role of epigenetics and other mechanisms in producing the Lansing effect reported herein should be a priority for future research.

3.4 ASSESSMENT OF LIMITATIONS

While this study provided clear answers to the objectives at hand, limitations were encountered. To begin, the sample size being analyzed was limited due to space constraints in the growth chambers. While the current sample size was more than adequate for Objective 1, fitting survivorship curves – especially in old age classes, when the sample size is small by definition – requires very large

samples. Thus, it would have been preferrable to have a larger sample size to assess the demographic patterns underpinning the Lansing effect more accurately (i.e., Objective 2). This study could not assess concretely the underlying physiological or cellular mechanisms that may contribute to the Lansing effect or other parental age effects. As such, much of the conjectures in section 3.3 pertaining to reproductive investment, output, or DNA changes are simply that – conjectures – and further analysis of physiological senescence pathways should be considered in future work.

The study species used was *Lemna turionifera,* which was ultimately an ideal model system due to its small size, short lifespan, and limited reproductive output, in conjunction with the lack of genetic variance present in single-genotype clones. However, it would be ideal to analyze multiple duckweed strains or species in a comparative approach using similar experimental methods to those used here. This would help to assess the prevalence of the Lansing effect in the genus *Lemna* and would allow an assessment of whether the Lansing effect occurs consistently, as with other components of duckweed senescence (Barks & Laird, 2018).

Finally, a key limitation of this study is common to all lab-based studies, i.e., whether the patterns observed would persist in the species' natural environmental conditions. Thus, in addition to multiple species of a single genus, consideration should be given to analyzing the effects of senescence in natural conditions as opposed to laboratory conditions. Although studying duckweed demography in natural conditions is very challenging (Hayden, 2018), doing so would be rewarding as it would truly assess the effects of parental age on offspring survivorship in an evolutionarily relevant context.

3.5 FUTURE DIRECTIONS

I propose three additional areas of future research. The first is a multi-generational (observing parents, offspring, and grandoffspring) approach in which the Lansing effect is the primary parental age effect being assessed. Multi-generational studies have previously been done in a multitude of

species, and I believe this current study would benefit from inclusion of older generations to assess the persistence and long-term consequences of the Lansing effect.

Secondly, it would be useful to examine additional birth orders (observing offspring from the same parent) above B5. Many of the changes to offspring quality in *Lemna* are more pronounced when examining offspring of even older parents than those examined here (e.g., Barks & Laird, 2015, 2016). As such, I propose a study in which the effects of B7 and B9 birth orders are also examined. I recommend using these birth orders because the likelihood of the parent frond producing many more offspring past this greatly decrease with age. These birth orders are also after B5, which was used in this study and provided an insight into the Lansing effect. It would be beneficial to use birth orders *after* this point to see the persistence of the effect in subsequent birth orders.

A third area of future research should focus on both the demographic patterns associated with the Lansing effect, and the underlying physiological and molecular mechanisms that generate them. To this end, I propose a study that would delve into the more surprising findings of this study, specifically examining the increase in fitness of older parents and the increase in size of offspring of older parents.

3.6 STUDY CONCLUSIONS

To recall, the objectives of this study were (1) to determine if the Lansing effect is present in *Lemna turionifera*, and (2) to determine whether the Lansing effect is the result of an increasing rate of mortality or an increased baseline mortality in offspring of older versus younger parents. My study showed that the Lansing effect is indeed present in *L. turionifera*, and, upon analysis of lifespan data and survivorship curves, it was determined to have been generated by an increase in baseline mortality associated with increasing parental age. In addition to answering the objectives of this study, Offspring of older individuals had reduced lifetime reproductive output, increased offspring size, and increased fitness because of a hastened reproductive effort in a shortened lifespan.

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