

**SENESCENCE IN *LEMNA TURIONIFERA*: THE INFLUENCE OF STRESS ON
FITNESS**

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ABSTRACT

Senescence is a process of physiological deterioration observed in individuals advancing in age which is characterized by decreasing rates of survival and reproduction. Selective pressures are thought to eliminate individuals before geriatric ages can be reached and the intensity of pressure believed to shape patterns of survival in a population. My thesis examines how simple environmental stressors may affect senescence in *Lemna turionifera*, an aquatic macrophyte, and explores the possibility of observing senescence under more realistic circumstances. In these experiments, I observed that survival and reproduction of *L. turionifera* fronds can be influenced by specific stresses, e.g., salinity, and resilient against others such as tissue damage. With respect to realistic observations of senescence, I attempted to construct an apparatus for such an undertaking that succeeded in early observation attempts.

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CHAPTER 1: GENERAL INTRODUCTION

Demographic senescence is characterized by the decrease in the rates of survival or fecundity with increasing age (Partridge and Barton 1993, Kirkwood and Austad 2000). It is a population-level phenomenon that stems from physiological deterioration at the level of the individual. Despite the maladaptive nature of senescence, selective pressure decreases with increasing age, i.e., few individuals in a population will reach advanced age classes where the effects of a beneficial or harmful mutation could be realized, creating an environment favouring success at earlier age classes. Expression of deleterious traits is often observed in individuals who are in or approaching an advanced age class; e.g., declining immune functions in various animals (Shanley et al. 2009), reduced rates of photosynthesis in plants (Munné-Bosch 2007), and the accumulation of harmful proteins in bacteria and yeast (Aguilaniu et al. 2003, Lindner et al. 2008, Coelho et al. 2014, Okada et al. 2017). How senescence has evolved in populations is an important question in evolutionary biology given its widespread occurrence, diversity of organism lifespan, and the interactive effects between an organism and its environment.

EVOLUTIONARY THEORIES FOR SENESCENCE

The observation that survivorship is a declining function of age, because all individuals of a given age must first pass through all younger age classes, led to the inference that the force of natural selection acting on age-specific vital rates (survival and reproduction) should also decline with age (Hamilton 1966). There is almost no selective benefit of a late-life-acting mutation that extends survival if only a small proportion of the population reaches that age-class, reducing the impact of selection for those of advanced age and creating an environment that favours higher fitness at earlier ages. Three major theories

for the evolution of senescence, mutation-accumulation (Medawar 1957), antagonistic pleiotropy (Williams 1957), and disposable soma (Kirkwood and Holliday 1979), base their arguments on this initial premise and create the foundation on which the evolution of senescence in populations can be better understood.

The mutation-accumulation (MA) theory posits that senescence occurs because of the accumulation of mutations with late-acting detrimental effects in the gene pool (Medawar 1957). With few individuals reaching advanced age these mutations are commonly unopposed by natural selection. The overall effect of a detrimental mutation during advanced age is less impactful on fitness than a mutation at an earlier age class (Medawar 1952). Neurodegenerative genetic disorders, such as Huntington's Disease (Rose 1991), are examples of a late-acting detrimental mutation in humans whose expression in past populations is speculated to have been limited as few would reach the onset age of the disease.

Continuing forward, the antagonistic pleiotropy (AP) theory proposes that the accumulation of temporally pleiotropic genes, specifically genes that are beneficial at a young age that become harmful in advancing age, are the cause of senescence (Williams 1957). A selective premium on the success of early-age classes exists if the force of natural selection declines with increasing age, i.e., survival to a reproductive phase is more beneficial for a population's growth than survival past the reproductive phase. In the wild there is no guarantee that an individual will reach an advanced age unless placed in an artificial environment (Comfort 1956) and an increase in early-age fitness is selected for, as the intensity of selection is greater in juveniles than geriatrics (Hamilton 1966, Partridge and Barton 1993). Therefore, antagonistically pleiotropic mutations that provide

a benefit at early ages may be selected for even at the cost of increased harm at old age. Another perspective on pleiotropy, positive pleiotropy/modified mutation accumulation hypothesis, is that natural selection may not select against minor deleterious alleles i.e., random mutations can have pleiotropic effects on all age classes and increase in effect with age (Houle et al. 1994, Maklakov et al. 2015).

The nature of pleiotropic genes is further examined in the disposable soma theory, the third and most recent of three major theories for the evolution of senescence. Disposable soma theory (DS) (Kirkwood 1977, Kirkwood and Holliday 1979) asserts that the cause of senescence is a result of differential allocation of finite resources between competing activities, e.g., reproduction, growth, and somatic repair. Given limited resources, investment in long-term gene survival, i.e., the germ-line and reproduction, would be favoured over somatic maintenance (Kirkwood and Rose 1991). In the DS theory, an antagonistic pleiotropic gene can be beneficial if it reduces investment in somatic activities in favour of reproduction. Differential allocation of resources could allow for decreased energy needs in somatic tissues and/or accelerated reproduction, with the negative being increased somatic deterioration, e.g., increased replication error (Kirkwood and Holliday 1979). Evidence for antagonistically pleiotropic genes with age-specific effects on fitness are mixed with relevant studies limited to *Drosophila* (Rose 1984, Leroi et al. 2005, Giaimo and d'Adda di Fagagna 2012, Khazaeli and Curtsinger 2013, Everman and Morgan 2018). Experimental and observational suggest that there are associated fitness costs with early reproduction on long-term survival and fecundity (Kirkwood and Westendorp 1998, Charmantier et al. 2006, Penn and Smith 2007). These

observed trade-offs support AP/DS theories at the physiological level and investigations continue to explore the underlying genetic mechanisms of senescence.

SENESCENCE IN PLANTS

Whole-plant senescence is defined as a total loss of homeostasis, where multiple systems deteriorate resulting in an intrinsic mortality event (Noodén and Leopold 1988). It is distinguished from the other use of the word ‘senescence’ in plants, e.g., abscission of leaves before winter, or specified differentiation as seen with xylem tissue. Some have posited that certain plants are effectively non-senescent because of indeterminate (i.e. continual) growth and regeneration via totipotent apical meristems (Silvertown et al. 2001, Roach 2004). Short-lived plants, such as annuals, have life histories that are more constrained and are typically dictated by seasonal changes and reproductive events.

Difficulties can present themselves when investigating demographic senescence in plants, primarily due to their lifespans and reproductive strategies. The longevity displayed in certain plants (Noodén and Leopold 1988), e.g., perennial angiosperms, can make tracking an individual from birth-to-death prohibitively time consuming, though the use of population projection matrices allows for inferences in these types of species (Silvertown et al. 2001, Baudisch et al. 2013). Asexual and sexual reproductive strategies in plants can raise questions as to which unit that natural selection is acting on (Buss 1983, Tuomi and Vuorisalo 1989). In clonal reproduction, selection could occur at the level of individual clones (ramets), a group of ramets from the same zygote (genets), or at both levels simultaneously (Pedersen 1995). Even with these complications surrounding studies into demographic senescence in plants, the range of life histories and unique

adaptations found in the Kingdom Plantae can provide ample material for examining the evolutionary processes of senescence.

ENVIRONMENTAL STRESS AND SENESCENCE

Heterogeneous environments, in space and through time, create the complex systems that influence the survival and reproduction of individuals (Wade and Kalisz 1990, Stearns 1992). The overall impact of selective pressure in an environment is thought to shape the patterns of aging in species, e.g., high-levels of extrinsic mortality result in rapid rates of senescence (Williams and Day 2003, Williams et al. 2006). The reliability theory of senescence (Gavrilov and Gavrilova 2001, Laird and Sherratt 2010) suggests that investment in cellular repair is finite, i.e., repair allowing for extreme longevity has little benefit on overall fitness. Redundant copies of genes and gene products act as a buffer against the effects of extrinsic damage/intrinsic failure, e.g., ultra-violet radiation, reactive oxygen species, replication error (mutations). The interactions that influence the survival and reproduction of an organism are intricate, with interspecific interactions and species-environment relations representing two sources of extrinsic stress shaping senescence.

Interactions between species in nature are multifaceted, i.e., mutualistic, predator/prey, competitive, etc., and create selective pressures on the individual (Thompson 1999, Morris et al. 2007, Urban 2011). Ecological function and community structure can be influenced by species abundance, scarcity, and introductions (Levin et al. 2006, Anderson and Rosemond 2007, Weidenhamer and Callaway 2010). Herbivory is a common event for almost all plants, which has led to adaptations to ensure their survival and reproduction (Maron and Crone 2006, DeGabriel et al. 2009, Agrawal et al. 2012,

Züst and Agrawal 2017). Manipulation of plant parts in the laboratory has demonstrated that the removal of vegetative-sink and reproductive tissue has the potential to rejuvenate or retard senescent plant tissue (Leopold et al. 1959, Noodén and Leopold 1988, Zavaleta-Mancera et al. 1999). Herbivory experiments have shown that prevention of insect grazing of certain tissues may increase fecundity (Maron 1998, Andersson et al. 2016), and that consumption can have mixed results, e.g., early feeding inducing a response that deters herbivores, increasing long-term survival but decreasing reproductive output (McArt et al. 2013). These experiments demonstrate that targeted damage can influence the overall fitness of plants with a definite lifespan and the potential cost of repair/maintenance can be negligible depending on the tissues damaged.

Species presence or absence can be determined by the availability of nutrients in an environment, light availability, and temperature gradient (Venterink et al. 2003, Shirima et al. 2016). Salt content of terrestrial and aquatic ecosystems is an abiotic factor in many environments that influences the variety of species present (e.g. marine vs freshwater), and increases in the level of salinity in sensitive environments pose a threat to those unable to acclimate (Sarma et al. 2006, Boetius and Joye 2009, Smith et al. 2009, Larson and Belovsky 2013). Plants are excellent candidates for studying the effects of salts and other natural and introduced chemical agents on fitness. Their sedentary growth forms, potential for bioremediation, and their importance as primary producers, both ecologically and economically make them viable specimens for research (Roach 1993, Costa-Pierce 1998, Salguero-Gómez et al. 2013, Adhikari et al. 2015). Many plant species have the potential to exhibit a lack of senescence via indeterminate growth of apical meristematic tissue or preservation of the genetic lineage in clonal species

(Silvertown et al. 2001, Roach 2004), i.e. the genet survives though individual ramets expire; however, if environmental conditions become unfavourable for growth, then species viability, range, and ability to compete can be reduced as a result.

The selective pressure present in an environment is a result of multiple factors acting in unison; the individual can be accosted by several stressors at one time. This accumulative effect is thought to increase extrinsic mortality within the population, decreasing the number of individuals reaching advanced age (Williams et al. 2006). Observations of senescence have been made in natural environments (Kawasaki et al. 2008, Sherratt et al. 2010), but difficulties can occur in tracking and monitoring individuals. Laboratory experiments create environments that allow for easy monitoring and controlled manipulation of variables. Individuals grown under such conditions, if allowed to complete their lifecycle, will often succumb to an intrinsic mortality event, i.e., internal failure. Internal deterioration is a cellular process that results in the failure of multiple systems, with increases in replication error, telomere attrition, and increased levels of reactive oxygen species (Monaghan et al. 2008). The interaction between the internal and external environments, i.e., cellular processes and environmental stress, is likely what shapes patterns of aging. We can manipulate the environmental conditions of a laboratory to better understand how specific stressors shape senescence though our interference separates the individual from the dynamic natural environment. Plants make suitable candidates for studying senescence under laboratory conditions and the versatility they display in variable environments allows the opportunity for field studies observing aging in a natural setting.

STUDY SPECIES

Lemna turionifera (Araceae) is a small aquatic plant from the subfamily Lemnoideae (the duckweeds). The simplified thallus of *L. turionifera* consists of a single frond and root and it floats on or just below the surface level of still water (Hillman 1961, Landolt 1986). *Lemna turionifera* reproduction is primarily asexual; offspring fronds are produced from two meristematic pockets located proximally on the parent frond and are released alternately over the lifespan of the frond. Fronds are also able to produce turions, small, rootless, starch-heavy fronds that overwinter and re-emerge when conditions favour growth (Landolt 1986). Investigations into senescence in *Lemna* species have shown that decreases in survival and fecundity with age are indeed observable and tend to be characterized by the decreasing size of offspring fronds in aging parents (Ashby and Wangermann 1951, Barks and Laird 2015, Ankutowicz and Laird 2018). *Lemna turionifera* is a good plant species for studying aging because of its small size (~1-5mm²), reproductive output (~5-15 offspring), distribution (temperate), and relatively short lifespan for an angiosperm (~1 month) (Barks 2015).

OBJECTIVES AND THESIS OVERVIEW

My thesis has two primary objectives. The first (Chapter 2) is to test for the potential costs of physical damage (simulated herbivory) in *Lemna turionifera* and determine if the physiological impairment decreases overall fitness. The second objective of my thesis (Chapter 3) is to expose *L. turionifera* to variable nutrient concentrations and a high-salt stress and test for significant differences in survival and fecundity. Included in my thesis is a supplementary chapter outlining possible methods for observing *L. turionifera* senescence in a (semi-) natural setting.

Objective 1: Simulated herbivory effects on senescence

Insect herbivory is a common interaction for many plants which has resulted in adaptations for both species. Plants are often quite tolerant of damaging events and may be able to regrow using intact meristematic tissue and coordinating specific growth via hormone controls. The influence exerted by herbivory on senescence can be negligible in many instances, but tolerances to damage may have thresholds that once crossed have significant consequences for growth and development.

At present, plant species are experiencing shifts in the level of herbivory with introduced insect species proliferating in new environments (Peccoud et al. 2008, Martorana et al. 2017) and decreasing insect-mortality with increasing winter/summer temperatures (Andrea et al. 2005, Robinet and Roques 2010, Safranyik et al. 2012, Kiritani 2013, Lemoine et al. 2017, McAvoy et al. 2017). The degree of herbivory present in an environment may significantly affect ecological landscapes with increases posing a potential threat to plants, e.g., gregarious phase locusts, and decreases allowing for increased competition between plant species. Herbivory is a common stressor in environments and how variation in herbivory affects senescence is worth investigation as ecosystems continue to evolve in human-influenced spaces.

In this experiment (Chapter 2), I performed an experimental manipulation on *Lemna turionifera* to test if a simulated herbivory event accelerated senescence and decreased overall fitness, i.e., survival and reproduction. How demographic senescence in short-lived plants is affected by damaging stresses may increase accuracy for future population projections in ecosystems with increasing herbivory and provide informative data pertaining to the potential fitness costs of physical damage in plants.

Objective 2: Nutrient/salinity variation effects on senescence

The sedentary nature of plants, e.g. *Lemna* species growing in still or slow-moving water, confines them in a specific location indefinitely. The abiotic materials present in the retaining medium, i.e., macronutrients and micronutrients, are essential for the development of functional living tissue. Field samples collected from sites around Alberta, Canada, were shown to have relatively low nutrient concentrations when compared to suggested laboratory levels (Barks 2015). Development under nutrient poor conditions likely decreases the overall fitness of the wild plant when compared to a typical, high-nutrient laboratory specimen. The effects of nutrient variation on senescence in plants can be examined in a controlled environment which is useful for determining the impact of potential change in dynamic environments.

Prolonged exposure to toxic substances and nutrient deficiencies/excesses can cause substantial shifts in the health and composition of plant communities. The survival of short-lived plants declines rapidly in response to deteriorating conditions and long-lived plants may persist, but their survival and reproduction can be jeopardized by the continuous impairment (Colling and Matthies 2006, Oliva et al. 2014).

In this experiment (Chapter 3), I cultivated *Lemna turionifera* (Landolt 1986) under six nutrient concentrations, with or without a supplemental salt (sodium chloride), to test for differences in senescence and overall fitness for fronds grown in varied abiotic circumstances. How demographic senescence in short-lived plants is affected by abiotic circumstances, e.g., nutrient availability and salinity, may uncover positives/negatives for population growth potential and increase accuracy of future population projections in disturbed, deteriorating, and recovering ecosystems.

Supplementary: Semi-natural observation study

Studies into senescence in the wild have been few but have shown that it is possible to track individuals over the course of their life in a natural setting (Kawasaki et al. 2008, Sherratt et al. 2010). Senescence in wild plants has been recorded with wild variants having lower survival than their greenhouse counterparts (Roach 2004). *Lemna turionifera* and other *Lemna* species make excellent candidates for studying senescence in wild plants given their short lifespans and global distribution. By observing aging under natural conditions, we adopt a more realistic approach than that of the laboratory. How senescence presents itself in these conditions may allow for a better understanding of its evolution and provide validation for popular theories.

In this supplementary chapter, I outline methods for creating a system for observing *Lemna turionifera*, and other aquatic macrophytes, in a natural setting. Results from field tests are included with recommended modification.

CHAPTER 2: DAMAGING STRESS EFFECTS ON SURVIVAL AND REPRODUCTION IN LEMNA TURIONIFERA

Abstract

Senescence is a process of physiological deterioration occurring in a wide spectrum of species and is characterized by decreases in survival and fecundity of organisms as they advance in age. Within the Kingdom Plantae there exists large variation in observed lifespans with some plants possibly being non-senescent due to the indeterminate growth of apical meristems. However, many plants do exhibit senescence, and the phenomenon may be prevalent in species with determinate growth. Trophic interactions may play a significant role in the evolution of senescence in plants communities. Plants are often tolerant of herbivory; utilizing intact meristematic tissue to regenerate lost tissue. Herbivory pressure has the potential to influence senescence in plants if the damage causes an imbalance in resource allocation between somatic repair and reproduction. To test for the effects of gross damage on senescence in plants I conducted an experiment simulating herbivory by applying a mechanically induced excision to an aquatic macrophyte, *Lemna turionifera*. Focal specimens were selected after reaching the desired birth-order ($n = 112$ in each of the excision and control treatments; initial total $n = 224$, final total $n = 209$), placed in a growth chamber with a 15:9 photoperiod at 25 °C, and given fresh growth medium every four days. Measures used to test for differences between control and excision samples included total lifespan, total offspring produced, daily offspring production, and the intrinsic rate of increase (I_r). Two-sample t-tests revealed no significant differences in selected fitness measures between treatment groups. Continued investigations into the effects of injury on plant fitness could take into

consideration possible intergenerational effects, i.e., the consequences of parental damage on offspring fitness and size.

Introduction

Decreases in survival and reproduction that accompany aging in a wide variety of taxa, is a common phenomenon defined as senescence (Partridge and Barton 1993, Kirkwood and Austad 2000). It is a population-level phenomenon that stems from physiological deterioration at the level of the individual. Despite the apparent maladaptive nature of senescence, selective pressure decreases with increasing age; i.e., few individuals in a population will reach advanced age where the effects of a beneficial or harmful mutation could be realized, creating an environment favouring success at earlier age classes (Medawar 1952, Williams 1957). Senescence represents an interesting challenge and opportunity for evolutionary biology given its widespread occurrence, diversity of organism lifespan, and the ecological interactions that shape patterns of aging (Kirkwood et al. 2000, Williams and Day 2003).

Interactions between species in nature are multifaceted, i.e., mutualistic, predator/prey, competitive, etc., and create selective pressures on the individual (Thompson 1999, Morris et al. 2007, Urban 2011). Herbivory is a common event for almost all plant species which has led to adaptations to ensure their survival and reproduction (Maron and Crone 2006, DeGabriel et al. 2009, Agrawal et al. 2012, Züst and Agrawal 2017). Manipulation of plant parts in the laboratory has demonstrated that the removal of vegetative-sink and reproductive tissue has the potential to rejuvenate or retard senescent plant tissue (Leopold et al. 1959, Noodén and Leopold 1988, Zavaleta-Mancera et al. 1999). Herbivory experiments have shown that prevention of insect

herbivory on certain tissues may increase fecundity (Maron 1998, Andersson et al. 2016), and that consumption can have mixed results, e.g., early feeding inducing a response that deters herbivores, increasing long-term survival but decreasing reproductive output (McArt et al. 2013). These experiments demonstrate that targeted damage can influence the overall fitness of plants with a definite lifespan and the potential cost of repair/maintenance can be negligible or severe depending on the tissue damaged and the extent of the damage. Herbivory, as it occurs in the wild, can have several detrimental outcomes, the entire plant itself can be consumed (extrinsic mortality event), the plant can experience decreased productivity with loss of photosynthetic tissue, and damage can leave the plant more susceptible to pathogens (Anten et al. 2003, Fischer et al. 2012, Rob et al. 2016).

At present, plant species are experiencing shifts in herbivory with introduced insect species proliferating in new environments (Peccoud et al. 2008, Martorana et al. 2017). There may also be decreasing insect-mortality with increasing winter/summer temperatures (Andrea et al. 2005, Robinet and Roques 2010, Safranyik et al. 2012, Kiritani 2013, Lemoine et al. 2017, McAvoy et al. 2017). The degree of herbivory present in an environment may significantly affect plant population and community dynamics with increased herbivory posing a potential threat to plant health (e.g., locusts in the gregarious phase), and decreased herbivory allowing for increased competition between plant species. Understanding how demographic senescence in short-lived plants is affected by damaging stresses may increase accuracy of future population projections in ecosystems with increasing herbivory and provide data pertaining to the potential fitness costs of physical damage in plants.

In this experiment, I performed an experimental manipulation on *Lemna turionifera*, a small, short-lived aquatic macrophyte, to test if a simulated herbivory event decreased overall fitness, i.e., lifespan and reproductive output. *Lemna* species are important primary producers in aquatic ecosystems; as such they are exposed to several types of trophic interactions with multiple species, such as waterfowl, fish, and arthropods (Scotland 1940, Landolt 1986). Species within the Lemnoideae subfamily are well-suited for testing senescence as studies have reported decreases in survivorship and reproduction with age (Barks and Laird 2015). Past studies have also demonstrated that *Lemna* species can continue to grow and reproduce after having significant portions of the frond and root removed (Ashby and Wangermann 1951). To test for differences in overall fitness (survivorship and reproduction) between treatment groups, it was necessary to observe fronds over their entire life and maintain a record of offspring production, total offspring, and total lifespan. The caveat to these fitness measures is that they do not capture the premium on early reproduction (i.e., in terms of population growth rate). A composite measure of fitness that incorporates the timing of reproduction with survivorship and fecundity, the intrinsic rate of increase (I_r), is a demographic approach that may be a better approximation of overall fitness (Stearns 1989, Partridge and Barton 1996). I hypothesize that by decreasing the total frond surface area in a simulated herbivory event, that damaged *L. turionifera* fronds will produce fewer offspring and live for less time than their control counterparts. If a significant area of photosynthetic tissue is removed, then the total reproductive output in individual fronds may be reduced, although tolerance to damage in aquatic macrophytes can be robust (Ashby and Wangermann 1951).

Materials and methods

STUDY SPECIES

The focal species for this experiment is *Lemna turionifera* (Araceae), a small aquatic plant from the subfamily Lemnoideae (the duckweeds) (Landolt 1986). The simplified thallus of *L. turionifera* consists of a single frond and root and it floats on or just below the surface level of still water (Hillman 1961). *Lemna turionifera* reproduction is primarily asexual; offspring fronds are produced from two meristematic pockets located proximally on the parent frond, and are released alternately over the lifespan of the frond (Hillman 1961, Landolt 1986). Fronds are also able to produce turions, small, rootless, starch-heavy fronds that overwinter and re-emerge when conditions favour growth. Investigations into senescence in *Lemna* species have shown that decreases in survival and fecundity with age are indeed observable and tend to be characterized by the decreasing size of offspring fronds in aging parents (Ashby and Wangermann 1951, Barks and Laird 2015, Ankutowicz and Laird 2018). *Lemna turionifera* is a suitable plant species for studying aging because of its size ($\sim 1\text{-}5\text{mm}^2$), reproductive output ($\sim 5\text{-}15$ offspring), distribution (temperate), and relatively short lifespan for an angiosperm (~ 1 month) (Barks 2015).

FITNESS MEASURES

To compare differences between treatments, several fitness measures were selected, i.e., latency to first reproduction, total lifespan, total number of offspring, and the intrinsic rate of increase. Lifespan and number of offspring are general fitness measures for survival and reproduction. The intrinsic rate of increase (I_r) is a composite fitness measure that

incorporates lifespan, offspring production, and timing of reproduction into a single demographic I_r -value; high I_r -values indicate a greater population growth potential than low I_r -values. Latency to first reproduction is the interval from focal frond detachment to the release of its first offspring frond; earlier reproduction is advantageous for population growth; therefore, shorter latency to first reproduction leads to increased fitness. Latency and the intrinsic rate of increase were later removed as a fitness measure in this study because the excision technique would sometimes damage the meristematic pocket of the parental focal frond inducing pre-mature release of an offspring frond.

PLANTS AND GROWTH CONDITIONS

The plants used in this study were derived from a clonal lineage that had been previously collected by Dr. Patrick Barks from Young's Point, Alberta, Canada (55 14° N, 117 57° W). *Lemna turionifera* was initially grown in stock cultures by aseptically transferring individual fronds from past single-frond cultures into autoclaved 125 mL Erlenmeyer flasks filled with 36 mL of half-strength Schenk-Hildebrandt (Sigma-Aldrich S6765, St. Louis, MO, USA) growth medium. Schenk-Hildebrandt (S-H) is a growth medium containing macro- and micronutrients (at half-strength (1.6 g L⁻¹); KNO₃ 1250 mg L⁻¹, (NH₄)H₂PO₄ 150 mg L⁻¹) required for plant growth. Added to the medium are a suite of compounds (sucrose 6.7 g L⁻¹, yeast extract 0.07 g L⁻¹, tryptone 0.3 g L⁻¹) to assist in detecting bacterial and fungal contamination.

The samples I studied were genetically homogenous; heterogeneity (both genetic and environmental) has the potential to mask true patterns of senescence (Zens and Peart 2003). Considering the possibility of parental age effects in *L. turionifera*, the 224 focal fronds (112 per treatment) were each first offspring of first offspring going back at least

three generations (Ashby and Wangermann 1951, Barks and Laird 2016). Progenitor plants were aseptically cultured in 60 x 10 mm Petri dishes containing 10 mL of half-strength S-H growth medium and kept inside a growth chamber set to 25 C with a 15:9 photoperiod (Environment Canada 2007). Placement of focal fronds in the growth chamber was determined using randomization.

To ensure environmental constancy we aseptically transferred each plant into a new Petri dish with 10 mL of fresh growth medium every four days. Once the desired birth-order was achieved, focal fronds marked for excision were treated, transferred to fresh medium, and observed for the duration of their life. Fronds were transferred to fresh medium and a new Petri-dish in the event of contamination (e.g. bacterial or fungal) and discarded if remediation attempts failed to mitigate the infection. Fifteen fronds were discarded in total (eight Control and seven Excision).

EXCISION TECHNIQUE

To simulate herbivore activity on *L. turionifera* fronds, a mechanical excision was applied at the widest point perpendicular to the long axis (**Figure 2-1**). The part of the frond containing the meristematic pockets was retained and the excised part was discarded. Roots were left intact.

Maintaining an aseptic environment for this technique was accomplished using a flow hood to maintain positive pressure in the workspace, sanitizing the area and gloves with 70% EtOH, and sterilizing contact equipment—loop ring, scissors, ink applicator—in an infrared BactiZapper™ (Benchmark Scientific, Edison, NJ 08818). After the necessary birth order was achieved, i.e., first offspring of a first offspring up to three generations, fronds selected for the excision treatment were aseptically removed from

their Petri dish, cut with sterilized scissors at the desired location, and returned to the Petri dish. Treated fronds were then placed in a growth chamber, monitored for infection, and allowed to grow for the duration of their life. Control fronds, those not selected for excision, were left intact and monitored under identical conditions.

DATA ANALYSIS

Statistical analysis of the data was done using two-sample t-tests (Control vs. Excision) to test for any significant differences in total number of offspring, and total lifespan (measured in days starting from the day a focal frond detached from its parent and ending when the last offspring detaches from the focal frond; inclusive). All analyses were conducted in R v. 3.4.3 (R Core Team 2017)

Results

SURVIVAL WITH AGE AND LIEFSPAN

Survival curves for excision and control treatments are given in **Figure 2-2**. There were no significant differences in total lifespan between treatments (**Figure 2-3**; Two Sample t-test, $t = 1.19$, $df = 207$, $p = 0.24$). The mean lifespan for control focal fronds was 24 days and excised fronds 23 days. Both treatments began experiencing declining survivorship 15 days after focal frond selection. Survival of focal fronds did not exceed 40 days.

REPRODUCTION WITH AGE

Number of fronds reproducing with age are given in **Figure 2-4**. There were no significant differences in total number of offspring produced between treatments (**Figure 2-5**; Two Sample t-test, $t = 1.86$, $df = 207$, $p = 0.065$). Focal fronds began reproducing within 5 days of being released from parental fronds and reproduction in both groups

started to decline after 15 days. Mean total offspring for control focal fronds was 11 offspring and excised fronds 10 offspring.

Discussion

EFFECT OF EXCISION ON FITNESS IN *L. TURIONIFERA*

All treated focal fronds survived the initial excision. Release of offspring fronds was observed on occasion, though whether this was an induced abscission or caused by meristematic pocket stimulation is unknown. Induced abscission has been observed as a response to abiotic stress in aquatic macrophytes (Henke et al. 2011). The simplified structure of aquatic macrophytes provides little space for the separation of parts (Landolt 1986 pp. 20-26) and the excision may have overlapped with basal tissues stimulating an abscission event.

In this experiment there were no statistically significant differences between the fronds treated with an excision and the control group. The p-values associated with lifespan and total number of offspring were relatively low which could suggest that there is an associated cost of physical damage. How the damage is applied, e.g., an excision vs. pin pricks, or increasing the degree of damage could have a more significant impact on the population. The focus of this experiment was on parental frond survival and reproduction which excluded potential shifts in the quality of offspring being produced.

Intergenerational effects may have occurred with the loss of photosynthetic tissue in parental fronds resulting altered fitness in future generations. Total frond surface area has been used as a measure of offspring quality, e.g. first offspring fronds are larger than fronds produced later, and differences in the size of offspring fronds could indicate an

unaccounted-for fitness cost associated with damage in parental fronds. Evidence suggests that parental effects on offspring frond fitness is limited within a few generations after which a process of rejuvenation occurs (Hillman 1961, Barks and Laird 2016). Avoiding parental-age effects by exploiting this rejuvenation is what necessitated using multi-generation first offspring as focal fronds in this experiment. Interestingly, Ashby and Wangermann (1951) demonstrated early-life offspring fronds that were pre-maturely removed had similar growth patterns to late-life offspring fronds that were naturally released. An intergenerational damage experiment could be conducted with control and excised focal fronds with an additional factor (pre-mature removal of offspring fronds) resulting in four total treatment groups (with, say, 15 fronds in each group). The first, third, and fifth offspring of these focal fronds would then be selected (180 fronds total) and observed to discern differences in fitness. To test for differences in the recovery period, the surface area of the initial frond treatment groups can be compared to the first, third, and fifth first-offspring's selected generation. The combination of damaging stress may result in an observable lag in recovery of frond health and allow for a better understanding of how injury affects future generations.

The excision administered on focal fronds sought to replicate a single damaging herbivory event but in nature herbivory is more likely a constant pressure than a one-off event. Aquatic macrophytes are targeted by multiple species across taxa (Landolt 1986) and some invertebrates have adapted to use *Lemna* species as a primary resource, e.g., *Lemnaphila scotlandae* (duckweed miner fly) and *Tanysphyrus lemnae* (duckweed weevil) (Scotland 1934). Herbivory in a natural setting also creates opportunities for foreign agents, e.g. bacteria, viruses, fungi, etc., to interact with the individual which are

absent under laboratory conditions. Dutch elm disease and blue stain fungi are examples of fungal agents that can be introduced via beetle herbivory. A controlled herbivory experiment could be conducted with *Lemna turionifera* and a selected species of insect to observe how constant herbivore pressure effects senescence. The multifaceted aspect of environmental interactions could mean that the effect of a single variable is negligible but when combined with other stressors, it may have a significant effect.

Physical injury is one aspect of environmental stress and it is possible that damage in combination with an abiotic stress, e.g., limited nutrient availability, could result significant shift the fitness in comparison with control fronds grown in similar conditions. Field samples collected from Alberta, Canada (Barks 2015) suggest that nutrient availability is much lower in a natural setting, compared to this study, and could be easily reproduced under laboratory conditions. Chapter 3 of my thesis examines the effect of nutrient variation and high-salt stress on fitness in *L. turionifera*, where observable differences occurred between treatment groups. Further investigations into the effects of combined stressors on demographic senescence may replicate real world conditions more accurately.

CONCLUSION

The versatility of the aquatic macrophyte *Lemna turionifera* as a model system was observed in this experiment. After a substantial portion of tissue was removed the fronds continued to reproduce and continue living with no observed adverse effects.

Experimental manipulation of plant parts has shown that damage can be negligible to plant health (Noodén and Leopold 1988) but these plants are often more compartmentalized, i.e. distinct root, shoot, and leaf systems, than the simple *Lemna*

frond. A single damaging event did not inhibit survival or reproductive output in a significant way in *L. turionifera*, though it is possible that cumulative herbivory events will have more impact. Continued investigations into how plants and their offspring react to damaging stresses could incorporate multiple feeding events, live herbivores, and varied nutrient concentrations.

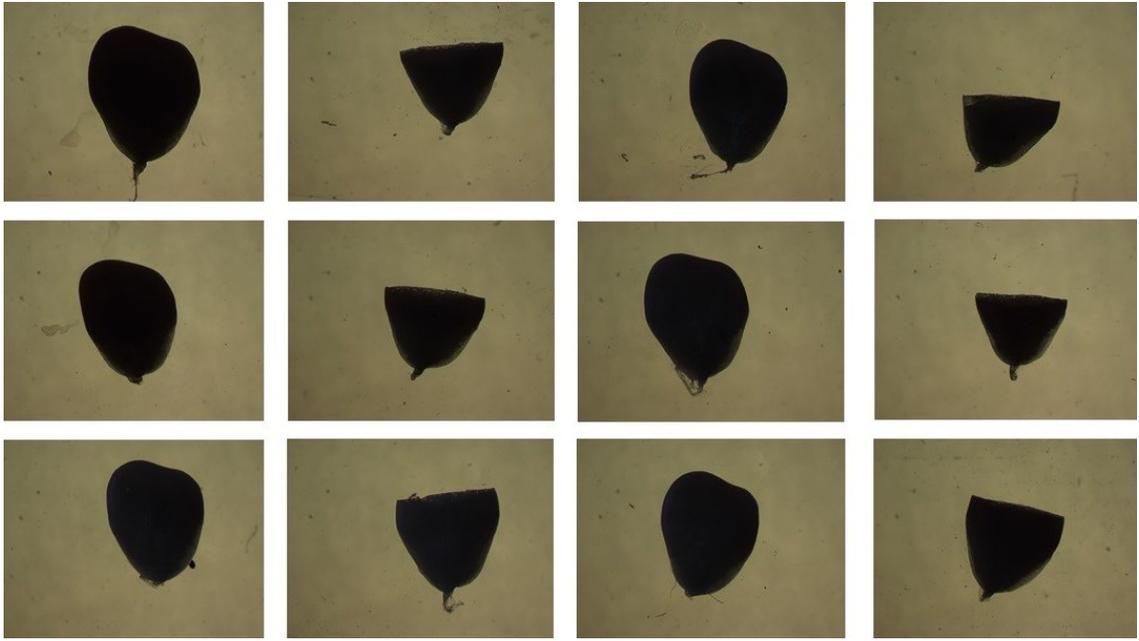


Figure 2-1. Comparison of control fronds (first and third columns) and excised treatment fronds (second and fourth columns).

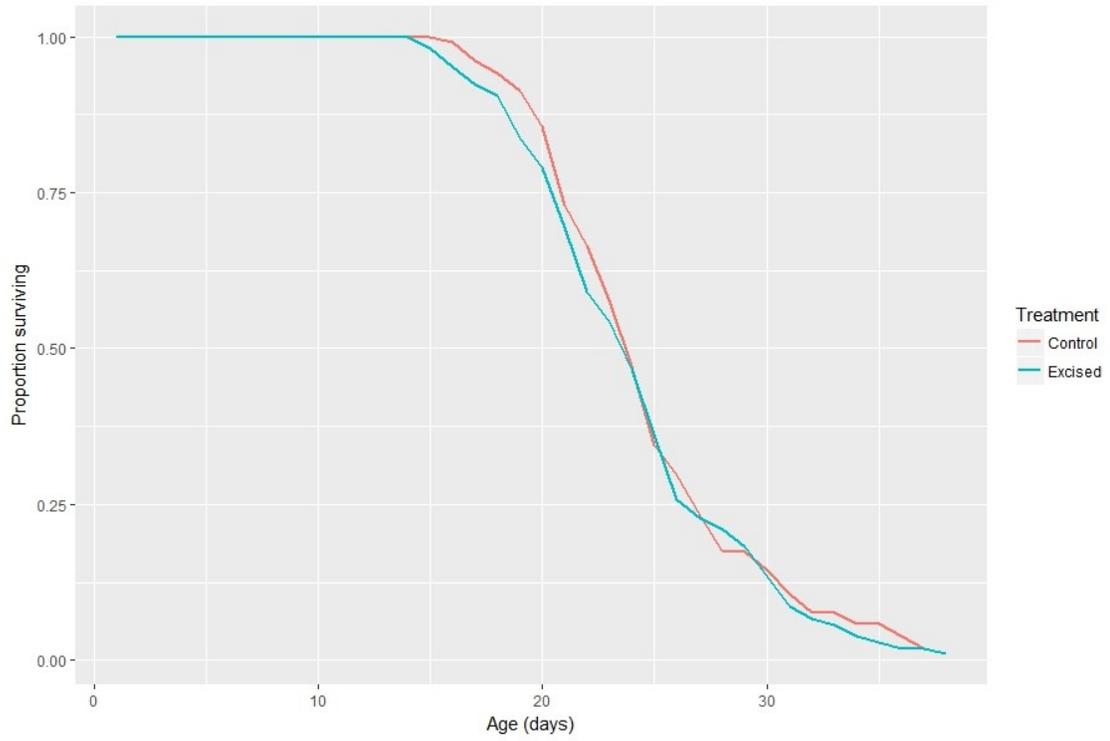


Figure 2-2. Proportion of fronds surviving with age in control and excised treatments.

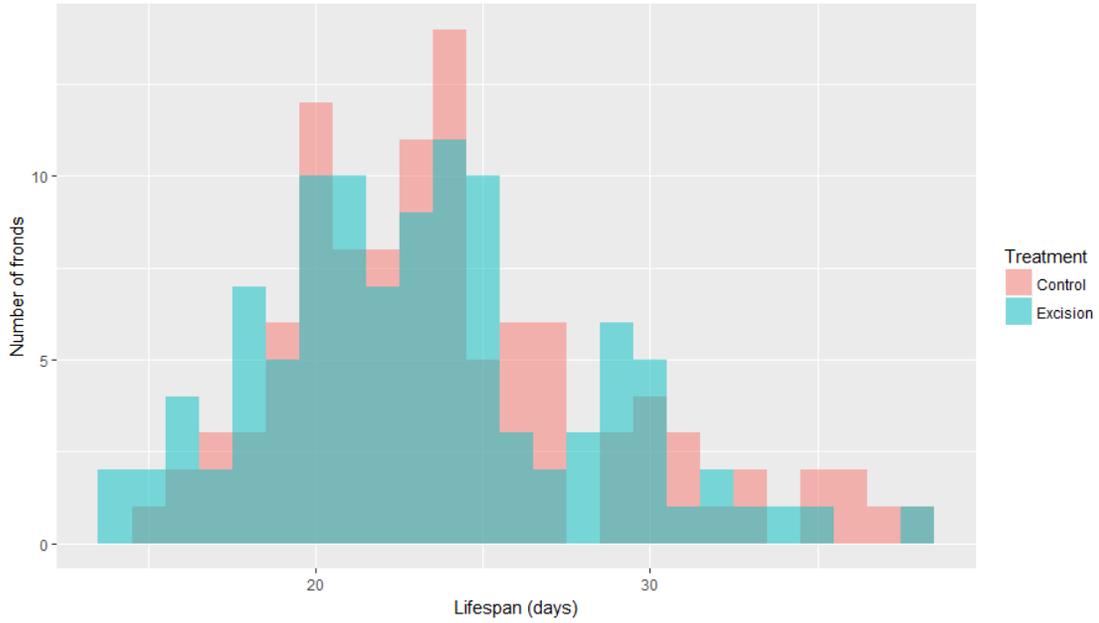


Figure 2-3. Distribution of frond lifespan (measured in days starting from day detached and ending when the last offspring detaches; inclusive) in control and excised treatments. Control mean \pm SEM = 24 days \pm 0.48, Excised mean \pm SEM = 23 days \pm 0.49.

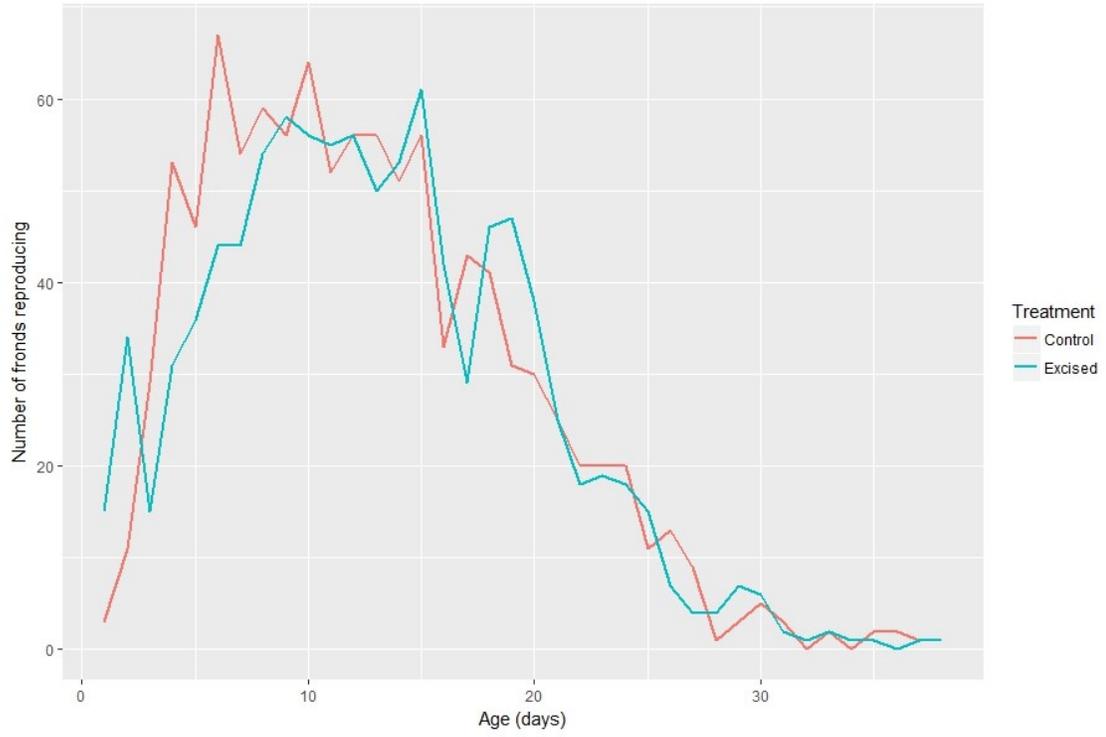


Figure 2-4. Number of fronds reproducing with age in control and excised treatment groups.

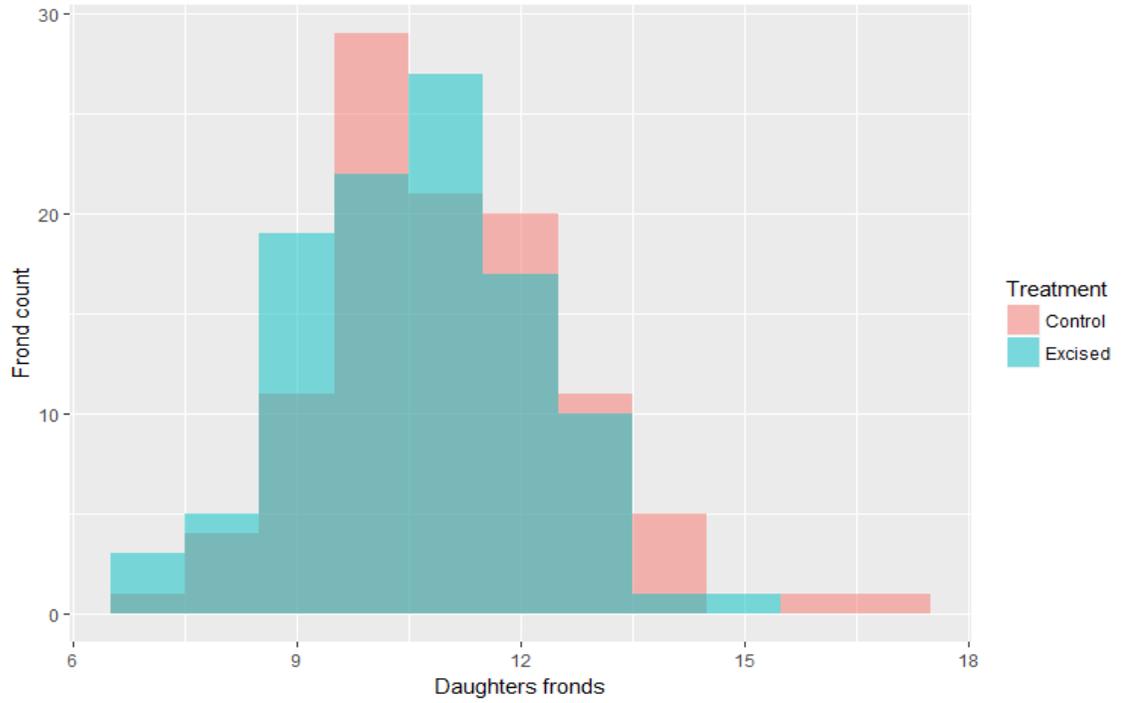


Figure 2-5. Distribution of total offspring fronds by treatment. Control mean \pm SEM = 11 ± 0.17 , Excised mean \pm SEM = 10 ± 0.15 .

**CHAPTER 3: EFFECTS OF NUTRIENT VARIATION AND HIGH SALINITY
ENVIRONMENTS ON SURVIVAL AND REPRODUCTION IN LEMNA
TURIONIFERA**

Abstract

The physiological deterioration observed in almost all life as it advances in age is known as senescence, a process characterized by decreases in survival and fecundity. Plant species display a wide-variety of life histories with some species appearing almost non-senescent due to the indeterminate growth of apical meristems. However, senescence can be detected in plants with determinate growth such as monocarpic plants and other short-lived species. The sessile mode of plants exposes them to the natural and artificial fluctuations in their abiotic environment, which can influence their senescence. To test for the effects of nutrient variation and high salt-stress on senescence in plants, I grew *Lemna turionifera* (an aquatic macrophyte) under six different nutrient levels, with or without a supplemental salt (sodium chloride). Focal specimens were selected after reaching the desired birth-order (18 fronds in each treatment x 12 treatments, 216 fronds total), placed in a growth chamber with a 15:9 photoperiod at 25 °C, and given fresh treatment medium every four days. The supplemental salt-stress, NaCl at 5 g L⁻¹, was detrimental to frond development below certain nutrient concentrations resulting in early mortality for many specimens in these treatments which resulted in heteroscedasticity issues between data sets. Measures to test for differences between treatment groups included; total lifespan, total offspring produced, and daily offspring production. A two-way fixed effect ANOVA revealed a significant interaction between nutrient variation and the salt-stress. Growth of focal fronds in a saline environment was conditional on the medium having a relatively

high nutrient concentration. Further investigation into the influence that environmental factors can have on senescence could uncover significant thresholds and tolerances for the potential growth of a population under similar conditions.

Introduction

Senescence is characterized by the decrease in survivorship and reproduction that accompanies aging (Partridge and Barton 1993, Kirkwood and Austad 2000). It is a population-level phenomenon that stems from physiological deterioration at the level of the individual. Few in a population will likely reach an age where senescence might be realized as external pressure and internal failure removes maturing/mature individuals (Medawar 1952, Williams 1957). The evolution of senescence in populations represents an interesting challenge and opportunity for evolutionary biology given its widespread occurrence, diversity of organism lifespan, and the ecological interactions that shape patterns of aging (Kirkwood et al. 2000, Williams and Day 2003).

Interactions with environmental abiotic factors has led to the evolution of various life strategies in species (Wade and Kalisz 1990, Stearns 1992). Species presence or absence can be determined by the availability of nutrients in an environment, light availability, and temperature gradient (Venterink et al. 2003, Shirima et al. 2016). Salt content of terrestrial and aquatic ecosystems is an abiotic factor in many environments that influences the variety of species present (e.g. marine vs freshwater) and increases in the level of salinity in sensitive environments pose a threat to those unable to acclimate (Sarma et al. 2006, Boetius and Joye 2009, Smith et al. 2009, Larson and Belovsky 2013). Plant species have been shown to be excellent candidates for studying the effects of salts, and other natural and introduced chemical agents, on fitness given their sessile growth

forms, bioremediation potential, and their importance as a primary producer, both ecologically and economically (Roach 1993, Costa-Pierce 1998, Salguero-Gómez et al. 2013, Adhikari et al. 2015).

Current aquatic environments are experiencing fluctuations of salt content, nutrients, and other toxic materials (Arts 2002, Braukmann and Böhme 2011, Woodward et al. 2012), likely a result of human development influencing/accelerating natural cycles (Ciais et al. 2013) which could significantly alter ecological landscapes in the absence of remediation (Cui et al. 2009, Riemann et al. 2016). Prolonged exposure to toxic elements and nutrient deficiencies/excesses can cause substantial shifts in the health and composition of plant communities. Short-lived plant survival declines rapidly in response to deteriorating conditions though long-lived plants may persist, but survival and reproduction can be jeopardized by the continuous impairment (Colling and Matthies 2006, Oliva et al. 2014). In disturbed or modified environments, non-native species can encroach and the loss of biodiversity through increased competition for resources may occur (Milchunas and Lauenroth 1995, Colling and Matthies 2006, Hautier et al. 2009, Oliva et al. 2014, Wang et al. 2017). How demographic senescence in short-lived plants is affected by abiotic circumstances e.g., nutrient availability and salinity, may uncover positives/negatives for population growth potential and increase accuracy of future population projections in disturbed, deteriorating, and recovering ecosystems.

In this experiment, I cultivated *Lemna turionifera* (Landolt 1986), a short-lived aquatic macrophyte, under six nutrient concentrations, with or without a supplemental salt (sodium chloride), to test for differences in overall fitness, i.e., survival and reproduction, in fronds grown in varied abiotic circumstances. Samples of *L. turionifera*

collected from sites around Alberta, Canada, were shown to be growing in relatively low nutrient concentrations (Barks 2015; Chapter 5), in comparison with recommended laboratory medium concentrations, which allowed for a large variation in nutrient concentrations to be explored in this experiment. A quarter-series was implemented using Schenk-Hildebrandt growth medium (Sigma-Aldrich S6765, St. Louis, MO, USA), full-strength to 1/1024, crossed with salt-stress presence/absence for a total of twelve treatment groups. *Lemna* species are able to tolerate salinity to some extent (Haller et al. 1974). To test for differences in overall fitness (survivorship and reproduction) between treatment groups it was necessary to observe fronds over their entire life and maintain a record of offspring production, total offspring, and total lifespan. The caveat to these fitness measures is that there is a premium on early reproduction. A composite measure of fitness that incorporates the timing of reproduction with survivorship and fecundity, the intrinsic rate of increase (I_r), is a demographic approach to individual fitness that may be a better approximation of overall fitness/population growth potential (Stearns 1989, McGraw and Caswell 1996, Partridge and Barton 1996). My hypothesis for this experiment is that fronds grown in high-nutrient medium will have longer lifespans, more offspring fronds, and greater I_r -values than fronds grown in low-nutrient medium. I predict that the salt-stress will have a significantly negative effect on all fitness measures independent of the medium type the fronds are grown in, i.e. salt-treatments will have fewer offspring, shorter lifespans, and lower I_r -values than their control counterparts.

Materials and methods

STUDY SPECIES

The focal species for this experiment is *Lemna turionifera* (Araceae), a small aquatic plant from the subfamily Lemnoideae (the duckweeds) (Landolt 1986). The simplified thallus of *L. turionifera* consists of a single frond and root and it floats on or just below the surface level of still water (Hillman 1961). *Lemna turionifera* reproduction is primarily asexual; offspring fronds are produced from two meristematic pockets located proximally on the parent frond, and are released alternately over the lifespan of the frond (Hillman 1961, Landolt 1986). Fronds are also able to produce turions, small, rootless, starch-heavy fronds that overwinter and re-emerge when conditions favour growth. Investigations into senescence in *Lemna* species have shown that decreases in survival and fecundity with age are indeed observable and tend to be characterized by the decreasing size of offspring fronds in aging parents (Ashby and Wangermann 1951, Barks and Laird 2015, Ankutowicz and Laird 2018). *Lemna turionifera* is a suitable plant species for studying aging because of its small size ($\sim 1\text{-}5\text{mm}^2$), reproductive output ($\sim 5\text{-}15$ offspring), distribution (temperate), and relatively short lifespan for an angiosperm (~ 1 month) (Barks 2015).

METHOD FOR CREATING AN AXENIC STOCK CULTURE

Following the recommended procedures (Barks 2015), fronds collected from a field site on campus (49° 40' 19" N, 112° 51' 44" W) were rinsed with diH₂O and pre-cultured for 24 hours in half-strength Schenk and Hildebrandt medium to encourage microorganism growth. Selected fronds were then rinsed a second time with diH₂O, submerged in a 5-15% v/v diH₂O of bleach for 1-5 minutes, rinsed, and placed into fresh half-strength S-H

medium for 10 days. Fronds were monitored for plant vitality and microorganism contamination; ~20% of fronds survived the bleaching process and had no signs of contamination. A single, vital, non-contaminated *L. turionifera* frond was then selected to initiate the sterile stock culture.

FITNESS MEASURES

To compare differences between treatments several fitness measures were selected; latency to first reproduction, total lifespan, total number of offspring, and the intrinsic rate of increase. Latency to first reproduction is the interval from focal frond detachment to the release of its first offspring frond; earlier reproduction is often considered advantageous for population growth (Medawar 1952, Williams 1957); therefore, cohorts expressing shorter latency to first reproduction will be more fit. Lifespan and number of offspring are general fitness measures for survival and reproduction. The intrinsic rate of increase (I_r) is a composite fitness measure that incorporates lifespan, offspring production, and timing of reproduction into a single demographic I_r -value; high I_r -values indicate a greater population growth potential than low I_r -values.

PLANTS AND GROWTH CONDITIONS

The plants used in this study were derived from a clonal lineage that had been obtained from a pond on the University of Lethbridge campus (*L. turionifera* (strain PwsA GenBank MG000404, MG000478); collected from University of Lethbridge, Alberta, Canada 49° 40' 19" N, 112° 51' 44" W) (Barks 2018). *Lemna turionifera* was initially grown in stock cultures by aseptically transferring three to four individual fronds from past cultures into autoclaved 125 mL Erlenmeyer flasks filled with 36 mL of half-strength Schenk-Hildebrandt (S-H) growth medium (Sigma-Aldrich S6765, St. Louis, MO, USA).

S-H is a growth medium containing macro- and micronutrients (at half-strength (1.6 g L^{-1}); KNO_3 1250 mg L^{-1} , $(\text{NH}_4)\text{H}_2\text{PO}_4$ 150 mg L^{-1}) required for plant growth that is added to distilled water. Added to the medium are a suite of compounds (sucrose 6.7 g L^{-1} , yeast extract 0.07 g L^{-1} , tryptone 0.3 g L^{-1}) to assist in detecting bacterial and fungal contamination.

The samples I studied were genetically homogenous; heterogeneity (both genetic and environmental) has the potential to mask true patterns of senescence (Zens and Peart 2003). Considering the possibility of parental age effects in *L. turionifera*, the 216 focal fronds (18 per treatment x 12 treatments) were each first offspring of first offspring going back at least three generations (Ashby and Wangermann 1951, Barks and Laird 2016). Progenitor plants were aseptically cultured in 60 x 10 mm Petri dishes containing 10 mL of half-strength Schenk-Hildebrandt growth medium and kept inside a growth chamber set to 25 C with a 15:9 photoperiod (Environment Canada 2007). Focal fronds were transferred to their designated treatment medium once the desired birth-order was achieved, randomly assigned a position inside the growth chamber, and observed for the duration of their life. To ensure environmental constancy we aseptically transferred each plant into a new Petri dish with 10 mL of fresh growth medium every four days. Fronds were transferred to fresh medium and a new Petri-dish in the event of contamination/infection (e.g. bacterial or fungal) and were to be discarded if remediation attempts failed. However, no fronds were discarded in this experiment from contamination complications.

DILUTION PROCEDURE

To obtain the desired medium concentrations for the experiment, a full-strength S-H solution was created (3.2 g L^{-1} ; sucrose was not added to any of the treatment medium as

studies suggest that the growth of aquatic macrophytes can be influenced by its presence (Landolt 1986), tryptone and yeast extract were also not added to mitigate any potential effects ,e.g., an unaccounted for interaction with the frond, salt, or nutrients) and diluted to 1/4, 1/16, 1/64, 1/256, and 1/1024 strength with deionized H₂O. Levels of total dissolved phosphorus and total dissolved nitrogen from natural habitats in Alberta, Canada; TDP: 0.008-5.0 mg L⁻¹, TDN: 0.4-4.2 mg L⁻¹ (Barks 2015; Chapter 5) were comparable, and often lower, to low-nutrient treatments. After dilution the medium was tested for pH and adjusted to a pH of ~4.6 using diluted NaOH and HCl. Medium marked for an additional salt-stress was pH balanced after adding 5 g L⁻¹ of NaCl. The salt content of this experiment at 5g L⁻¹ (0.5%) was selected as a high-stress level, evidenced by previous studies investigating aquatic macrophytes (Haller et al. 1974, Panda and Upadhyay 2003, Thouvenot et al. 2012).

DATA ANALYSIS

Statistical analysis of the data was done using a two-way fixed effect ANOVA (Nutrient Level x Salinity) to test for any significant differences in total number of offspring, latency to first reproduction, total lifespan (measured in days starting from the day a focal frond detached from its parent and ending when the last offspring detaches from the focal frond; inclusive), and the intrinsic rate of increase (I_r). Focal fronds grown with a salt-stress in medium 1/16 strength and below often produced no offspring and had data sets that were not normally distributed or highly skewed. Fronds in these treatments were classified as ‘non-reproducing’, i.e., no offspring fronds produced after start of life. These data sets were analyzed in the following ways for comparisons to be conducted for the following fitness measures:

- i) *Lifespan/survival*: lifespan defined as one day
- ii) *Latency/intrinsic rate*: one offspring frond added 30 days after start of life to non-reproducing fronds (low productivity at old age)

Focal fronds grown at full-strength and 1/4 strength medium with the supplemental salt met the assumption of normality. An analysis of the data was also done using the unmodified data, but these evaluations are less statistical and more for identifying trends. All analyses were conducted in R v. 3.4.3 (R Core Team 2017)

Results

SURVIVAL WITH AGE AND LIFESPAN

Survival curves for all experimental treatments are given in **Figure 3-1**.

- i) Total lifespan decreased significantly with decreasing nutrient concentration ($F_{5,204}=229.03$, $p<0.05$), and significantly decreased in the presence of high salinity ($F_{1,204}=294.90$, $p<0.05$). Moreover, the negative effect of high salinity was especially prevalent at low nutrient concentrations, as evidenced by the significant interaction ($F_{5,204}=45.38$, $p<0.05$) (**Figure 3-2**). Focal fronds were grown in all nutrient levels in the experiment with no immediate impact on health, i.e., mortality from toxicity. Fronds grown in low nutrient medium (1/16-1/1024x) had shorter lifespans, fewer offspring, and appeared foliose i.e., a single leafy mass; parental fronds would often retain offspring fronds well into maturity. Loss of green colouration was observed in fronds grown in relative nutrient concentrations below 1/4 strength in the presence of a salt-stress.

REPRODUCTION WITH AGE

The number of fronds reproducing with age is given in **Figure 3-3**.

ii) Latency to first reproduction was significantly affected by salinity ($F_{5, 158}=60.50$, $p<0.05$), nutrient concentration ($F_{5, 158}=14.29$, $p<0.05$), and the interaction between the two factors ($F_{5, 158}=42.66$, $p<0.05$) (**Figure 3-4**). Latency to first reproduction was relatively consistent between control treatments; I_r -values decreased with decreasing nutrient concentration. Total number of offspring fronds produced increased significantly with increasing nutrient concentration ($F_{5,204}=310.30$, $p<0.05$), and was significantly decreased by salinity ($F_{1,204}=683.80$, $p<0.05$). Fronds in high salinity/low nutrient environments produced fewer offspring, evidenced by the significant interaction ($F_{5,204}=20.20$, $p<0.05$) (**Figure 3-5**). Intrinsic rate of increase (I_r ; composite fitness measure) was significantly higher with increasing nutrient concentration ($F_{5,204}=115.48$, $p<0.05$), and significantly lower in the presence of salinity ($F_{1,204}=968.82$, $p<0.05$). High salinity proved to be harmful at low nutrient concentrations decreasing intrinsic rates of increase, seen by the significant interaction ($F_{5,204}=25.34$, $p<0.05$) (**Figure 3-6**).

Discussion

INTERACTIVE EFFECT OF SALINITY AND NUTRIENT VARIATION ON *L.*

TURIONIFERA

High-nutrient availability was pre-requisite for growth in *L. turionifera* exposed to salt in my experiment and the interaction between the two factors was critical for the frond development. Increasing nutrient availability in saline environments has been shown to encourage growth and development in plants (Levine et al. 1998) whereas increasing salinity in constant nutrients deters growth (Ravikovitch and Yoles 1971). Control and salt treatment I_r -values indicated that the potential fitness/growth of a population is

significantly hindered in the presence of salt even at high nutrient concentrations (**Figure 3-6**).

In saline, low-nutrient treatments focal fronds would often not produce offspring fronds, or if an offspring frond was in development before transfer to treatment medium, it would be released pre-maturely when transferred (induced abscission) (Henke et al. 2011). Focal fronds grown at a high nutrient level, full-strength and 1/4, with a salt-stress experienced a significant delay to first reproduction and had fewer offspring fronds in comparison to their non-salt counterparts. Lower I_r -values (intrinsic rate of increase) were observed across all nutrient treatments in the presence of salt, with high mortality at early age classes in low nutrient medium and delayed time to reproduction in high-nutrient medium underlying the decreases in growth potential of the population.

Chlorosis (loss of green colouration) was observed in focal fronds grown in low nutrient levels (below 1/4 strength) in the presence of a salt-stress after five days in some treatments. Decolouration in plants is attributed to protein and nutrient deficiencies (Hanaoka et al. 2002). The lifespan of the focal fronds grown in high nutrient environments was not significantly affected by the salt stress and low nutrient environments were generally inhospitable to frond development. Studies into salt-stresses in aquatic macrophytes and terrestrial plants have demonstrated that the response of plants to salts can be detrimental to development; i.e., leading to decreases in biomass, reproductive output, and survivorship (Brock et al. 2005, Macek and Rejmankova 2007, Merino et al. 2010, Gilbert and Fraser 2013). The response exhibited by *L. turionifera* resulted in decreases in overall fitness in treatments able to tolerate the salt-stress. With our samples being genetically homogenous, i.e., all fronds gathered from a single clone, the plasticity of response highlights the possible variation in expression from a single

genotype under stress. Further investigations into the effects of prolonged salt exposure on *Lemna* species, specifically intergenerational, may prove valuable in the future for projecting shifts in populations under changing selective pressures.

In aquatic macrophytes it has been observed that low levels of essential macronutrients, nitrogen and phosphorus, can result in increases in frond surface area, decreases in total number of offspring, and limit growth and survivorship (Wangermann and Lacey 1955, Landolt 1986). Decreases in overall fitness were observed in focal fronds grown in low-nutrient medium although the growth patterns may be more comparable with fronds found in the wild given the limited nutrient availability (Barks 2015). Investigations into senescence in natural populations may give a better understanding of how ecological interactions shape patterns of aging in stressful environments and provide data for evolutionary models.

CONCLUSION

I observed, in a controlled laboratory experiment, that overall fitness of *L. turionifera* increases under increasing nutrient availability and was negatively impacted in the presence of a salt-stress. The effects of salinity and nutrient variation on growth in *L. turionifera* were interactive, i.e., relatively high nutrient availability was a necessity for relative success in saline environments. The response of *L. turionifera* was congruent with other studies investigating variable nutrient-salinity environments on aquatic macrophytes (Macek and Rejmankova 2007, Suthar 2015). Salt presence in aquatic environments under stable nutrient states (constant levels of macronutrients) can be detrimental to the development of plants that are unable to acclimate to salinity. Tolerances to salt and herbicides has been a focus for agriculture sciences resulting in the development of crops with increasing resilience to abiotic environmental stresses

(Flowers 2004, Funke et al. 2006, Ashraf et al. 2008). Natural plant populations that are not directly under management can be indirectly affected by human development e.g., agriculture, forestry, and mining. In the absence of selective controls, i.e., active human prevention/intervention, plants exposed to harmful excesses or deficiencies of nutrients and salts in an environment may experience significant shifts survival and fecundity. Continued investigations into how abiotic stresses affect senescence in plants could be extended to an intergenerational perspective to observe how offspring fitness may be affected by continued stress or if recovery/rejuvenation after multiple generations will occur in a neutral environment.

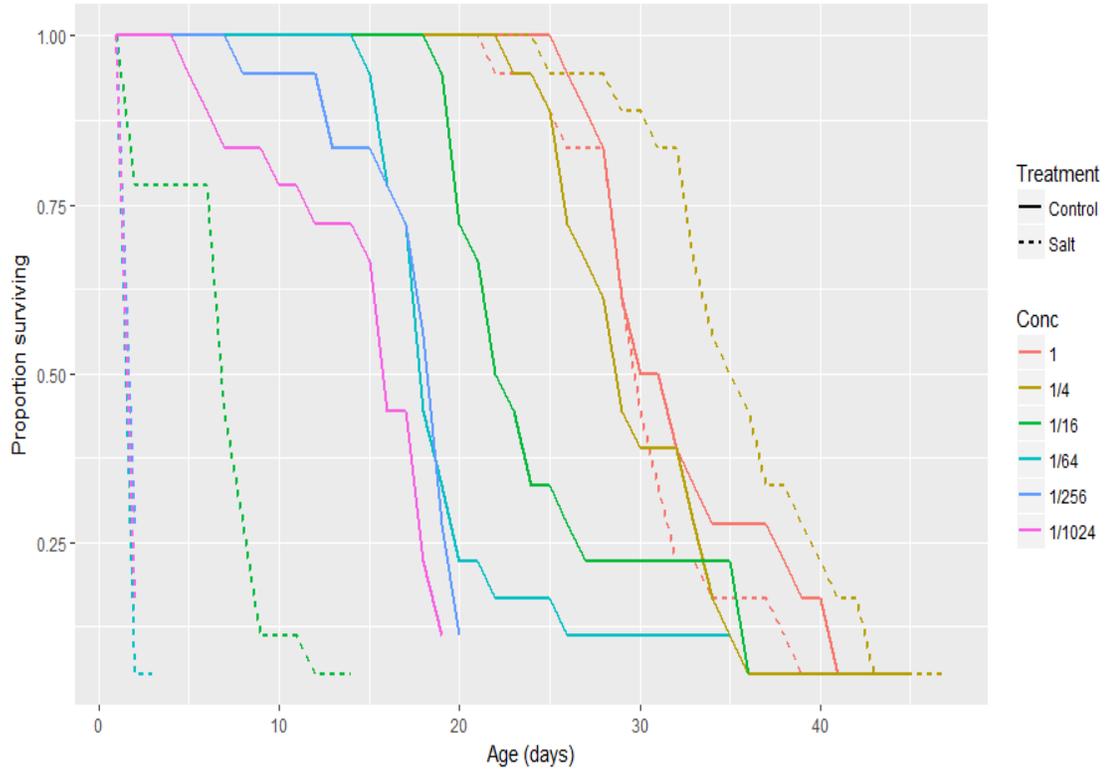


Figure 3-1. Proportion of fronds surviving with age (days) for twelve treatments groups. Treatments exposed to a salt-stress are represented by the dashed line and control treatments by the solid lines. Nutrient concentration is expressed by colour. All treatments demonstrate a decreasing probability of survival with increasing age.

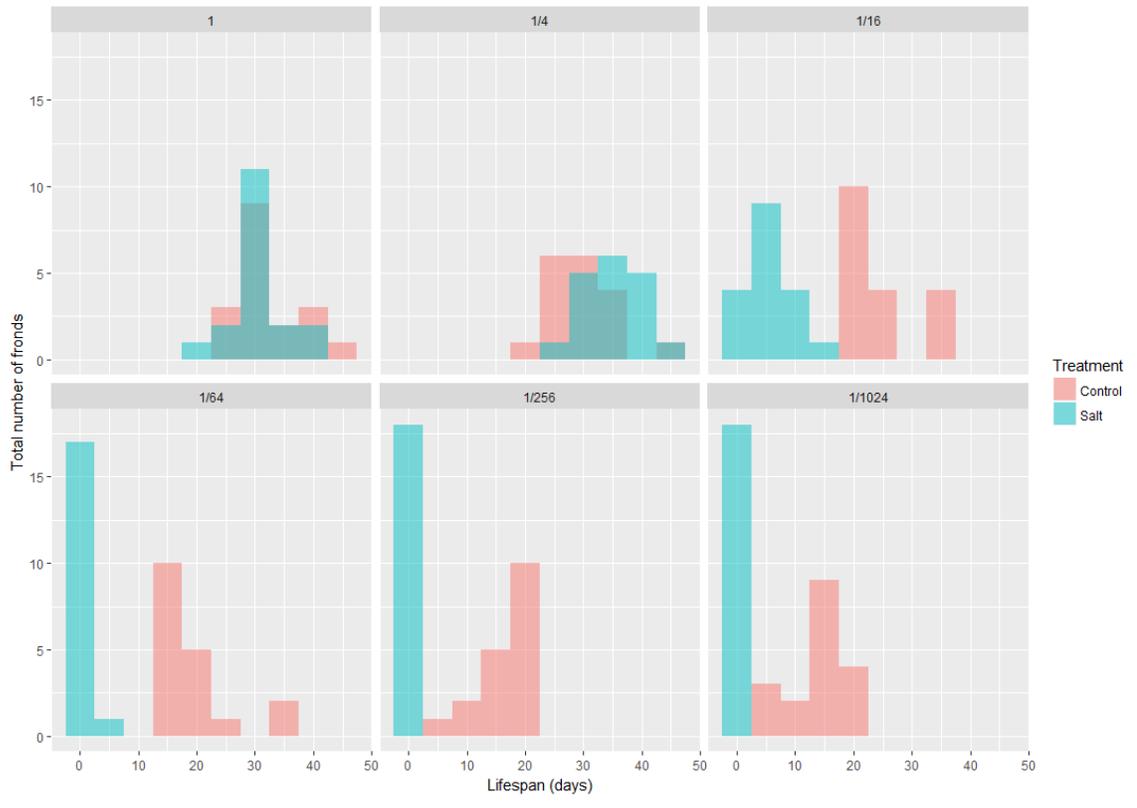


Figure 3-2. Distribution of lifespans by relative strength of SH-medium

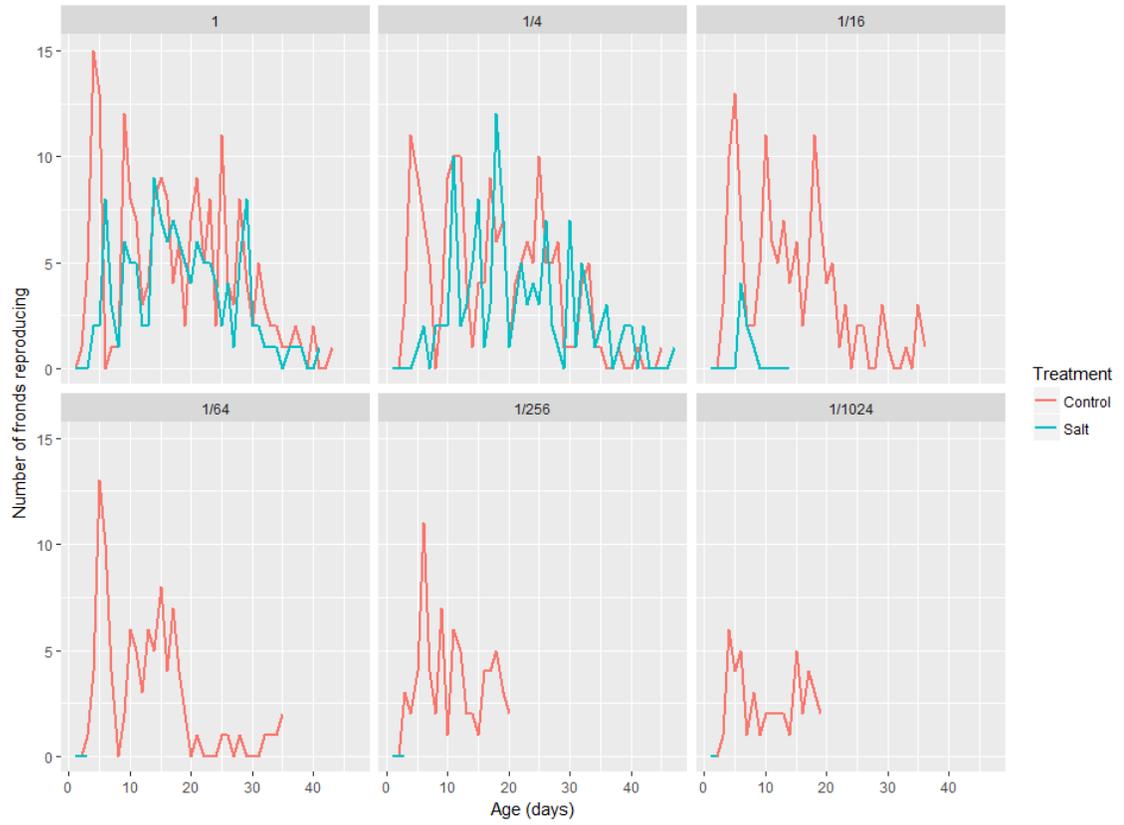


Figure 3-3. Number of fronds reproducing with age by relative strength of SH-medium

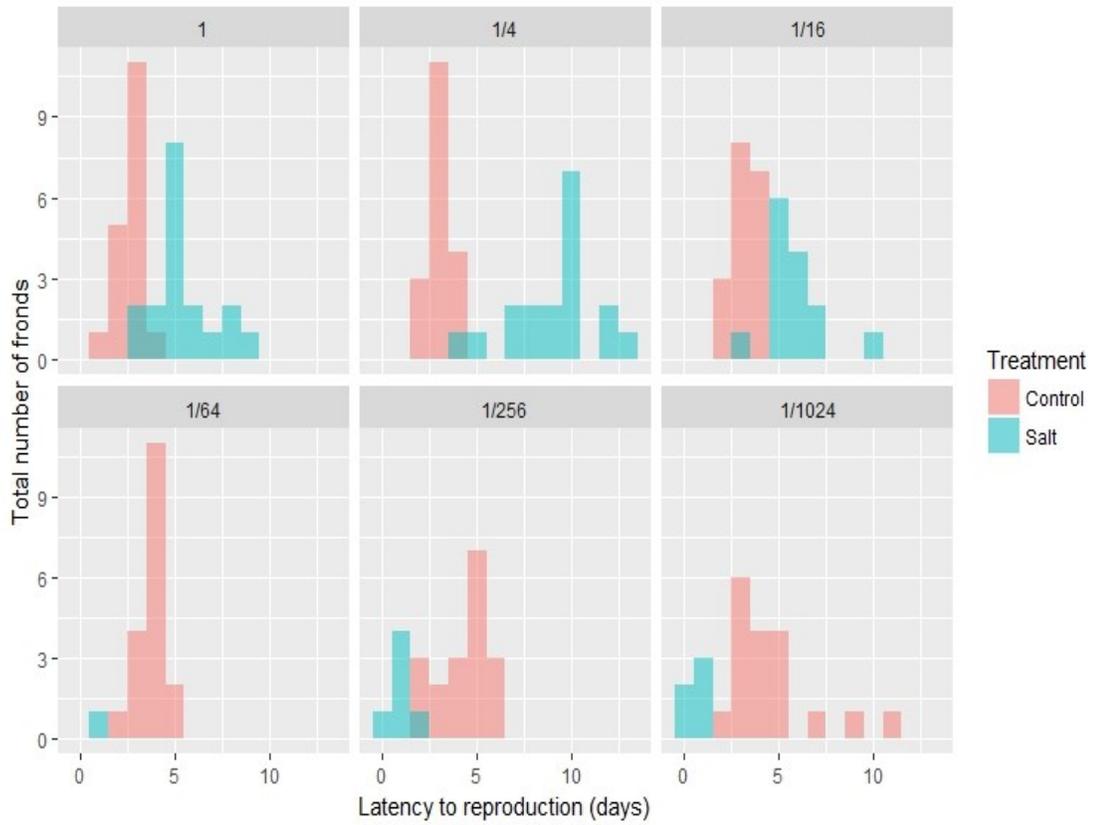


Figure 3-4. Distribution of latency to first reproduction by relative strength of SH-medium

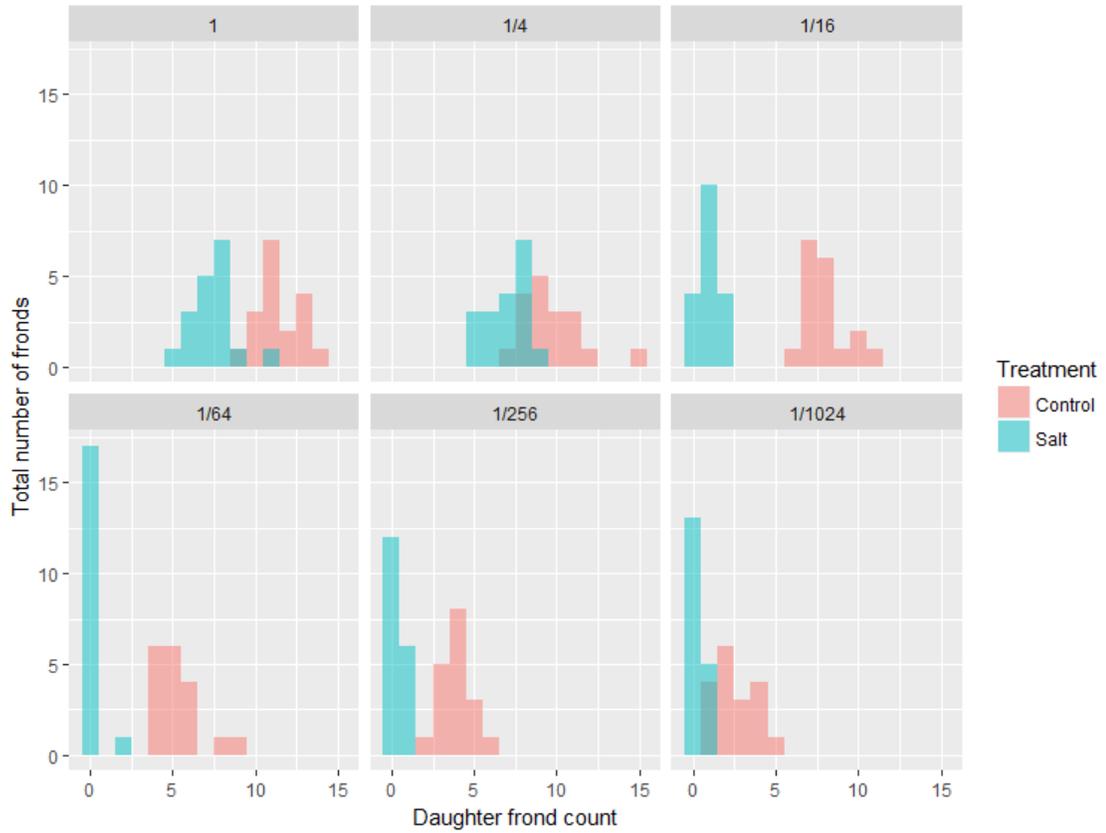


Figure 3-5. Distribution of number of offspring by relative strength of SH-medium

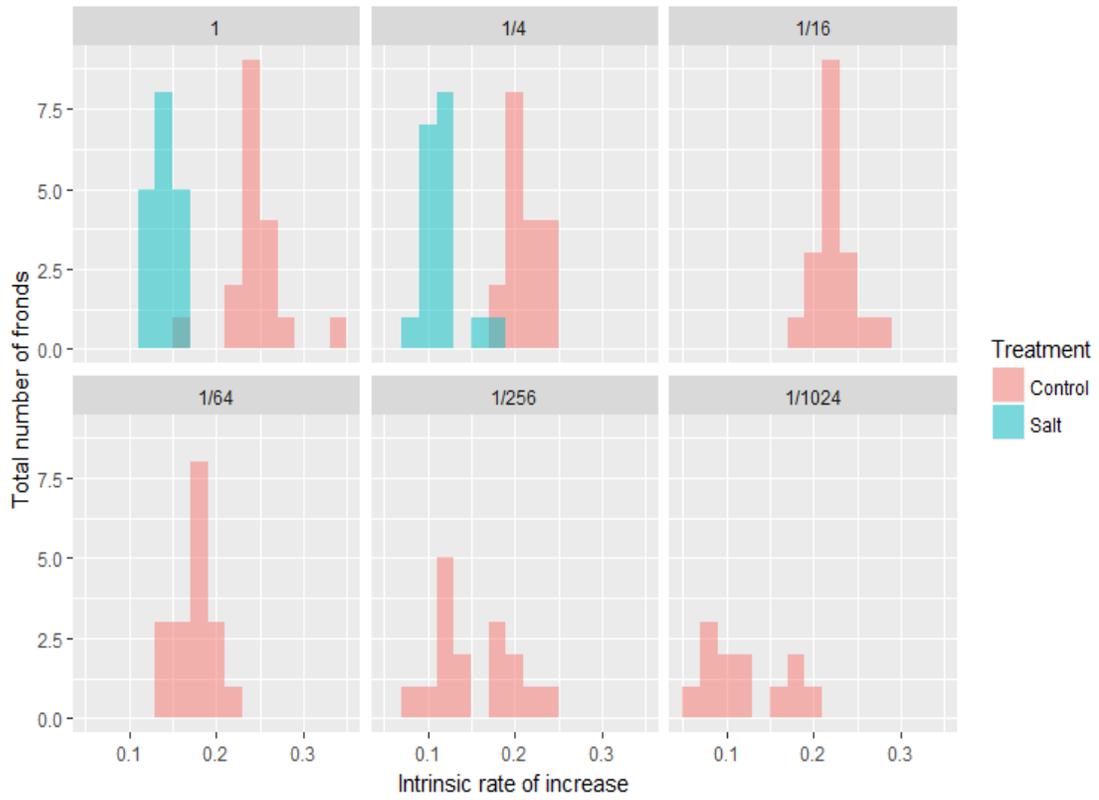


Figure 3-6. Distribution of the intrinsic rate of increase by relative strength of SH-medium

CHAPTER 4: GENERAL DISCUSSION

THE INFLUENCE OF STRESS ON SENESCENCE

Dynamic environments, heterogenous in space and through time, have been recognized for influencing patterns of survival and reproduction in individuals (Stearns 1992). Variation in multiple selective factors, e.g., nutrient availability, temperature, population density, etc., create the selective pressures in an environment. The patterns of aging that are observed in species have been attributed to the intensity of selective pressure in an environment (Williams and Day 2003, Williams et al. 2006). Declining force of selection favours earlier reproduction in a population even at the potential cost to survival later in life (Hamilton 1966). In a natural setting there is no guarantee of survival to old age; potential for positive population growth is frequently contingent on the success of early age classes (Kirkwood and Rose 1991). How stress influences senescence is an important topic in plant demography, ecology, and evolution.

Removal of antagonizing or stressful factors from an environment can allow the individual to live to an advanced age (Comfort 1956). Domesticated animals, barring any fatal chance events, will reach old age then succumb an intrinsic mortality event, i.e., internal failure. Exposure to a natural environment creates opportunities for extrinsic death, e.g., predation, starvation, and infection. Evolution has led to the adaptations that allow individuals to survive to reproductive maturity in these variable environments (Stearns 1989). Functioning ecosystems can be influenced by human development; disrupting equilibriums with extractions or influxes of natural and artificial resources (Ciais et al. 2013, Collins et al. 2013, Kirtman et al. 2013). How senescence is influenced by the variation of external pressures, i.e., extreme, neutral, and rare scenarios, can be

explored in a controlled environment. Understanding how an isolated stressor effects senescence, i.e., a population's trajectories of survival and reproduction, provides insights into the significance of a stressors influence and at what levels it can become a significant deterrent or accelerant to population growth. In Chapters 2 and 3, I examined the influence that specific stressors have on survival and reproduction in *Lemna turionifera*. A supplementary chapter that pursued the objective of monitoring *L. turionifera* in a natural setting was included in my thesis with preliminary field results and recommendations for apparatus improvements.

Simulated herbivory stress

The stressor applied in Chapter 2 was an excision that sought to replicate a single herbivory event on an *L. turionifera* frond. Herbivory is a common interaction for many plants; the effect on the plant is often negligible as regrowth is possible via intact meristematic tissue (Noodén and Leopold 1988). *Lemna turionifera* fronds are relatively simple in structure, i.e., no distinct stem and leaf systems (Landolt 1986), and the loss of limited photosynthetic tissue through an excision was predicted to induce a fitness cost, e.g., decreased reproductive output and shorter lifespans. Despite this prediction, *L. turionifera* fronds proved tolerant of the applied excision and no significant differences in fitness measures were detected between treatments (**Figure 2-3, Figure 2-5**). This single simulated herbivory event had no measured immediate consequences on parental frond health but there is a possibility that offspring quality, i.e., their survival, reproduction, and size, could have been affected (Barks and Laird 2015). Continued investigations into the intergenerational effects of damage could highlight potential costs to offspring quality from *Lemna* fronds experiencing injury. Multiple damaging events or the use of live

herbivores could also be utilized to test for the effects of increasing levels of damage/herbivory on senescence.

Salinity and nutrient variation stress

The focus of Chapter 3 was nutrient variation, both high and low, and how a high salt stress influenced survival and reproduction in *L. turionifera*. The sedentary mode of plants subjects them to the variation of abiotic materials in the environment through time; with natural processes and stochastic events retaining, recycling, and introducing elements into the medium. *Lemna turionifera* can be found in Alberta growing in water with low nutrient concentrations, compared to suggested lab culture growing concentrations, and low salinity (Barks 2015). Salinity was selected as a primary stress in this experiment as salts are naturally occurring elements in environments, are essential for biological processes, detrimental to health in their extremes, and influenced by human activities, e.g., road salts (Jackson and Jobbágy 2005, Kaushal et al. 2005, Boetius and Joye 2009). I predicted that high salinity, 5 g L^{-1} of NaCl, would be detrimental to the survival and reproduction of *L. turionifera*. At low nutrient concentrations the high salinity proved to be extremely detrimental to frond survival (**Figure 3-1**). Fronds grown in high nutrient medium appeared more robust, salt and control treatments at these levels were not significantly different from one another displaying similar lifespans and total number of offspring (**Figure 3-2, Figure 3-5**). In this experiment, I observed that high nutrient concentration was essential for the survival of *L. turionifera* fronds in a saline environment. How low nutrient concentrations and varied salinity, high to low, affects senescence in *L. turionifera* could be explored further to test for tolerances and thresholds of abiotic stress.

Natural observations of senescence

As observations of senescence are not limited to the laboratory (Bonduriansky and Brassil 2002, Sherratt et al. 2010), the supplementary chapter of my thesis explores methods for monitoring *L. turionifera* fronds in the wild. Observing *L. turionifera* in a natural setting involved the construction of an apparatus, the floating *Lemna* observation apparatus (FLOA), designed for isolating and maintaining fronds in a body of water. The FLOA needed to be buoyant, stationary and stable, and contain multiple fronds for observation. Early deployment of the FLOA was initially positive though complications arose later in the field season with decreasing water levels. The primary concern for the FLOA, aside from natural events, was the overall weight; the materials used to construct the apparatus were bulky and a lighter, more streamlined FLOA may be the solution for the successfully monitoring *L. turionifera* in a natural setting. Observing senescence in wild plants is a novel concept and *L. turionifera* provides a suitable candidate species given its lifespan and reproductive mode.

CONCLUSIONS

Senescence is a potentiality, realized when the individual has successfully progressed to a relative old age. Selective pressures in a natural environments act as the filter that an individual must pass through to approach advanced old age. The influence that these stressors have on a population's patterns of survival and fecundity is thought to shape patterns of aging. In a controlled environment, I conducted experiments to observe how growth and development in *Lemna turionifera* was affected by isolated stressors. Fronds proved tolerant of a single injuring event with no apparent impairment to survival or fecundity though it is possible that offspring quality may have been affected. Exposure to

high saline environments was shown to be fatal for fronds grown in low nutrient concentrations with high nutrient concentrations a requirement for survival and reproduction. Many challenges present themselves when attempting to observe senescence in the wild but with the proper tools and methods it is possible. Dynamic environments contain multiple types of stressors that act upon the individual in unison, shaping patterns of survival and reproduction in the process. Understanding how specific stressors influence senescence in plants can allow for increased accuracy of predictive population models in dynamic environments and identification of threshold stresses for growing populations.

SUPPLEMENTARY: FIELD STUDY INVESTIGATION TECHNIQUES; APPARATUS AND TRIAL OUTCOMES

Introduction

Senescence is defined as the decrease in survival and fecundity observed in individuals as they advance in age (Partridge and Barton 1993, Kirkwood and Austad 2000). Reaching a geriatric age-class is thought to be an unlikely occurrence in nature given an organism must progress to old age, i.e., survive all previous age classes. Observational studies of senescence in natural environments have shown that individuals can be seen to undergo a senescent process and may appear accelerated in comparison to laboratory counterparts (Bonduriansky and Brassil 2002, Roach 2004, Kawasaki et al. 2008, Sherratt et al. 2010). Observing aging in the wild presents challenges, e.g., species longevity, maintaining specimen ID, and sample loss through chance events can make tracking individuals through time difficult. However, in examining senescence under natural conditions, we can obtain a more realistic environment than could be easily replicated in the lab. How aging presents itself in the wild may allow for a better understanding of its evolution and provide validation for popular theories.

In this study, I attempted to monitor *Lemna turionifera* (Landolt 1986), an aquatic macrophyte, under natural conditions to determine if senescence could be observed; i.e., decreases in survival and reproduction with age. A field site was selected on the University of Lethbridge campus that was found to contain *L. turionifera* fronds. An apparatus was constructed in the lab and transported to the field site where fronds were selected *in situ* and marked for observation. To test for decreases in survival and reproduction in focal fronds, it was necessary for specimens to be observed over their

entire life and to maintain a record of offspring production. I hypothesized that *L. turionifera* fronds could be isolated and observed undergoing senescence in the wild.

Materials and methods

STUDY SPECIES

Sample fronds collected from the field site in the previous summer were genetically identified as *Lemna turionifera* (Araceae), a small aquatic plant from the subfamily Lemnoideae (Landolt 1986). The simplified thallus of *L. turionifera* consists of a single frond and root and it floats on or just below the surface level of still water (Hillman 1961). *Lemna turionifera* reproduction is primarily asexual; offspring fronds are produced from two meristematic pockets located proximally on the parent frond, and are released alternately over the lifespan of the frond (Hillman 1961, Landolt 1986). Fronds are also able to produce turions, small, rootless, starch-heavy fronds that overwinter and re-emerge when conditions favour growth. Investigations into senescence in *Lemna* species have shown that decreases in survival and fecundity with age are indeed observable and tend to be characterized by the decreasing size of offspring fronds in aging parents (Ashby and Wangermann 1951, Barks and Laird 2015, Ankutowicz and Laird 2018). *Lemna turionifera* is a suitable plant species for studying aging in the wild because of its small size (~1-5mm²), reproductive output (~5-15 offspring), distribution (temperate), and relatively short lifespan for an angiosperm (~1 month) (Barks 2015).

FLOATING *LEMNA* OBSERVATION APPARATUS

The apparatus for observing aquatic macrophytes in the wild required certain properties. It had to be lightweight and buoyant, contain many fronds, be stable and stationary, and protected from any large disturbances, e.g., hail storm, random debris, etc. A recycled plastic barrel that had been partitioned into two cross-sections was chosen as be main

containment vessel (**Figure 5-1**). Attached to main vessel were two foam flotation devices that provided the necessary buoyancy, a mesh net as a barrier to incursion by wandering fronds from the environment, and an internal and exterior temperature monitor (**Figure 5-2**). Within the main containment vessel were the individual observation cells; two containment vessels with 90 observation cells for 180 total specimens. During the study, additional foam was added to the observation cells to prevent intrusion from wandering fronds (**Figure 5-3**). The containment vessel was kept in one place by leashing it to a stationary post in the pond and protected from disturbances by constructing a transparent roof above the study site.

Results

FLOA PERFORMANCE

The water level within field site pond was not as constant as initially thought. During the summer field season, due to activities by the University of Lethbridge Facilities Management, the water in the pond continued to decrease to the point that the FLOA itself had contacted that bottom (**Figure 5-4**). Fronds continued to be monitored under such conditions until the pond experienced a resurgence of water, now flooding over the FLOA and dispersing the samples (**Figure 5-5**). The FLOA achieved all its required goals; buoyant, stable and stationary, contain isolated fronds, and protected from most large-scale disturbances. Isolated fronds could be observed in the observation cells, but many had to be replaced over the experiment and few reached the desired focal generation before the FLOA failure.

Discussion

Observing senescence in the wild is difficult for many reasons and for this study it proved to be a site-specific issue, i.e., pond water management. The FLOA accomplished its

primary purpose in isolating *L. turionifera* fronds though improvements to the apparatus could be made: i) constructed from lightweight plastic to decrease weight, and ii) smaller dimensions for easier deployment. Selection of a field site with *Lemna* species that is less subject to disturbances may also allow for stable observations. With the proper modifications, the FLOA could be used for observing *Lemna* species and other aquatic macrophytes under natural conditions.

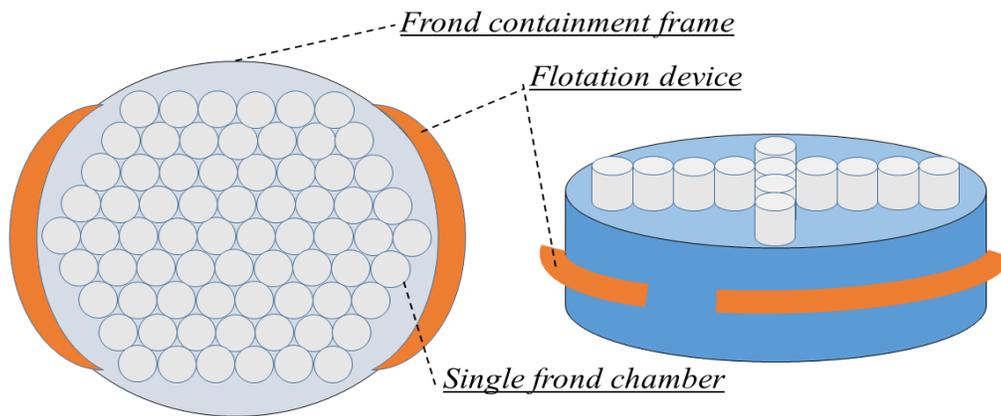


Figure 5-1. Conceptual design for the floating *Lemna* observation apparatus (FLOA). The finished apparatus contained 90 individual units.



Figure 5-2. FLOA early deployment



Figure 5-3. FLOA with additional foam in observational cells. The foam was added to prevent random fronds from emerging into the observational cells.



Figure 5-4. FLOA during low water level



Figure 5-5. FLOA submerged after increase in water level. The mesh-barrier on the FLOA became inundated with mud after contacting the bottom, weighing down the apparatus.

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