DO MALES REALLY HAVE IT EASIER? THE COSTS OF REPRODUCTION IN *SAGITTARIA LATIFOLIA* (ALISMATACEAE)

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ABSTRACT

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Darwinian fitness is determined by the outcome of allocations of energy and nutrients to plant growth, survival, and reproduction. Allocations to reproduction differ from allocations to growth and survival because the products of reproduction (pollen, ovules, and fruits) are dispersed away from the plant and so the resources used to produce them cannot be re-allocated. As a result, and because the total resource pool available to a plant is limited, allocations to reproduction can reduce future growth, reproduction, and/or survival, yielding fitness costs of reproduction. This study provides a novel approach to detecting the costs of reproduction through multiple reproductive currencies, including both biomass and nitrogen allocations and their temporal effects on current and future plant performance. My results demonstrate the environmental dependence of the costs of reproduction and show that under certain conditions, males can bear greater costs than females, contrary to the prevailing view.

KEYWORDS

Cost of reproduction; life-history trade-offs; sexual dimorphism; dioecy; photosynthetic machinery; nutrient availability; flower removal

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Chapter 1: General Introduction

1.1 The costs of reproduction and life-history trade-offs

To grow, to survive, and to reproduce are fundamental to fitness in angiosperms, but plants must face trade-offs between these important life-history variables (Reznick 1985). These trade-offs occur when plants allocate to plant growth, survival, and reproduction from a pool of fixed resources (Bawa 1980; Reznick 1985; Obeso 2002). While plant growth - and for perennial plants, survival - are essential components of plant life-histories, reproduction is the process that determines plant fitness (Calvo and Horvitz 1990; Dorken and Van Drunen 2012). Because the total amount of resources available to plants is limited, resources (energy, minerals, and biomass) allocated to reproduction results in trade-offs, with effects on future reproductive fitness (Obeso 2002). Fitness in plants is generated through the production of ovules and pollen - the routes by which genes are transmitted to future generations. When plants allocate finite resources to reproduction, they cannot be re-mobilized or re-allocated to other life-history components, like growth or survival (Reznick 1985). Unlike growth and survival, reproductive structures (seeds, fruits, and pollen) are dispersed from the parent plant, along with the resources invested in those structures. Allocations to reproduction should, therefore, have demographic consequences because they reduce the amount of resources that can be allocated to survival, growth, and/or future reproduction (*i.e.*, there are 'costs' to the investment in reproduction; Bawa 1980; Reznick 1985; Obeso 2002). As a result, reproductive allocations determine the degree to which fitness is determined by current versus future reproduction, and responses to natural selection on these allocations shape life-history evolution in the flowering plants.

It is generally thought that the costs of reproduction are greater for female function than male function (*e.g.,* Obeso 2002). However, male and female function have been documented to involve different resource types, including different demands for elemental nutrients like carbon and nitrogen (*e.g.*, *Sagittaria latifolia*; Dorken and Van Drunen 2012). These differential resource costs may be linked to divergent morphologies (Harris and Pannell 2010), life-histories (Dorken and Van Drunen 2012), and physiological processes in plants (Wright and Dorken 2014). The objective of this study was to assess the fitness and resource costs of reproduction via female and male sex functions considering biomass and nitrogen as separate currencies and measuring direct (instantaneous and/or short-term) and indirect (demographic) consequences for the investment into reproduction. We grew Broadleaf Arrowhead (*Sagittaria latifolia*), a dioecious clonal plant in a common garden over two growth periods and experimentally manipulated nitrogen availability and reproductive investment. We measured the direct costs of reproductive allocations using assessments of plant physiology (including photosynthetic rates) and the carbon and nitrogen content of perennating structures, and indirect costs as the investment in clonal growth, and post-reproductive growth and survival.

1.2 Direct and indirect costs of reproduction

The last comprehensive review of the costs of reproduction in plants (Obeso 2002) highlighted the importance of linking current reproduction and physiology to demography and life-history evolution, yet most studies today (more than 20 years later) have not taken the opportunity to do so. Upon review of the literature (1950-2022), most studies concerning the costs of reproduction have only measured direct costs, and while the number of indirect studies is

growing, there have been few studies that link direct costs to their demographic consequences (Fig. 1.1).

Commonly used ‘currencies’ for the measurement of reproductive costs include carbon or biomass of fruits, seeds, and/or clonal structures (*e.g., Oxytropis sericea* in Ida *et al.* 2009), the nitrogen content of pollen, leaves, and clonal structures (*e.g., Sagittaria latifolia* in Dorken and Van Drunen 2012 and Wright and Dorken 2014), and less commonly, some studies have included assessments of phosphorus content (*i.e., Rubus chamaemorus* in Agren 1988). Allocations of these various currencies to reproduction can limit their availability in vegetative tissues and constrain physiological processes (*e.g.,* photosynthesis, water-use efficiency, etc), inhibiting growth and increasing mortality (Case and Ashman 2005). Also referred to as the “somatic costs of reproduction” in a current growth period (Obeso 2002), direct costs are the most extensively studied aspect of this discourse of cost of reproduction in the literature to date (see Fig 1.1). It has become common practice to assess direct costs in terms of the allocation of elemental currencies (carbon/biomass, nitrogen, and phosphorus) to reproductive structures, and to measure phenotypic trade-offs of reproduction versus growth (clonal and vegetative) and survival (Van Drunen and Dorken 2012). Direct costs of reproduction can also be detected via indirect measures of physiological performance, which is useful when costs are not always observable (Case and Ashman 2005). Measuring direct costs provides useful insights for the extent to which organisms invest resources into reproductionbut are not sufficient estimates for determining costs to plant fitness over longer temporal scales (*i.e.,* more than one growing season). Although a variety of currencies have been measured, the vast majority of studies have used carbon as the only measure of reproductive costs.

When plants allocate nitrogen, phosphorus, carbon, and other resources to produce and maintain reproductive structures, there may be a direct cost that inhibits the physiological processes responsible for plant growth (Obeso 2002; Knops *et al.* 2007). The idea that reproduction and vegetative growth compete for nutrients from the same pool of resources is not new, and the majority of these studies have considered the trade-offs that occur when allocations to reproduction occur at the expense of plant growth. This is in fact one of the most documented trade-offs and serves as an effective approach to determining the costs of reproduction in a current growth period. Many studies have detected inverse relationships between growth and the production of fruit and seed, meaning that the partitioning of resources to these two life-history components are perhaps some of the most obvious direct costs of reproduction for some plants (Obeso 2002). For example, in the species *Corema album*, reproductive allocation to both male and female function resulted in decreased resource availability for the production of vegetative biomass throughout other parts of the plant, especially in leaves (Avarez-Cansino *et al*. 2010). These findings were consistent with that in the species *Rubus discolor*, as the removal of floral buds from reproductive canes increased the size of juvenile canes that arose from clonal sprouting, demonstrating a trade-off between current reproduction and current growth (Lambrecht-McDowell & Radosevich 2005).

In some cases, reproduction can be relatively inexpensive (Harper 1977), because pollen production and acquisition may be constrained by resource availability (Koenig and Ashley 2003), reproductive organs may be partly self-sustaining (Bazzaz *et al*. 1979), and photosynthetic rates may be upregulated to compensate for increases in the plants carbon demand during reproduction (Gehring and Delph 2006). Thus, trade-offs are still occurring between growth and reproduction in iteroparous species (plants that reproduce several times

throughout the life-history), but the effects of such partitioning may be less pronounced compared to semelparous species (plants that reproduce once, resulting in fecundity). The direct costs of reproduction and trade-offs to plant growth are not always easily detectable, because plants have compensatory mechanisms that allow them to balance the costs by taking up more nutrients to compensate for increased allocations to flower production and maintenance (Obeso and Grubb 1993; Delph and Meagher, 1995; as reviewed in Obeso 2002 and; Banuelos and Obeso 2004). Thus, environmental conditions (*i.e.*, the availability of nutrients) could determine the extent to which trade-offs occur between allocations to reproduction versus growth in some species, while modularity and size could also play an important role.

The acquisition and mobilization of nutrients and energy throughout plants is controlled at the physiological level and is fundamental to understanding the costs of reproduction (Obeso 2002; Case and Ashman 2005; Wright and Dorken 2014). Many studies have included experiments that directly or indirectly measure physiological costs of reproduction. Some studies have documented water uptake and water use efficiency by above and below-ground structures (*i.e*., water pressure and retention in leaves, stems, and roots) (Gassmann 2004, Álvarez-Cansino *et al.* 2010; Euler *et al*. 2012). For example, Alvarez-Cansino *et al*. (2010) performed bud-removal in the dioecious shrub *Corema album* and documented that carbon allocation to male function yielded physiological costs in the form of decreased shoot length and water uptake potential, demonstrating the costs of reproduction are at least to some extent mediated via physiological processes. Other studies have measured photosynthate allocation (sugars produced by photosynthetic reactions) to reproductive structures (Harris and Pannell 2008; Hua *et al.* 2012; Jacquemyn *et al.* 2010; Ida *et al.* 2012). The experiment by Ida *et al*. (2012) reported that reproduction in *Oxytropis sericea* was physiologically costly, as photosynthate allocation

increased by 190% to fruits, thus demonstrating high C-demands during reproduction. This study also reported that C-reserves in taproots could be re-mobilized during reproduction, as taproot biomass declined by nearly 30%, and demonstrated that female function can be versatile (Ida *et al*. 2012). When resources are mediated via physiological processes (including allocations to reproduction), there are therefore less nutrients available for future growth, survival and reproduction. The implications of the underlying physiological mechanisms have implications concerning male function too.

Measures of leaf photosynthetic rates (and/or components of the photosynthetic 'machinery', *e.g.,* amounts of chlorophyll *a*) and respiration rates (*i.e.,* leaf gas exchange rates and stomatal conductance) can be an effective approach to detecting physiological costs (Schultz 2003; Aschan *et al.* 2005; Leigh *et al.* 2006; Álvarez-Cansino *et al.* 2010; Jacquemyn *et al.* 2010; Wright and Dorken 2012).Wright and Dorken (2014) compared N-content in leaves and photosynthetic rates between male and female plants in a natural population of dioecious *Sagittaria latifolia*, but found no significant difference in photosynthetic rates between the sexes, potentially due to high nutrient availability and/or compensatory mechanisms. Empirical approaches to measuring the effects of allocations of nutrients and energy to reproduction on physiology have largely failed to reveal different physiological costs of reproduction between the sexes (Dorken and Van Drunen 2012).

It has been hypothesized that nutrient availability could be a key determinant of the extent to which trade-offs between life-history components are expressed (Liu *et al*. 2009). Stearns (1992) argued that life-history trade-offs will only be detectable when resources are limited. In terms of clonal reproduction, the costs of reproduction are more easily detected when

nutrient availability is limited, but compensatory mechanisms and the re-mobilization of stored reserves are thought to make short-term assessments difficult (Dorken and Van Drunen 2012). It may also be that when nutrient availability is high, trade-offs may be too subtle to detect (Liu *et al*. 2009). Some studies have assessed the costs of reproduction by limiting nutrient availability, but still only measured carbon (biomass allocation) costs. Liu *et al.* (2009) assessed the trade-offs of these two components in an emergent aquatic herb native to East Asia (*Sagittaria pygmaea*) under three different nutrient conditions: low, medium, and high. At low nutrient levels, weak trade-offs between sexual reproduction and clonal mass were detected at the end of the growth period. However, at high nutrient levels, there were no-trade offs detected between sexual reproduction and clonal growth, indicating that reproductive costs are context dependent (Liu *et al*. 2009).

Investment in reproduction entails risks to survival, future growth and fecundity (Bell, 1980; as reviewed in Obeso, 2002; Lambrecht-McDowell and Radosevich 2005; Lin *et al.* 2016). As a result, there are also indirect, or demographic, costs of reproduction, because the implications of reproductive investment should often have effects in the future. Indirect costs can be measured at different temporal scales, from short term (*i.e.,* survival costs of short-lived perennials Aragon *et al.* 2009) and extended time periods (*i.e.,* survival costs in long-lived herbs over many decades, Edelfeldt *et al*. 2019). Studies considering costs over shorter time periods typically involve experimental manipulations, often of short-lived perennial plants. For example, short-term studies have considered how reproductive effort in a previous season affected growth, reproductive output, or even survival (*i.e.*, Shefferson and Simms 2007; Aragon *et al.* 2009; Toivonen and Mutkikainen 2012; Lin *et al.* 2016; Villellas and Garcia 2018). Long-term studies typically assess the costs of reproduction over several generations (*i.e.,* Knops *et al*. 2007; Santos

and Climent 2014; Hacket-Pain *et al.* 2017). These long-term studies sometimes include matrix models that estimate the demographic consequences of reproductive investments and include assessments of trees and shrubs (*i.e.*, Pico and Tetana 2008; Villellas and Garcia 2018; Edelfeldt *et al*. 2019). Studies of indirect costs of reproduction are important for determining the consequences of reproductive investments for organismal fitness and provide insights into life-history evolution. The indirect costs of reproduction have not been as thoroughly studied as direct costs of reproduction (See Fig 1.1).

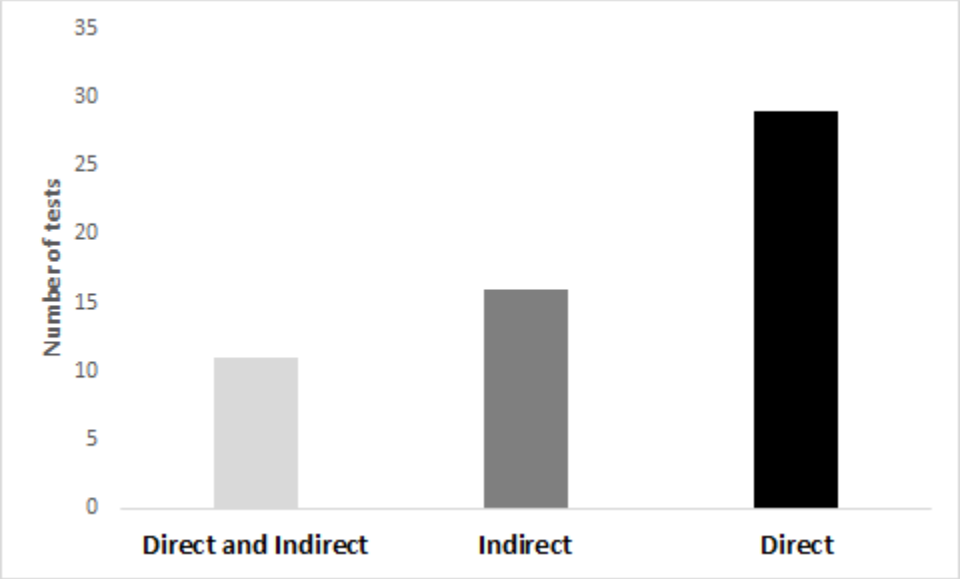

Figure 1.1. The distribution of studies that assessed direct and indirect costs of reproduction, and the proportion of those which considered both direct and indirect costs.

Many angiosperms combine sexual reproduction with clonal growth (Fischer and van Kluenen 2002; Vallejo-Martin *et al.* 2012; Dorken and Van Drunen 2012). Clonal growth occurs through the production of vegetative structures that - like sexual reproduction - can lead to the dispersal and establishment of shoots (ramets) at new locations (Vallejo-Martin *et al.* 2012). Both modes of reproduction can be costly and have been documented to trade-off against one

another (Otto, 2009). While plants display a vast range of reproductive strategies, it is estimated that approximately 80% of flowering plants combine the dual reproductive mode of producing both sexual structures (flowers) and clonal (or vegetative) structures (Fryxell 1957; as reviewed in Vallejo-Martin *et al*. 2012). Clonal growth and sexual reproduction draw on the same pool of resources, and it is becoming well-documented that these two modes will trade-off against one another in terms of biomass and nutrient composition (Dorken and Van Drunen 2012).

1.3. Male and female function

Male function involves the production of pollen (male gametophytes) and fertile stamen (Roulston *et al*. 2000; reviewed in Obeso 2002; Dorken and Van Drunen 2012; Barrett and Hough 2013). Pollen is protein-rich and contains high concentrations of nitrogen (N) and phosphorus (P) (Roulston *et al.* 2000). Male function can therefore entail substantial allocations of these nutrients, and these allocations are known to trade-off against life-history components. Wind-pollinated (anemophilous) plants involve intensive N-costs from the production of pollen (*i.e.*, *Mercurialis annua* in Tonnabel *et al.* 2017) (Harris and Pannell 2008; reviewed in Tonnabel *et al*. 2017). Animal-pollinated plants (zoophilous plants) tend to produce fewer pollen grains, but the total mass of pollen can be greater when compared to wind-pollinated plants (*e.g.*, Zoophilous pollination in *Cucurbita pepo* in Lau and Stephenson 1993). Although these two reproductive strategies may involve different allocations of N to reproduction (*i.e.*, proportional differences), both animal- and wind-pollinated plants may experience high costs of reproduction via their male function (*i.e.*, via pollen production).

Nitrogen is a key biological element that is utilized by plants in the production of proteins, nucleic acids, pigments, and organic molecules (Vitousek *et al*. 1997; reviewed in Chen

et al. 2014). N-content in leaves may be mobilized and allocated to reproductive organs, resulting in the inhibition of metabolic processes responsible for photosynthetic N-use efficiency (PNUE) (*e.g.*, Rubisco enzymes) (Feng *et al*. 2009; Millard and Way 2011; reviewed in Chen *et al*. 2014). PNUE is achieved by allocating N to foliar tissues, and this can upregulate intracellular CO_2 concentrations and thus leaf-photosynthesis rates (Case and Ashman 2005). In non-hermaphroditic plants, the absence of male function in females can be associated with upregulated photosynthetic rates, because N that would typically be allocated to pollen production is available for use by photosynthetic machinery or by female function (Case and Ashman 2005).

As already noted, most studies of the costs of reproduction use carbon as the sole currency for measuring costs, potentially ignoring the costs to male function (reviewed in Obeso 2002; Case and Ashman 2005; Dorken and Van Drunen 2012; Wright and Dorken 2014). In the monoecious species *Pinus meziesii*, the allocation of resources to pollen production accounts for less than 10% of the reproductive carbon budget, but more than 25% of the reproductive nitrogen budget (McDowell *et al* 2000; Case and Ashman 2005). Similarly, in *Sidalcea oregana*, pollen production accounted for less than 7% of the total floral carbon allocationbut was found to have high phosphorus concentrations (Hemborg and Karlsson 1999; reviewed in Case and Ashman 2005). Allocations of nitrogen to male function in monoecious and dioecious *Sagittaria latifolia* have shown similar patterns, as pollen production directly traded off against N-content stored in clonal structures, demonstrating that male function is costly in terms of the overall N content of plants (Dorken and Van Drunen 2012).

Many pollen-producing angiosperms (both males and hermaphrodites) can incur high opportunity costs if they begin to invest N to produce pollen early in the flowering season

(Eckhart and Seger 1999; reviewed in Case and Ashman 2005). This early allocation to male function may result in trade-offs that divert resources from maintaining metabolism in foliar tissues and photosynthetic mechanisms (Case and Ashman 2005). This suppression of growth can result in the decreased ability to take up and retain water and nutrients, resulting in indirect, or demographic, costs of reproduction that decrease the resources available for future growth (Case and Ashman 2005). Additionally, pollen-producing plants have been documented to have increased risk and exposure to herbivory during flowering, because the attraction of herbivores to forage pollen and nectar may result in damage to (or the complete removal of) floral and vegetative structures (Agren *et al*. 1999; Ashman 2002; Case and Ashman 2005). This can increase the costs of reproduction as herbivores may influence plants to bear high repair or replacement costs (reviewed in Case and Ashman 2005). Thus, flowers bearing male reproductive organs may withstand increased demographic costs in the presence of predatory organisms, due to their attraction to pollen resources.

The development and maintenance of ovules, seeds, and fruits is associated with female sex function, and requires large allocations of carbon to do so (Schultz 2003; Case and Ashman 2005). Female function differs from male function because seeds and fruits require resource allocations for development, even after flowers have senesced (Schultz 2003). In many cases, the production of fruits and seeds has been documented to enhance leaf respiration rates because the reproductive structures associated with female function can sometimes be capable of self-regulation (*i.e.,* they can respire), and do not require heavy allocations of carbohydrates to maintain (*i.e., Sidalcea hirtipes* in Schultz 2003). Delph and Laporte (1996) found that fruiting females had 30% higher light-saturated leaf-photosynthesis rates by 28 days after flowering than females that were not setting fruit. They found that female reproductive structures are longer-

lived and contribute more carbon to their own support than male reproductive structures and suggested that females have a sink-regulated mechanism of photosynthesis that allow compensation for variations in fruit set (Delph and Laporte 1996). Carbon demand in female function (fruit and seed production) could have a compensatory effect on the total carbon acquisition if allocation to seeds reduces photoinhibition from high concentrations of starch in foliar tissues (Delph and Laporte 1996).

Allocations to female function usually span longer time frames than do allocations to male function. Female function may entail additional costs because these longer time frames can require investments in defensive compounds that deter herbivores and protect developing seeds and fruits (Case and Ashman 2005). For instance, female function in *Pastinaca sativa* (wild parsnip) has been documented to involve the production of furanocoumarins when floral and vegetative structures are damaged by insects, and this can reduce predation by generalist herbivores (Zangerl and Berenbaum 1990; reviewed in Case and Ashman 2005). Female function may also incur costs to construct and maintain support structures (stalks) for developing fruits (*i.e.*, thickening inflorescence stalks to support heavy fruits) (Case and Ashman 2005). Seed dispersal strategies by some plants may also require increased nutrient and energy allocation to occur, especially for armored, fleshy, and ornate pericarps (Case and Ashman 2005).

1.4 *Sagittaria latifolia* - study system

To study the costs of reproduction, Broadleaf arrowhead (*Sagittaria latifolia*, Alismataceae), provides an ideal model. Arrowheads are perennial semi-aquatic macrophytes found in many North American wetlands (Sarkissian *et al*. 2001). This flowering plant is unusual

among the angiosperms in that both monoecious and dioecious populations co-occur, often within the same regions (Wooten 1971; Dorken *et al*. 2001). Thus, *S. latifolia* genets (genetically identical individuals) can be hermaphroditic, male, or female (Wooten 1971). Reproduction occurs both sexually and asexually via the production of clonal propagules (corms) which are also the perennating structures (of which plants invest in for survivability through to the next season) for the plant. Corm production by plants is size-dependent (Dorken and Barrett 2004). Flower production is also size-dependent (Sarkissian *et al*., 2001) and flowers are produced on whorled racemes and pollinated by insects (zoophilous).

Sagittaria latifolia has been used in previous studies of the costs of reproduction (Dorken and Van Drunen 2012; Wright and Dorken 2014). Natural populations of dioecious *S. latifolia* have been sourced for experimental evaluation of reproductive allocations and life-history ecology and evolution. *S. latifolia* has been used to measure how the nitrogen status and content in leaves of male and female plants affects leaf-photosynthesis rates (Wright and Dorken 2014). An earlier study detected a 1:1 trade-off of carbon investment in biomass by female function for fruit/seed production and investment in clonal propagation (Dorken and Van Drunen 2012). While females invested heavily in carbon, they also stored significantly more nitrogen in the overwintering propagules (clonal genets) compared to male plants (Dorken and Van Drunen 2012). This resulted in reduced nitrogen availability stored in males overwintering propagules (Dorken and Van Drunen 2012). While these data show the differential requirements for male and female function in this species, little is known about the implications of lower N availability for plant survival in future generations. This study species (sourced from dioecious populations) is ideal for measuring the costs of reproduction because differences in outcomes of reproductive allocations to male and female function are easily disentangled.

1.5 Objectives

The objective of my thesis was to assess the direct and demographic costs of reproduction in male and female *S. latifolia* plants. I investigated the different reproductive costs of *S. latifolia*, and how these costs subsequently affect male and female growth (*i.e.*, leaf-photosynthesis as a proxy for growth and C-gain), and survivability (*i.e.*, growth and performance in future growth periods). Since background information on the costs of reproduction in dioecious *S. latifolia* is available, I expected that, following reproduction, male plants will be larger than females. Due to the high demand of carbon in females, I predicted that females would be smaller than males, and while males might be larger, they might be of poorer quality due to the expenditure of nitrogen reserves to pollen production. Nitrogen allocation to pollen was further predicted to reduce photosynthetic abilities in males. In this study I experimentally manipulated nitrogen availability through two growth seasons, (1) from seed to plant, to an overwintering propagule and (2), from propagule to plant. In this controlled common garden experiment, I sourced *S. latifolia* seeds from 7 natural populations to produce several male and female plants with a diverse genetic background. Through these efforts, I documented how nutrient availability (particularly nitrogen) affected male and female function and their ability to photosynthesize, produce flowers, and grow, including in the year following reproduction. Overcoming mate limitation through male function could entail reproducing over longer periods, more frequently, and/or expending more resources that yield potentially equivalent demographic costs as for females (Dorken and Van Drunen 2012). I assessed demographic costs by considering post-reproductive growth and survival, and post-reproductive leaf-health in male and female plants *S. latifolia*. There are few studies that have combined

resource gradients, multi-year experimentation, assessment of direct (including physiological) measures with fitness/demographic costs of reproduction. This kind of multifaceted approach will provide new insights into the understanding of the costs of reproduction in plants.

Chapter 2: Investigating the direct and indirect costs of reproduction in *Sagittaria latifolia* under varying environmental conditions

Abstract

Plant fitness is determined by the outcome of allocations to growth, survival, and reproduction. Reproductive allocations can be further divided into allocations to female versus male function and it is generally thought that female function entails greater costs and stronger trade-offs with growth and survival. However, most previous studies have had limited scope, both in terms of the 'currencies' (carbon, nitrogen, etc.) and timescales used to measure reproductive costs. The long-term, demographic costs of allocations to male function remain poorly understood. The objective of this study was to assess the direct and indirect (demographic) costs of reproduction via female and male sex functions using biomass and nitrogen as currencies. We grew Broadleaf Arrowhead (*Sagittaria latifolia*), a dioecious clonal plant in a common garden over two growth periods and experimentally manipulated nitrogen availability and reproductive investment. We measured the direct and indirect costs of reproductive allocations via female versus male function using assessments of plant physiology (including photosynthetic rates), the carbon and nitrogen content of perennating structures, investment in clonal growth, and indices of survival. The costs of reproduction via female and male sex functions depended on the environments in which plants were grown. Under nitrogen-limited conditions, males experienced much higher direct and indirect costs than females. The reverse was true when plants were grown in nitrogen-supplemented conditions, particularly in terms of indirect (demographic) costs - females produced fewer clonal offspring than males under these conditions. This is the first study to demonstrate the environmental dependence of the costs of reproduction and we show that under certain conditions males bear substantially greater reproductive costs than females. Previous findings that females pay greater reproductive

costs might - to some extent - be an artefact of conducting experiments using plants grown under nutrient abundant conditions.

2.1 Introduction

Organisms must expend resources to attain fitness, but the allocation of resources to reproduction results in trade-offs with plant growth and survival (Charlesworth and Charlesworth 1978; Charlesworth, 1989; Ashman, 1994; Obeso, 2002). When resources allocated to reproduction reduce allocations to other life-history components, particularly those affecting fitness (survival and future reproduction) the result is the so-called costs of reproduction. Costs of reproduction involve direct and indirect effects (Obeso, 2002). The allocation of nutrients and energy to reproductive tissues leads to *direct* costs that may limit physiological performance (*e.g.*, reduced photosynthesis, water-use efficiency, etc.). Moreover, because allocations of nutrients to reproduction cannot be recovered and re-mobilized, and nutrients are typically limited in many environments (Saulnier and Reekie 1995), investment in reproduction entails risks to survival, future growth, and fecundity (Bell, 1980; as reviewed in Obeso, 2002; Lambrecht-McDowell and Radosevich 2005; Lin *et al*. 2016), yielding *indirect* (or *demographic*) costs. Sexual reproduction is a pervasive feature of plants (and other eukaryotes). Clearly identifying reproductive costs will improve our understanding of organismal ecology and evolution.

For anisogamous organisms (including plants and animals) reproduction entails separate allocations to female or male sex functions. Female function in plants has long been known to involve large allocations of energy (carbon, biomass) to the production and maintenance of ovules, fruits, and seeds (Ashman 1994). By contrast, male function only directly entails pollen

production (though flowers are often also included as part of male allocations, even for hermaphroditic taxa; Lloyd and Webb 1977; Cipollini and Wingham 1994; Obeso 2002). Because male function involves smaller allocations of biomass (Teitel *et al.* 2015), it has become widely accepted that female function usually comes at a greater cost to growth and survival (reviewed in Obeso 2002). However, biomass is not the only resource involved in reproductive allocations - male function is associated with substantial allocations of nitrogen (Harris and Pannell 2008; Dorken and Van Drunen 2012; Lankinen *et al*., 2013; Teitel *et al*., 2016). Importantly, nitrogen is a key element that often limits overall plant growth and therefore the amount of biomass available for allocation to reproduction (Obeso, 2002). Few studies have assessed these differential reproductive currencies and have only measured costs using biomass (or carbon) (Obeso 2002). Thus, the empirical evidence for the cost of reproduction, particularly as it pertains to male function, remains poorly understood.

Theoretical studies regarding the costs of reproduction have contributed to the idea that costs are greater for female function than for male function. Key assumptions of sex-allocation theory in particular, are that (1) allocations to female and male function are derived from a single pool of resources and that (2) an equivalent allocation of resources to female and male sex functions need not yield an equivalent gain in fitness (*i.e.*, there are distinct fitness gain curves for the two sex functions Figure 2.1; Charlesworth and Morgan 1991). In relation to the second point, it is usually assumed that the gain curve function is steeper for female than for male function, corresponding with Bateman's principles (Bateman 1948; Moore and Pannell 2011). In particular, fitness gains via female function are thought to be primarily resource limited and fitness gains via male function are thought to be mate limited (Bateman 1948). However, even if Bateman's principles apply, the optimal allocations of resources, and the ensuing costs to

reproduction also depend on the first assumption listed above - that there is a single pool of resources shared by both sex functions. If the resources allocated to female function are fundamentally different from those that are allocated to male function, the life-history consequences of resource allocations to reproduction depart significantly from those expected under life-history theory (Silvertown 1987, Dorken and Van Drunen 2018).

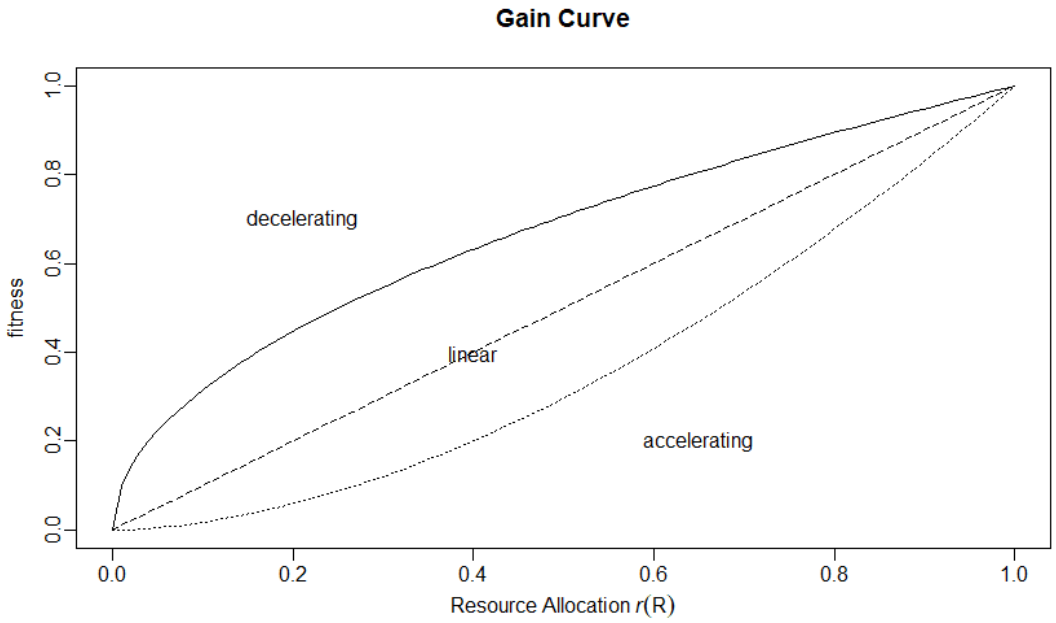

Figure 2.1: Fitness gain curves, demonstrating decelerating, linear, and accelerating fitness returns as functions of resource allocation to reproduction. Here *R* is the total resource pool, of which a fraction, r, is allocated to reproduction. Female function is typically associated with linear fitness gains (corresponding with resource limitation of reproductive fitness), and male function is typically associated with decelerating fitness curves (corresponding with mate limitation of reproductive fitness). Accelerating curves are rare and may only apply to dioecious populations (Charlesworth 1998).

Many studies have indicated that the volume of resources allocated to female function in plants are different from those that are allocated to male function (reviewed in Obeso 2002). In spite of this, previous research on the costs of reproduction have not usually accounted for these

differential currencies involved with male and female function. Sexually dimorphic reproductive currencies could mean that, where a particular resource is environmentally limited, the realized costs of reproduction might be greater for one sex (function) than the other. For example, if male function requires large investments of N, plants in N-limited environments may have greater realized costs of reproduction via male function than plants growing in N-enriched environments. There are few studies that have investigated the costs of reproduction across gradients of resource availability, and therefore whether the costs of reproduction might vary across environments.

To date, no studies have jointly addressed the physiological (direct trade-offs with plant growth or performance in a current growth period) and demographic (indirect or ultimate outcomes) costs of reproduction for male and female sex functions across a resource gradient. Additionally, very few studies have considered multiple reproductive currencies. I hypothesized that the costs of reproduction are determined by the environmental availability of nutrients. Here I addressed the question of whether the cost of reproduction is greater for male than for female function, at least under select environmental conditions. I also predicted that the costs of reproduction (both direct/physiological and indirect/demographic) depend on the environmental availability of the nutrients associated with reproductive investment in female or male function. Tied with this, I predicted that the costs of reproduction would have a temporal component; and that the competing nitrogen demands between pollen production and photosynthesis would yield decreased photosynthetic rates during flowering for males but not females. As a result, under nutrient-limited (N) conditions the demographic costs of reproduction for males might exceed that of females. To test my predictions, I utilized an experimental manipulation of resource availability and reproductive investment to assess the costs of reproduction in a dioecious

perennial, *Sagittaria latifolia*. Physiological costs of reproduction were evaluated by measuring photosynthetic (PS) rates before, during, and after flowering and the N content of plants after flowering. To examine the demographic costs of reproduction, I measured the number, growth and performance of perennating structures and grew them under nutrient-limited conditions. Through these measures, I was able to relate how instantaneous costs scale-up and (or to) might affect growth and survival in subsequent growth periods. I found that allocations of nitrogen to male function under low nutrient conditions can yield greater direct and indirect reproductive costs for males than for females.

2.2 Methods

2.2.1 Study Species – *Sagittaria latifolia* Willd. (Alismataceae)

Broadleaf Arrowhead (*Sagittaria latifolia* Willd.) is a zoophilous, clonal emergent aquatic native to many North American wetlands (Sarkissian *et al*. 2001; Dorken *et al*. 2002). This plant is unusual among angiosperms in that natural populations may be either dioecious or monoecious (Wooten 1971; Dorken *et al.* 2001). As a result, three sex phenotypes occur in natural populations of *S. latifolia*: hermaphrodite plants (in monoecious populations), and females and males in dioecious populations (Wooten 1971).

The flowers on *S. latifolia* inflorescences are formed in whorled racemes, with three flowers per node. Flowers are open for a single day and during the flowering season, reproductive ramets (clones) can produce multiple inflorescences that develop in sequence. Each flower has three showy white deciduous petals, and inflorescences typically contain 3-6 whorls, but the number of flowers produced is highly variable and strongly size dependent (Sarkissian *et al.* 2001). The flowers are commonly visited by non-specialist pollinators, including hover flies, wasps, and solitary bees. In the region around Peterborough, Ontario, Canada, flowering in dioecious populations typically occurs from July through early-September, and fruits and seed maturation lags flowering by approximately four weeks. Male *S. latifolia* flowers contain 20-40 yellow pollen-bearing stamens and 2-10 rudimentary (and non-functional) carpels (Kaul, 1967). Female flowers develop rounded green domes of unfused gynoecia that later mature into hundreds of individual achenes (Kaul, 1967) (See Fig 2.2).

Figure 2.2: Male and female inflorescences of dioecious *Sagittaria latifolia* plants. Note the yellow pollen-bearing stamens in the left hand panel, which is absent in the right hand panel.

Populations of *S. latifolia* are maintained via sexual and asexual (clonal) reproduction. Clonal propagation occurs through the sprouting of daughter ramets at the terminal end of axillary stolons. Later in the summer, corms (the perennating structure) are formed at the ends of the stolons, typically after peak flowering. The spread of vegetative and dormant (corms) ramets results in the localized spread of ramets within habitats (Holt *et al.* 2020; Stephens *et al.* 2021). At the end of the growing season, all vegetative biomass dies back leaving seeds and corms as the only types of living plant tissue (see Fig. 2.3).

Figure 2.3: Corm produced by female *Sagittaria latifolia*, grown in the summer of 2020 in Lakehurst, ON. Corms are the sole perennating structure.

2.2.2 Source Populations

Samples of open-pollinated seeds were collected from seven dioecious populations of *Sagittaria latifolia* in southern Ontario (Supporting Information, Table 1). Source populations were located across southern Ontario, predominantly at sites within 2 km of the shores of Lakes Erie, Ontario, and St. Clair (from dioecious populations). Seeds were sampled in 2017 from female plants separated by at least 2 m apart to avoid re-sampling seeds from the same genet (*i.e.,* from the same clone where genet refers to the genetic individual arising from a single

mating event). I germinated seeds from a total of 160 different maternal parents (see number of maternal parents in Table 1).

Table 2.1. Locality information for the seven source populations of *Sagittaria latifolia*, including the number of maternal seed families used for generating the maternal seed used in the experiment.

Site Location	Site Code	Latitude	Longitude	*n*	Habitat
Actinolite	ATL	N44.54853	W77.32333	32	shallow pond
Deerbrook	DER	N42.29376	W82.62192	64	agricultural ditch
Ecole St. Paul	ESP	N42.30619	W82.54784	98	roadside ditch
Long Point	LTP	N42.58022	W80.44023	68	roadside ditch
Point Pelee	PPL	N42.00621	W82.49547	47	agricultural ditch
Stoney Point	STP	N42.30486	W82.53218	25	roadside ditch
Stromness	STB	N42.88404	W79.55177	51	shallow pond

2.2.3 Plant Material and Propagation

In February of 2020, I placed 30-50 seeds from each maternal parent in 1.5 ml microtubes filled with deionized water for three weeks to break seed dormancy. In March, I took 20 seeds from each parent plant (3,200 seeds total) and placed them into 200-cell plug trays (one seed per cell; T.O. Plastics Inc.) that had been pre-filled with an equal mixture (by volume) of play sand (Premier™) and professional growing mix (Mix #15, Sun Gro Horticulture®, Agawam, MA, USA). Most seeds germinated within 1 week. Germination took place under greenhouse conditions at Trent University, Peterborough Ontario. To encourage establishment, seedlings were given nutrient applications (Plant-prod 20-20-20 Classic, Plant-Prod Inc.) on a

weekly basis (0.1% of a 50 mL solution 20:20:20 N:P:K). In April, I selected the largest 390 plants and transplanted them into larger 2-inch square nursery pots (T.O. Plastics Inc., Toronto, Ontario) using the same soil/sand mixture. After 12 weeks of growth, these plants were repotted into 8-inch azalea pots (same soil/sand mixture), placed into 5 L buckets and moved outside and positioned at random into a 12 x 6-meter grid in a research garden located in Lakehurst, ON, Canada on May, 24 2020. Pots were watered using treated and filtered well water alongside Pigeon Lake, ON and water levels were maintained at a standard of 4 L. On June 1st, I measured the mid-vein length (the length of the leaf blade from its attachment to the petiole to the leaf tip, hereafter referred to as MVL) of every plant to account for variation in size prior to experimental manipulations. MVL is strongly correlated with ramet size (Sarkissian *et al.* 2001; Dorken and Barrett, 2003).

2.2.4 Experimental Manipulation and Design

Nutrient Availability

I manipulated the availability of nutrient resources for all genets by placing individuals in a resource gradient of fertilizer treatments. I utilized a fully factorial design using the R package DoE.base (Grömping, 2018) to assign plants to positions in the garden, and to levels of the nutrient-addition treatment. Nutrients were added to plants using dilutions of one of three stock solutions of water-soluble Plant-Prod Classic 20:20:20 N:P:K nutrient fertilizer (Plant-Prod Inc., Toronto, ON, CA): (1) Low: 2 g per 6.5 L (0.03% solution), (2) Medium: 20 g per 6.5 L (0.3%), and (3) High: 195 g per 6.5 L (3%). Each week, 50 mL of the corresponding stock solution was applied to each plant, starting on June 2, 2020, until August 19th, 1 week after peak flowering.

Flower Removal

To manipulate reproductive investment through male and female function, I removed flowers to prevent allocations of nitrogen and biomass to reproduction. To do this, beginning in mid-July, I tagged and recorded the sex of each genet as soon as inflorescences emerged. All plants were randomly assigned to flower-removal treatments (henceforth referred to as 'neutered' and 'intact' genets) before flowering occurred. Because the sex of *Sagittaria latifolia* is determined by simple Mendelian segregation of alleles at a single locus (Dorken and Barrett 2004), I expected an approximately even number of males and females in each treatment group. Flowering occurred for 80 days and plants were monitored every other day during this timeframe (June-August). For each genet in the intact group, I recorded the total number of flowers produced.

Table 2.2: Total number of genets (sample size = N) used in the three nutrient treatment levels, including the flower removal treatment groups.

Plant Gender Class	**N = Low**	**N = Medium**	**N = High**	**Total**
Male Intact	22	38	35	95
Male Neuter	38	32	23	93
Female Intact	18	15	27	60
Female Neuter	17	25	17	59
Un-sexed Plants	37	25	29	91
Total	132	135	131	398

Photosynthetic rates

I measured photosynthetic rates (*i.e.,* net carbon assimilation in $\mu mol \cdot m^{-2} \cdot s^{-1}$) to document how reproductive allocations under different nutrient treatments affected physiological performance. I measured photosynthetic rates using a LI-6400XT (LI-COR inc, Lincoln, Nebraska, USA) portable photosynthetic meter (1) before, (2) during, and (3) after peak flowering. Measurements for neutered plants were recorded on the same days before, during, and after peak flowering for intact plants. Additionally, I measured light saturation curves in a random subset of plants from males and females in each treatment level before, during and after flowering occurred (5 males and 5 females from each nutrient treatment level in each time period, total $n = 30$). The response curve readings were used as visual descriptions of the response of each sex to nutrient limitation during each reproductive stage. I conducted measurements between 0800 hours to 1100 hours local time on calm days with full sun-exposure (non-cloudy) and air temperatures between 24 and 32 °C, following best-practice procedures for the measurement of photosynthetic rates (PrometheusWiki, 2013). The youngest, entirely unfurled leaf large enough to fit within the gas-exchange chamber was used for each measurement. The LI-6400XT is an open-flow system, whereby standardized ambient conditions were set for each PS measurement. I set the chamber conditions in the LI-6400XT to the following: 400 $\mu mol\ CO_2 \cdot mol^{-1}$; 65% RH (relative humidity); 1500 $\mu mol \cdot m^{-2} \cdot s^{-1}$ PAR (photosynthetically active radiation); 30 °C air temperature; and 200 $\mu mol\ CO_2 \cdot mol^{-1}$ intracellular CO_2 (Ci) (Prometheus Wiki, 2013).

Post-reproductive harvest

To measure trade-offs between reproduction and total plant growth, I harvested fruits and corms from all genets. As fruits matured, I collected, counted, and weighed all fruits produced by female genets (August through September) and then summed these values to obtain a total fruit

count and mass for each genet. Fruits were placed into labeled paper bags, air dried in a ventilated room and then sealed in air-tight containers. Once all aboveground biomasses had died back (after the first hard frost in October), I harvested, cleaned, counted, and weighed the fresh mass of all corms from each genet. Corms were then stored in labeled plastic zip-lock bags. The harvesting of corms took place over 7 days. I then stored all corms at 5 °C in a cold room at Trent University until analysis, approximately 5 months later.

Elemental analysis of corms

To measure the nitrogen content of corms, I sampled the largest corm produced by each genet for elemental analysis. Each corm was dried at 60 degrees Celsius for 48 hours in the Binder BD 720 oven (Binder GmbH inc, Tuttlingen, Germany). Dried corms were ground to a fine powder using a coffee bean grinder. I then took 50 mg of the homogenous powder mixed with 75-100 mg tungsten (a combustant) and ran samples through an Elementar vario MACRO cube CNS (carbon, nitrogen, and sulfur) analyzer (Elementar Americas, Inc., Mt. Laurel, New Jersey). The data are reported here as % mass of the subsample; these values were then scaled by total corm mass to estimate total C, N, or S (Carbon, Nitrogen, and Sulphur) per corm, and then multiplied by total corm mass per genet to estimate total nitrogen content per genet.

Post-reproductive plant performance

Starting in March of 2021, I grew daughter ramets (corms) from the plants harvested in 2020. Ramets were grown under experimental conditions over a 75-day period until May 2021. For each genet, I selected the largest remaining corm produced by each genet (after selecting corms for CNS analysis) and grew them out in a greenhouse at Trent University. I placed each corm at a 6 cm depth in 4” round pots filled with an equal mixture (by volume) of play sand

(Premier™) and professional growing mix (Sun Gro Horticulture®, mix #2, Brantford, Ontario, Canada), and provided weekly watering with municipal tap water. Plants were randomly placed in water-filled trays, each containing nine plants). During the entire duration of the trial plants were not given any supplemental nutrient fertilizer. At the end of the trial, on May 15, 2020, I measured the length of the newest entirely unfurled leaf to estimate the size of the largest ramet per pot. On the same day, I sampled leaf Chlorophyll content using a SPAD 502 Plus meter (Minolta ®, Azuchi-Machi, Chuo-ku, Osaka, Japan). This tool is non-destructive to foliar tissues and is used in agricultural practices to detect how Chlorophyll and Rubisco content in leaves is affected by the status of nitrogen by an individual plant simply by reading the degree of greenness on a single leaf (Kumagai *et al.* 2009).

2.2.5 Statistical Analyses

I conducted analyses using R v. 4.1 within R studio v. 1.3.1093 (R Core Team 2021; RStudio Team 2021). To measure variation in photosynthetic rates among female and male genets subjected to the flower removal and nutrient addition treatments, I utilized a fixed-effects linear regression model using the 'lm' function from the lme4 package (v.1.1-25, Bates *et al.*, 2012). The fixed effects included nutrient treatment (low, medium, high), flower removal (neuter and intact), time (before, after, and during flowering for leaf-photosynthesis measures), and sex (male, female). For this analysis, source population was excluded as a random effect because its inclusion yielded an overfit model. I tested for the significance of fixed effects using a type "III" analysis using the 'Anova' function from the 'car' package (v. 3.0-10, Fox & Weisberg 2018). A post-hoc test (Tukey's HSD) was then performed using the 'emmeans' function from the 'emmeans' package (v. 1.5.2-1, Lenth *et al.*, 2018) to assess pairwise comparisons between females and males across levels of the nutrient-addition treatment.

To evaluate whether patterns of corm production differed among genets depending on sex, flower-removal, and nutrient treatment, I utilized a generalized linear mixed model (GLMM). For this analysis, the total number of corms produced by each genet was the response variable, sex, treatment level, and flower-removal were included as fixed effects, and source population was included as a random grouping effect. I specified a poisson error distribution because the number of corms produced was count data. I then utilized type III SS using the 'Anova' function to test the significance of the highest-level interactions. The 'emmeans' function was again used to perform post-hoc (Tukey's) test of comparisons.

To detect whether patterns of corm mass were affected by differences among genets in terms of their sex, treatment level, and flower-removal status, I employed a linear mixed-effects model using the 'lmer' function. Sex, treatment level, and flower-removal status were specified as the fixed effects, while source population was the random grouping effect. A type III test was then utilized on the corm weight model using the 'Anova' function to test the highest-level interactions, followed by a post-hoc (Tukey's) test using the 'emmeans' function to assess pairwise comparisons of the means.

To measure whether patterns of stored nitrogen content in corms was affected by sex, treatment level, and flower-removal status, I utilized a linear mixed-effects model using the 'lmer' function. For this model, corm nitrogen content was log-transformed to meet assumptions of normality and homoscedasticity. Sex, treatment level, and flower-removal status were specified as the fixed effects while source population was included as a random grouping effect. A type "III" SS 'Anova' test was then used on the nitrogen content in corms to test the highest-level interactions, followed by a post-hoc (Tukey's) test using the 'emmeans' function to assess pairwise comparisons.

To test whether future growth performance and survival was affected by reproduction and nutrient availability in the previous growing season among female and male genets, I utilized linear mixed-effects models (lmer). For these tests, plant size at the end of the 2021 growth period (measured via MVL) and chlorophyll content in leaf tissues were the response variables. For both analyses, sex, treatment level, and flower removal status were included as the fixed effects with the source population as the random grouping effect. I then used type III SS using the 'Anova' function on both the MVL and chlorophyll models to test the highest-level interactions. A post-hoc (Tukey's) test was then performed using the 'emmeans' function to assess pairwise comparisons.

2.3 Results

2.3.1 Direct costs - Physiological Responses

Leaf-photosynthetic rates varied in a complex manner that depended on nutrient levels, sex, and whether photosynthesis was measured before, during, or after flowers. Patterns of light-response curves taken from intact plants before flowering show that the nutrient treatment did not have strong effects on photosynthetic responses to light availability, particularly for females (Figure 2.4A). This is not surprising - at this early stage of the experiment plants had only been subject to differences in nutrient applications for 2 weeks. During peak flowering, photosynthetic rates appeared to plateau at lower light availability in the low and medium nutrient availability treatments, particularly for male genets under medium-nutrient conditions (Figure 2.4B). However, comparison with maximum photosynthetic rates from a larger sample of plants under the same conditions (Figure 2.5) indicates that this result, which was driven by two plants with low photosynthetic rates, may not have been representative. The clearest difference in light-response curves between the sexes came after flowering for intact male plants grown under low nutrient availability (Figure 2.5C). Under these conditions, males had markedly lower photosynthetic rates across the range of light availability. The difference in leaf PS was much less apparent under medium nutrient levels and disappeared completely under high nutrient levels (Figure 2.4C).

Patterns of photosynthesis rates (PS rates) among plants were consistent with my prediction that the direct costs of reproduction would depend on environmental conditions. Before flowering, photosynthetic rates were similar between the sexes and were mainly influenced by nutrient availability. In particular, there was a general increase in PS rates from the low through medium to high nutrient treatments (Figure 2.5A). During peak flowering, the sexes

did not differ strongly in PS rates. Both intact and neutered genets (across all nutrient treatments) experienced a general reduction in PS rates in comparison with PS rates before and after flowering (Figure 2.5B). By contrast, after peak flowering, both plant sex and nutrient levels affected PS rates. Male genets that had flowered (intact males) in low nutrient conditions had the lowest PS rates (Figure 2.5C). This pattern was also evident - to a more limited extent - under medium-nutrient conditions where intact males tended to have lower PS rates than females. Females, by contrast, maintained relatively high PS rates across nutrient treatments. As happened before flowering, there was also a general trend for greater PS rates across the nutrient treatment gradient, with highest PS rates for plants in high-nutrient conditions (Figure 2.5).

As a result of these complex patterns of leaf-photosynthesis over time, and in response to plant sex and nutrient availability, there was a statistically significant three-way interaction between plant sex, nutrient availability, and time ($F_{4,824} = 4.38$, $P < 0.01$; Fig. 2.4). Intact male genets had broadly similar PS rates compared to other plants grown under low-nutrient conditions before and during flowering but, after flowering occurred, PS rates for intact males under low nutrient conditions were significantly inhibited. After flowering, intact males grown in nutrient starved treatments had 57% lower average PS rates on average than did intact females in the same nutrient treatment group (mean PS rate for intact males = 5.55 $\mu mol \cdot m^{-2} \cdot s^{-1}$ ± 1.82 SD vs. intact females = 12.8 $\mu mol \cdot m^{-2} \cdot s^{-1}$ ± 4.3 SD; *t*-ratio = 4.18, $P = 0.01$, See Fig 2.5C). These intact males grown in low-nutrient conditions also had significantly lower PS rates (by 59% on average) than neutered males in low nutrient treatments (mean PS rate for intact males = 5.55 $\mu mol \cdot m^{-2} \cdot s^{-1}$ ± 1.82 SD vs. neutered males = 13.6 $\mu mol \cdot m^{-2} \cdot s^{-1}$ ± 5.1 SD; *t*-ratio = -5.52, $P = 0.0001$ See Fig 2.5C). PS rates for intact males in low nutrients were significantly lower than intact males in high nutrients after flowering by 73% on average (mean PS rate for intact males =

5.55 μmol·m^{-2}·s^{-1} ± 1.82 SD vs. high intact males = 17.9 μmol·m^{-2}·s^{-1} ± 4.87 SD; *t*-ratio = -8.23, *P* = 0.0001, See Fig 2.5C). Similarly, after flowering, intact males in the medium nutrient treatment also had significantly lower PS rates than intact males in high nutrients by 34% on average (mean PS rate for intact males in intermediate nutrients = 11.9 μmol·m^{-2}·s^{-1} ± 3.7 SD vs. high intact males = 17.9 μmol·m^{-2}·s^{-1} ± 4.87 SD; *t*-ratio = -4.56, *P* = 0.003, See Fig 2.5C).

While PS rates for intact males that flowered in low nutrient conditions were significantly lower than for all other treatment groups, there were other general differences in PS rates between the sexes before and after flowering that contributed to the strong 3-way interaction described in the previous paragraph. For example, under intermediate and high nutrient conditions, males and females had broadly similar PS rates before and during flowering, but after flowering, males tended to have somewhat lower PS rates than females under intermediate nutrient conditions. Specifically, intact males in intermediate nutrient treatments had 28% lower average PS rates than intact females in this group (mean PS rate for intact males growing in intermediate nutrient conditions = 11.9 μmol·m^{-2}·s^{-1} ± 3.70 SD vs. medium intact females = 16.55 μmol·m^{-2}·s^{-1} ± 6.53 SD), and 32% lower average PS rates than neutered females (17.8 μmol·m^{-2}·s^{-1} ± 4.80 SD). Under high nutrient conditions, both groups of males (intact and neutered) had on average 14% higher PS rates than females.

2.3.2 Trade-offs with Clonal Nitrogen Storage

Males and females responded in a similar manner to the nutrient addition and flower-removal treatments in terms of their total N content after flowering (measured as corm N content), with no significant 2-way interactions between the sex and either the nutrient-addition treatment (χ^2 = 0.28, *df* = 1, *P* > 0.05) or the flower-removal treatment (χ^2 = 0.32, *df* = 1, *P* >

0.05). In general, corm N content increased with the amount of nutrients added (χ^2 = 40.61, *df* = 2, *P* < 0.001; See Fig 2.6A). Under high-nutrient conditions plants had 16% more stored N per unit mass in their corms than plants grown in medium nutrients, and 22% more N than plants grown in low nutrient conditions (%N in corms of low nutrients = 4.25% ± 0.73 SD, medium nutrients= 4.51% ± 0.7 SD, high nutrients = 5.4% ± 0.85 SD). There was also a significant statistical interaction between the nutrient addition and flower-removal treatments (χ2 = 6.5, *df* = 2, *P* < 0.05), and Post-hoc revealed that was driven by a significant reduction in stored N for neutered females grown in high nutrients, which had 12% less stored N than intact females (%N content in corms of intact females = 5.51% ± 0.51 SD, neutered females = 4.87% ± 1.3 SD). Under low-nutrient conditions, females, and neuter females in particular, had an average of 1.5% more N content than males (% N content in the corms of low-nutrient females = 4.26% ± 0.75 SD, low-nutrient males = 4.2% ± 0.72 SD). By contrast, under high-nutrient conditions, females tended to have the lowest N content and had an average of 8% less N than did male genets in high nutrients (%N content in corms of females= 5.19% ± 0.9 SD, males = 5.6% ± 0.78 SD).

2.3.3 Trade-offs with Clonal Biomass

As expected, the average biomass of clonal propagules produced by males and females (both intact and neutered) was significantly driven by nutrient treatment level, and those grown under high-nutrients conditions had significantly more biomass than plants grown in low- and medium-nutrient conditions (χ^2 = 37, *df* = 1, *P* < 0.001; See Fig 2.6C). Surprisingly, sex was not a significant determinant in the outcome of average corm biomass (χ^2 = 0.72, *df* = 1, *P* > 0.05). I expected there to be a strong relationship between sex and flower removal treatments, but this interaction was not statistically significant (χ^2 = 0.09, *df* = 1, *P* > 0.05).

2.3.4 Trade-offs with Future Growth (corm count)

Nutrient availability, combined with flower production, had strong effects on corm production. The average number of corms produced by each plant was significantly driven by an interaction between nutrient treatment level and the flower-removal treatment (χ^2 = 6.6, *df* = 2, *P* < 0.05). Unexpectedly, plants that produced flowers produced more corms than plants that were neutered. Post-hocs tests revealed that intact plants produced more corms on average than did those that were neutered. Under high-nutrient conditions, intact males produced 10% more corms on average (mean corms/plant= 31 ± 13.60 SD) than neutered males in the same nutrient treatment group (mean = 28.2 ± 19.4 SD, *z*-ratio = 8.74, *P* = 0.0001, See Fig 2.6B). Additionally, intact males grown under high-nutrient conditions produced 15.3% more corms (mean corms/plant = 31 ± 13.60 SD) than intact females (mean corms/plant = 26.3 ± 15.40, *z*-ratio = -5.57, *P* = 0.0001) and 34% more corms than neutered females (mean corms/plant = 20.2 ± 11 SD, *z*-ratio = -5.57, *P* = 0.0001). Thus, the interaction between nutrient treatment levels and flower removal revealed that flowering genets produced more corms on average than neutered genets.

Leaf Nitrogen Content (leaf greenness)

Leaf N differed between females and males, particularly under high-nutrient conditions, yielding a significant two-way interaction between plant sex and nutrient treatment levels (χ^2=7.12, *df* = 2, *P* < 0.01). This interaction was driven by higher average leaf-greenness for males versus females under high nutrient treatments (Figure 2.7A). To a lesser extent, a difference between the sexes in the other direction, with males having lower leaf greenness, was

evident under low nutrient treatments. Post-hoc (Tukey's test) revealed that intact males previously grown in low nutrient conditions had 84% lower leaf-greenness (mean greenness: 10.1 ± 5.13 SD) compared to intact males in medium nutrients (mean ± SD: 18.6 ± 9.17, *t*-ratio = -4.19, *P* = 0.01), 191% lower than intact males in high nutrients (mean ± SD: 29.4 ± 6.5, *t*-ratio = -9.54, *P* = < 0.0001), 84% lower than neutered males in low nutrients (mean ± SD: 18.6 ± 7.61, *t*-ratio = -4.18, *P* = 0.01), and 210% lower than neutered males in high nutrients (mean ± SD: 31.3 ± 6.87, *t*-ratio = -5.18, *P* = 0.0002). These intact males in low nutrients also had 105% lower leaf-greenness than did intact females in medium nutrients (mean ± SD: 20.7 ± 8.79, *t*-ratio = -3.91, *P* = 0.04), 153% lower than intact females in high nutrients (mean ± SD: 25.6 ± 8.4, *t*-ratio = -7.10, *P* = < 0.0001), and 167% lower than neutered females in high nutrients (mean ± SD: 27 ± 7.64, *t*-ratio = -6.82, *P* = < 0.0001). Surprisingly, intact males and females in low nutrients were not significantly different from one another (*t*-ratio =1.76, *P* = 0.999), although intact male leaf N-content was observably lower on average (see Fig 2.7A)

Plant Size After 75-days

Plant size (measured as leaf midvein-length) after the 75-day growth trial showed that as predicted, intact males that flowered in low-nutrient conditions were smallest compared to males and females across nutrient treatment groups (Fig. 2.7B). In the medium nutrient treatment group, intact and neutered females had the largest average MVL. In the high nutrient treatment group, intact and neutered males were both smaller than intact and neutered females. This contrasts with the corm mass results that indicate females produced corms of lower average biomass and indicates that females may have a greater tendency to start growth from corms in future generations. Additionally, there is a general effect of nutrient treatment in previous seasons growth and the outcome of plant size after 75-days, whereby plants were able to grow to

be larger with the greater amount of nutrients they had in the previous growth period. This supports the prediction that males withstand survival costs in a different way than females do, as nitrogen availability had an influence on plant fitness in a subsequent growing season.

Plant size (measured as leaf mid-vein length, MVL) was affected by previous reproduction and plant sex, yielding a significant two-way interaction between sex and the flower-removal treatment for plant size ($F_{1,266} = 16.25$, $P < 0.007$). This interaction was driven by stronger reductions in plant size for intact males versus intact females. For example, intact males grown under high-nutrient conditions in the previous year were 30% smaller on average than intact females in the same nutrient treatment group (MVL for high-nutrient intact males = 5.57 cm ± 1.88 SD; high-nutrient intact females = 7.41 cm ± 1.59 SD; Tukey pairwise comparison *t*-ratio = 4.229, $P = 0.002$). Plant size was also significantly determined by plant sex ($F_{1,266} = 17.4$, $P < 0.05$), with intact males being generally smaller than intact females across nutrient treatment levels. For example, In the high nutrient treatment group, intact males were 22% smaller than intact females (MVL for high-nutrient intact males = 5.73 cm ± 1.88 SD; high-nutrient intact females = 7.41 cm ± 1.59 SD; Tukey pairwise comparison *t*-ratio = 4.229, $P = 0.002$).

Figure 2.4 Light saturation curves before, during, and after flowering.

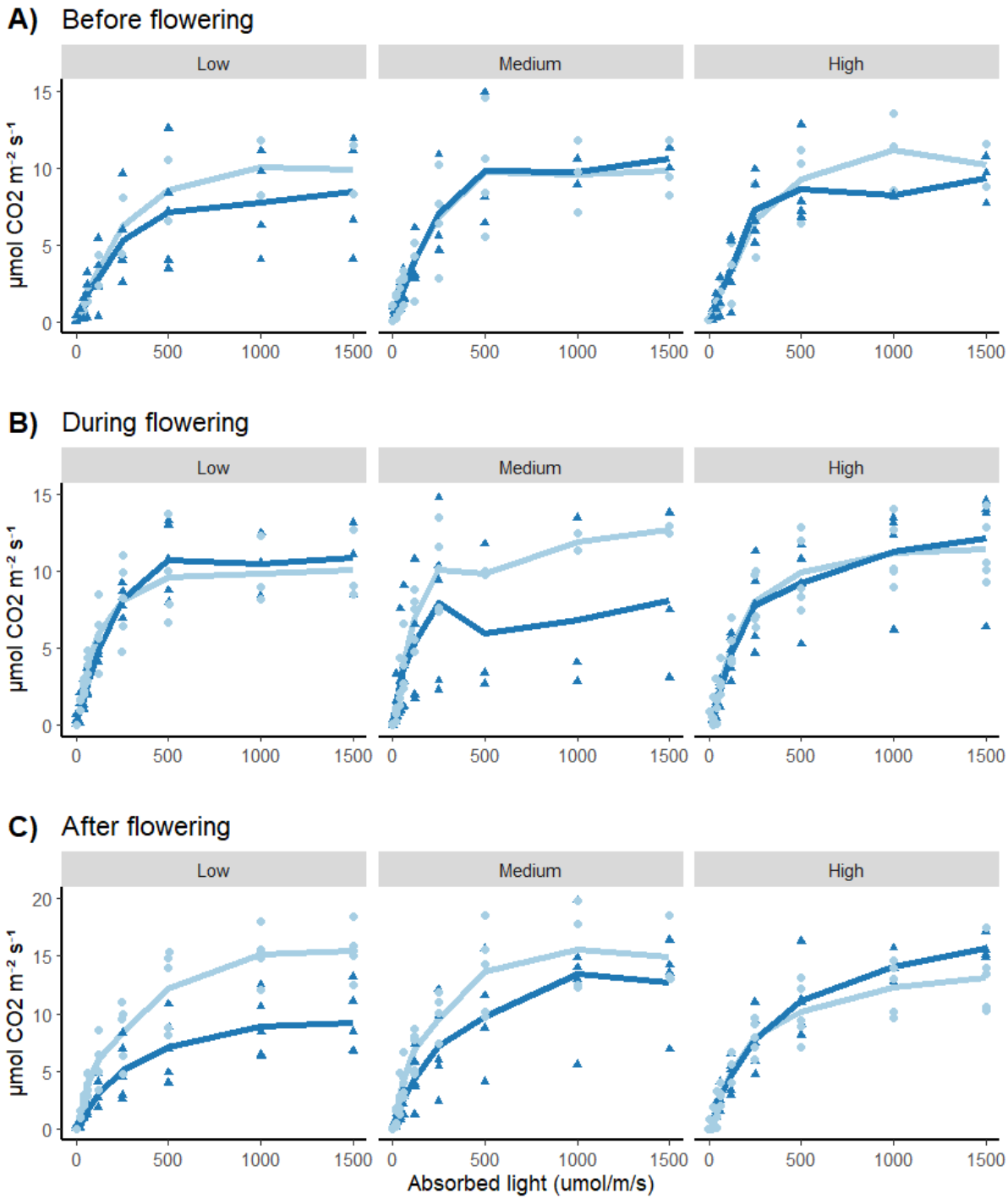

Figure 2.4: Light saturated response curves (increases in photosynthesis (µmol/m^{-2}/s^{-1}) as the intensity of photons (µmol/m^{-2}/s^{-1}) is absorbed by leaf tissues) before, during, and after flowering occurred (intact genets). Data are plotted on a response scale indicated by mean values.

Figure 2.6 Leaf PS before, during, and after flowering

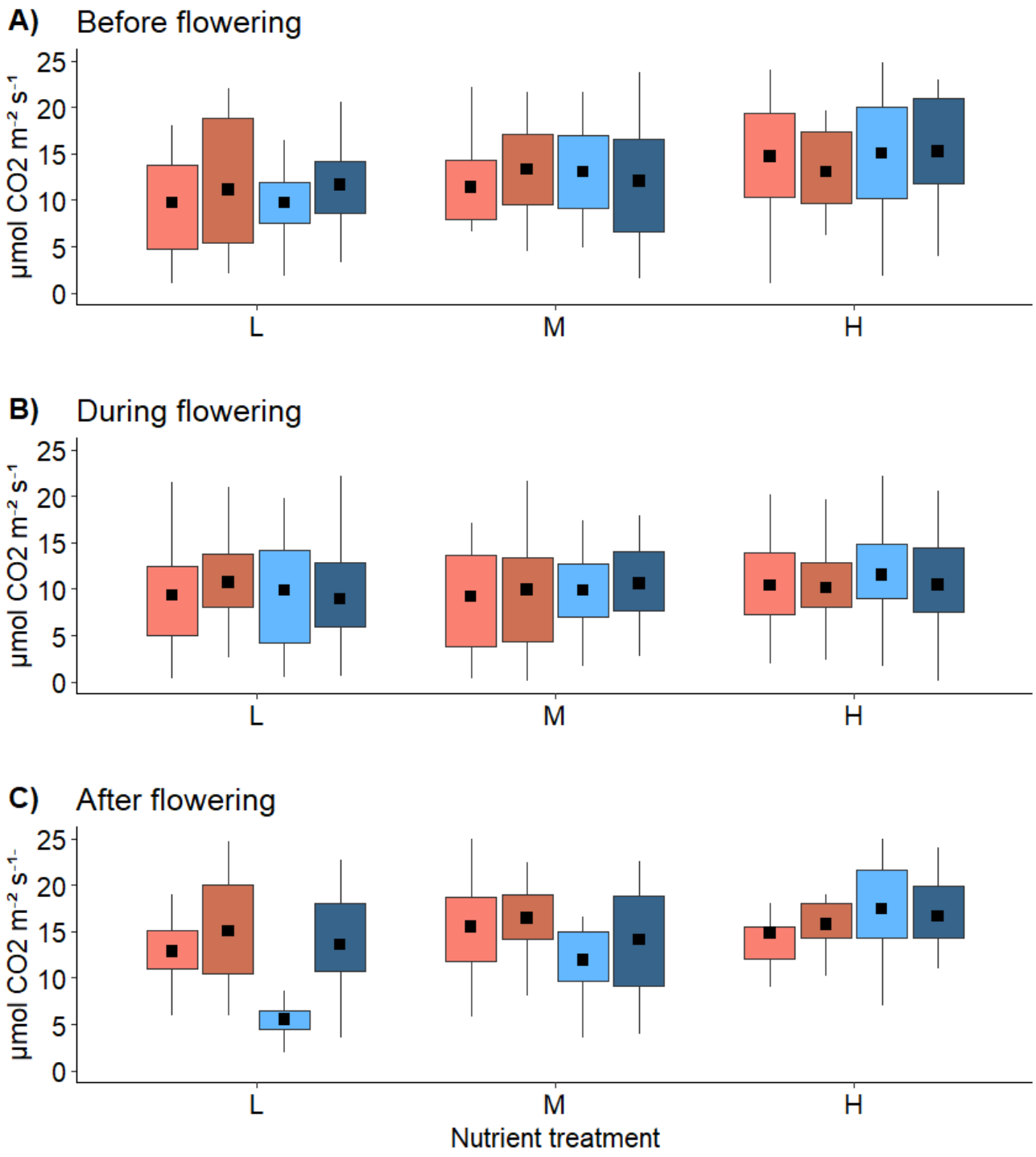

Figure 2.5: Boxplot comparisons for leaf photosynthesis (µmol/m^{-2}/s^{-1}) rates between intact and neutered male and female genets in low (L), medium (M), and high (H) nutrient treatment levels before, during, and after flowering had occurred.

2.6 Genet nitrogen content, count, and mass

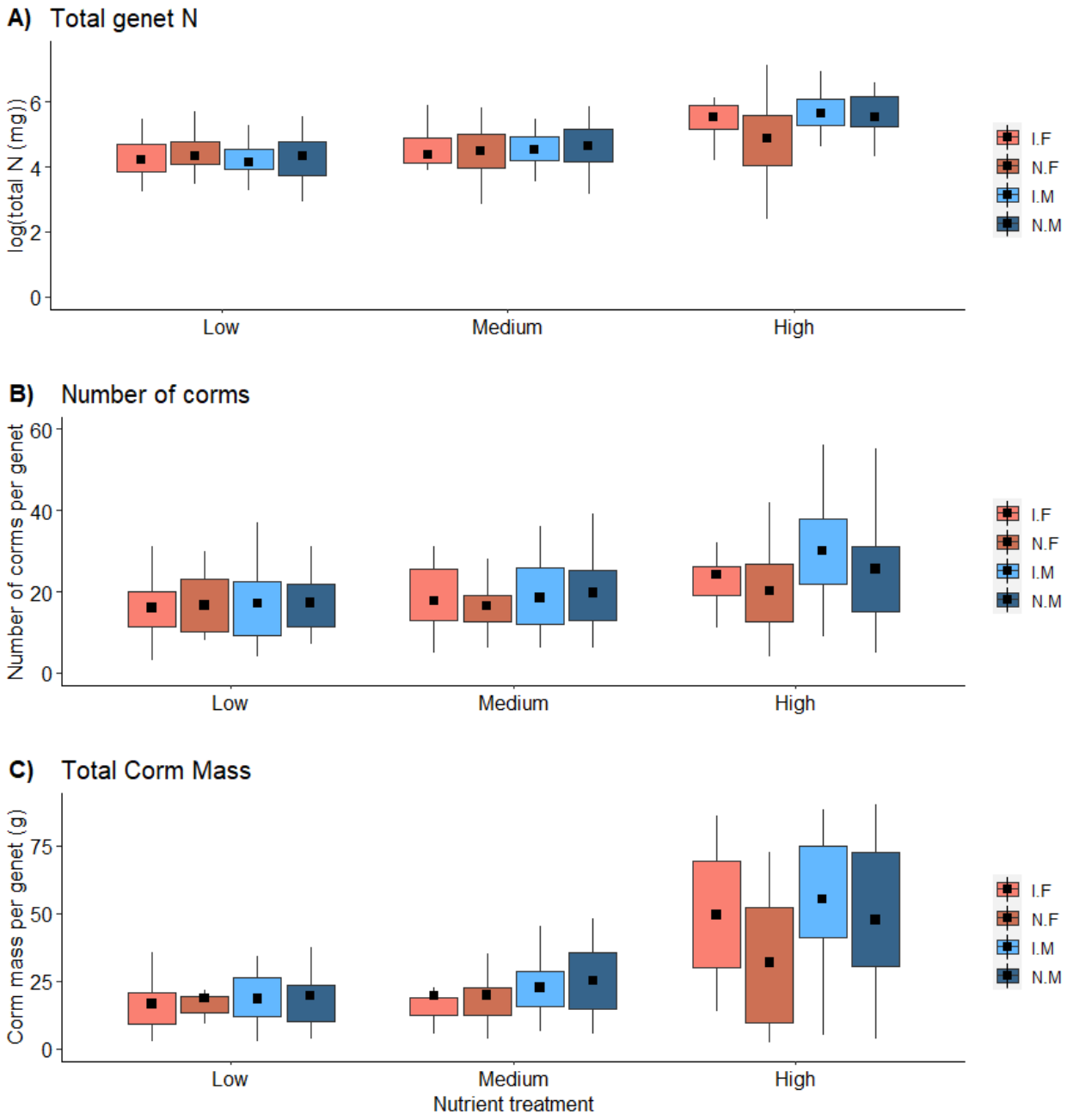

Figure 2.6: A) Boxplot comparisons for the mean log-transformed total nitrogen (mg) content stored in corms of the intact and neutered males from low, medium, and high nutrient treatment groups. B) Comparisons of the mean mass (g) of corms generated by intact and neutered males and females from low, medium, and high nutrient treatment groups. C) Comparisons of the mean corm numbers produced by intact and neutered males and females from low, medium, and high nutrient treatment groups.

2.7 Leaf greenness and plant size

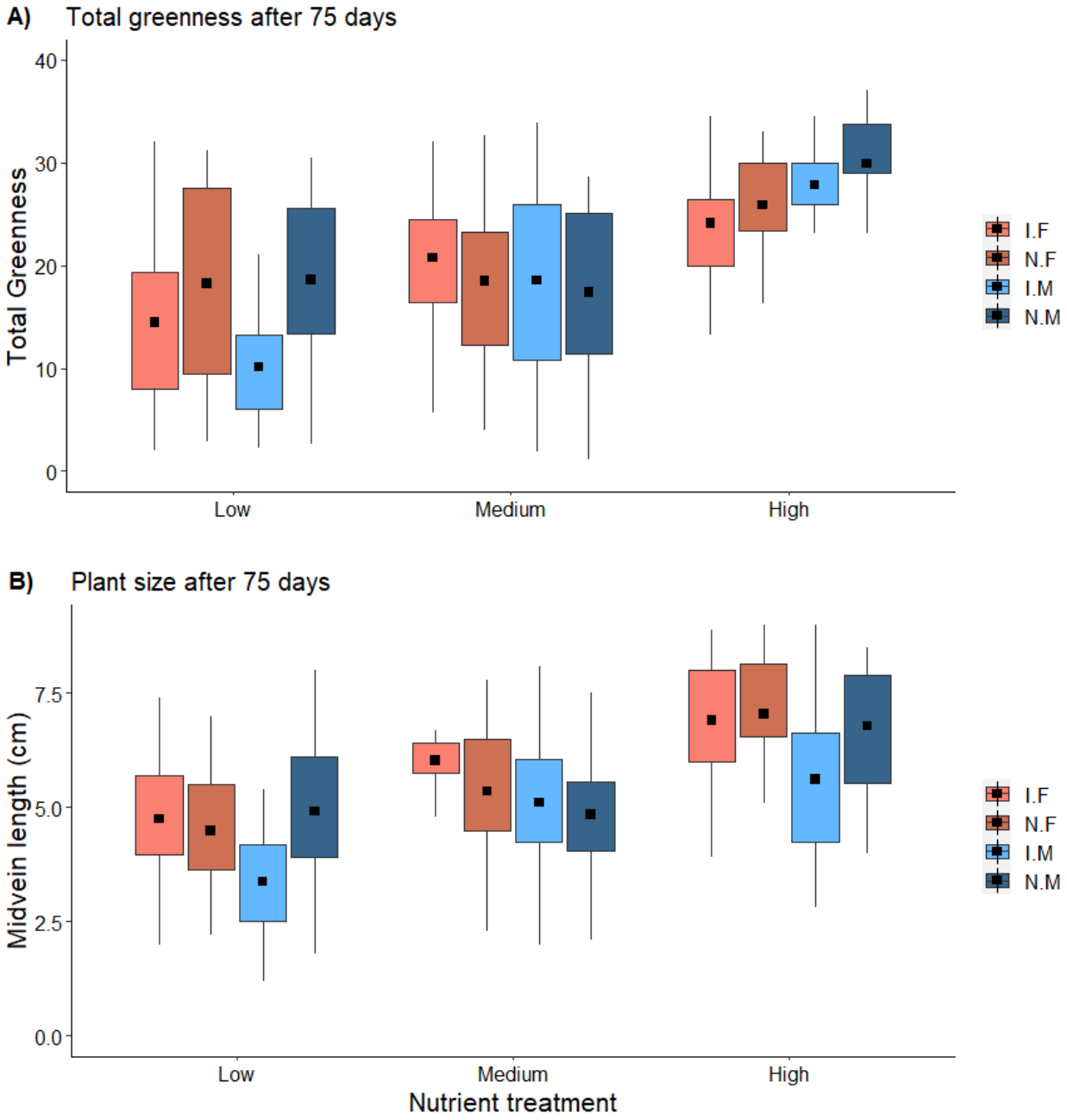

Figure 2.7: Boxplot comparisons of the mean leaf greenness (µmol per m^2 of leaf tissue) values for intact and neutered males and females of low, medium, and high nutrient treatment levels following the 75-day survival experiment. Plot B) indicates the mean midvein lengths (cm) for neuter and intact males and females grown in low, medium, and high nutrient treatment levels following the 75-day survival experiment.

2.4 Discussion

2.4.1 Investigating the direct and indirect costs of reproduction

My study is among the first to look at the costs of reproduction over a range of time periods (*i.e.*, instantaneous physiological costs, within-season costs, and indirect/demographic costs). This study is also among the first to consider multiple resource currencies (*i.e.*, nitrogen and biomass) to measure the costs of reproduction. Additionally, this study is among the first to examine the costs of reproduction across a resource-availability gradient, particularly for nutrient (including N) availability. As predicted, the costs of reproduction — both physiological/direct and indirect — were dependent on the environmental availability of nutrients. Under low nutrient conditions intact males had significantly lower photosynthetic rates (P) and capacities (Pmax) and had impaired growth the following year. By contrast, under high-nutrient conditions intact males did not incur any observable costs. Patterns for females were strikingly different, with similar measures of direct and indirect costs of reproduction, particularly under low- and medium-nutrient conditions. Female function entailed higher end-of-season costs expressed as greater reductions in corm biomass than males, particularly under low- and medium nutrient levels. I discuss the sexually dimorphic costs of reproduction for male versus female function and argue that, under nutrient-limited conditions, male function can entail greater costs of reproduction than female function.

2.4.2 Environmentally determined reproductive costs

My results indicated that resource availability affected the costs of reproduction. I found that the costs of reproduction for both male and female function in *Sagittaria latifolia* were driven by nutrient availability. Literature has demonstrated that the environment can affect the

outcomes of reproduction (*i.e*., sexual fecundity), but few studies have demonstrated this using a gradient of resource availability. It is well-known that plants adjust their allocation to reproduction versus growth in response to environmental conditions (Obeso, 2002). For example, a 2-year study of *Primula farinosa* (Dudley 1996) investigated the effects of water availability on fruit production and found observable costs of reproduction at low and high moisture levels, thus indicating environmental stress (drought and flooding events/influx of minerals and nutrients) can increase reproductive costs (see also Aragon, 2009). Similarly, other studies have manipulated carbon allocation to floral structures by performing leaf-removal treatments. A 4-year study of *Cypripedium acaule* (Primack and Hall 1990) found that leaf-removal yielded no observable costs of reproduction for the first two years of the study, but there were strong effects of limited carbon availability for future fruit reproduction during the last 2 years of the study. Another study involving environmental manipulation involves *Sagittaria pygmaea* (Liu and Chen 2009) where they manipulated reproduction by providing plants with a nutrient treatment gradient. They found strong trade-offs between sexual and clonal reproduction under moderate nutrient availability with sexual reproduction yielding a 37% decrease in clonal propagation (Liu and Chen 2009). Additionally, one study of *Oxytropis sericea* (Harder and Kudo 2011) used shading and defoliation to measure the effects on carbon allocation to nectar, seeds, and fruits. Harder and Kudo (2011) found that such stressors can yield high physiological costs, as well as costs to taproot biomass. All these studies suggest that a myriad of environmental conditions can alter reproductive allocations, but few demonstrate the outcomes of allocating nutrients across a gradient of resource availability. I was able to identify clear evidence for environmentally determined reproductive outcomes in *Sagittaria latifolia,* expressed as a relationship of decreases

in reproductive costs for those plants grown in resource abundant conditions (high nutrient treatments).

2.4.3 Evidence for direct trade-offs

During the first growth period, *Sagittaria latifolia* showed strong direct costs of reproduction, expressed as reduced photosynthetic rates (Pmax for both males and females during flowering at all nutrient treatment levels). Most notably, males that flowered in low-nutrient conditions had significantly lower leaf photosynthesis rates. Such direct physiological costs arising from reproductive allocations is one of the most well documented aspects of the costs of reproduction, and it is widely understood that female function entails greater direct costs (than male function) due to the allocation of energy and nutrients to ovule, fruit, and seed production (Reznick, 1985; Obeso, 2002). My findings also clarify results from a previous study of photosynthetic rates for *S. latifolia* that found no significant differences in PS rates between the sexes (Wright and Dorken, 2014). The study by Wright and Dorken (2014) occurred near an agricultural site, which, they speculated, might explain similar leaf-photosynthesis rates between the sexes (as males may not have been limited by nitrogen). Another study with the dioecious *Populus euphratica* found that leaf-photosynthesis for females was, on average, greater than males. Contrarily, the study by Wheelwright and Logan (2004) found that leaf-photosynthesis in *Ocotea tenera* was significantly higher for males than females. This indicates that differences in leaf-photosynthesis between the sexes is not static and can vary among species and their subjected environments. My study provided a nutrient gradient to better understand how reproductive allocations under nutrient limiting conditions would directly affect leaf-photosynthesis and there was a markedly clear inhibition for males that flowered in low nutrients. This was likely caused by minimal nutrient availability to take up for compensating

allocations to pollen production. Males that flowered in medium nutrients also had lower leaf-photosynthesis rates than did females after flowering occurred, but in high nutrients, males had greater leaf-photosynthesis rates than females, which could be attributed to there being no nutrient deficit.

However, not all of my results were consistent with the occurrence of direct costs of reproduction. Previous findings involving *S. latifolia* indicated that the average corm costs the equivalent of 1400-2500 seeds (Dorken and Van Drunen, 2012). My data were partially consistent with this previous finding. On one hand, total plant biomass (measured from the biomass of corms, the only living vegetative biomass at the end of the growing season) for females tended to be lower than that for males. This finding is consistent with previous findings from *S. latifolia* (Dorken and Van Drunen 2012) and with broader evidence for greater direct biomass trade-offs for females than for males (Obeso, 2002). On the other hand, intact females did not tend to have lower corm biomass than neutered males, as previously found by Dorken and Van Drunen (2012). These results for corm N and biomass are inconsistent with previous results on *S. latifolia*, and they differ between the sexes. Dorken and Van Drunen (2012) measured nitrogen content and found four times more nitrogen content per unit mass in females than males, which correlates with the decreased N-availability in leaves as found by Wright and Dorken (2014). In this study we did not identify a pattern of reproductive costs for N-allocation to clonal propagules, and we expect that intact genets would have less N stored if there is a cost for this currency. Under low-nutrient conditions, females had slightly higher stored N in corms, which is partially consistent with the findings in Dorken and Van Drunen (2012), but this small difference can only be interpreted as an absence of a difference. Under high nutrients, females had lower average N-stored in corms than did males, which is contrary to the prediction that

males may have greater N-demands. In this study, flowering males grown in low nutrient conditions had only modest differences in N-content than flowering females grown in low nutrients.

This finding is difficult to interpret given the clear difference in rate of biomass accumulation (C-assimilation) between intact and neuter plants, and between intact males and females, particularly under low and medium nutrient conditions. Unmeasured factors may have erased (or masked) differences in plant size and/or N content stored in corms. This experimental garden did withstand herbivory from aphids and caterpillars (*Pyrrharctia isabella*), and it could be possible that preferential herbivory occurred on females (for sugars from female leaves, inflorescences and fruits), which could have affected plant size and or N-content stored in corms. This could affect (or even have erased) end-of-season signals of the cost of reproduction. For example, preferential herbivory was found in *Valeriana edulis,* where females had 78% more natural enemies and 117% more ants than did males (Mooney *et al*., 2012). If female *S. latifolia* in this study were heavily browsed by herbivores, this could have resulted in a masking effect, whereby nitrogen content was less available for corms by females and resulted in both males and females having similarly low concentrations in the low nutrient treatment group. If plants allocate high proportions of nitrogen to leaves and inflorescences, herbivores can disrupt nitrogen balances, which may result in less nitrogen available for allocation to below ground biomass, and carbon can be replaced in leaves at a faster rate than nitrogen (Karban and Myers, 1989).

Dorken and Van Drunen (2012) measured nitrogen content and found four times more nitrogen content (represented as a %) per unit mass for corms produced in females than males, which correlates with the decreased N-availability in leaves as found by Wright and Dorken

(2014). In the current study we did not identify a pattern of reproductive costs for N-allocation to clonal propagules, and we expect that intact genets would have less N stored if there is a cost for this currency. There seems to be a clear story in the shoots, however as leaf N-content where neutered males had much higher N than intact males, particularly under low Nutrients. Males appear to benefit much more than females under high nutrients. Under low-nutrient conditions, females had slightly higher stored N in corms, which is partially consistent with the findings in Dorken and Van Drunen (2012). Under high nutrients, females had lower average N-stored in corms than did males, which is contrary to the prediction that males may have greater N-demands. In this study, flowering males grown in low nutrient conditions had only modest differences in N-content than flowering females grown in low nutrients.

On balance, my results are consistent with the importance of direct costs of reproduction, which demonstrate that underlying plant physiology, driven by N-availability can determine the performance of plants in a current growth period. Previous studies of leaf PS rates and within-season biomass costs have found that males and females can both have higher photosynthetic capacity (or there are no significant differences), which is also linked to differences in plant size for males and females. Although not all aspects of plant growth that I measured appeared to have been affected by whether plants reproduced or not, I conclude that reductions in plant size and photosynthetic capacity for *S. latifolia* in this study can be linked to direct limitations of resource environment (nutrient availability).

2.4.4 Implications for future growth

The bulk of studies on the costs of reproduction have considered only short-term (direct) costs, and this leaves the longer-term consequences (particularly survival costs) of reproduction

still poorly understood (Obeso 2002, Miller *et al.*, 2012). My study underlines the importance of measuring direct reproductive costs in plants, while also studying plants over multiple growth periods to examine the underlying effects of plant life-history trade-offs on the potential demographic effects of reproduction (as suggested in Seger and Eckart 1996; Obeso, 2002; Dorken and Van Drunen 2012). This study detected indirect costs of reproduction that were evident as stunted growth (measured in leaf MVL and N-content) for intact compared to neuter plants, particularly for males growing in low-nutrient conditions. To some extent, corm production (the number of corms per plant) was also consistent with the existence of reproductive costs, or at least that the costs of reproduction differed between the sexes. In low-nutrient conditions, males and females produced a similar number of corms, but in medium and high nutrients, males produced more. Moreover, my data showed that it is possible that plants with lower corm N are of lower quality and grow less vigorously in subsequent year(s) (and see Dorken and Van Drunen 2012). Another study with *Helianthemum squamatum* (a short-lived perennial), identified significant survival costs of reproduction over multiple years using yearly experimental bud removal treatments (Aragon *et al*., 2009; 2010). This species appeared to prioritize high reproductive output to enable increased fitness at the expense of a short lifespan, in a Mediterranean habitat of high environmental stochasticity and extrinsic mortality (Aragon *et al*., 2009; 2010). Here we found that male *S. latifolia* grown under low-nutrient treatments struggled to grow and become healthy in the absence of nutrients in the subsequent growth period. These findings align with the study on *Helianthemum squamatum* (Aragon *et al*., 2009; 2010) as it appears allocations to reproduction (and clonal propagation) for males was prioritized at the cost of future survival. My study elucidated that corms of poorer quality are indicative of

poor current growth conditions and may translate to impeded reproductive output in subsequent seasons.

Contrary to the findings in my study, Wheelwright and Logan (2004), investigated how previous-year reproduction affected leaf photosynthesis in male and female individuals in *Ocotea tenera* (neotropical trees), finding that female trees had generally greater PS inhibitions following reproduction. They found that female trees, which typically invest heavily in reproduction, exhibited reduced photosynthetic capacity if they had reproduced extensively in the previous year (Wheelwright and Logan 2004). This reduction in photosynthetic capacity was not observed to the same extent in male trees. The reduced photosynthetic capacity in females was associated with slower lifetime growth compared to males. Overall, the study suggests that the costs of reproduction have a greater inhibitory effect on leaf photosynthesis and long-term growth in female individuals compared to males.

2.4.5 Male function can entail greater costs than female function

The classical assumption of shared resource requirements for male and female function has resulted in a legacy of studies largely considering biomass as a currency of reproductive costs for the two sex functions (Charnov, 1982). Many studies have indicated that considering different currencies of reproduction is important to understanding reproductive costs in plants, and to better understand allocations to sex-specific plant functions (Ashman, 1994; Obeso, 2002; Harris and Pannell, 2008, McKone *et al*., 1998; Dorken and Van Drunen, 2012; Wright and Dorken 2014). Although I found no clear differences in corm N or biomass content between intact and neutered plants, the nutrient-availability gradient revealed striking differences in sex-specific costs, particularly for measurements made immediately after reproduction (PS rates) and

those made long afterwards (next-season greenness and growth). Both photosynthetic rates in the first growth period and reduced greenness and growth performance in the following growth period for males (particularly under low-nutrient conditions) are consistent with the high nitrogen costs of pollen production (Lau and Stephenson 1993; Roulston *et al*., 2000) trading off against allocations of N to the light-harvesting apparatus (Zhao *et al*., 2017; Rotundo and Cipriotti 2017). Thus, this supports that the allocation requirements of N for male function may be substantially greater than for female function, at least in some angiosperms.

2.4.6 Conclusions

I have expanded current understanding of the costs of reproduction by measuring short-term (direct) costs (physiology, nutrient partitioning to clonal propagules, and biomass trade-offs) in combination with the long-term (indirect or demographic) costs (measuring the number of clones produced, future growth and survival) while accounting for biomass as a currency of female function and nitrogen as a currency of male function. Based on previous theoretical and empirical evidence, the costs of reproduction for female function almost always exceeds the costs for male function (Charnov, 1982; Obeso, 2002). These studies overlook the importance of measuring costs over multiple growth periods, and through multiple resource currencies under varying environmental (in this case nutrient availability) conditions. This study supports that male and female function draw from a pool of resources for reproduction, but the volume or efficiency use of C and N by males and females is very much different. These differential requirements can yield greater costs for male function where nutrient conditions are sub-optimal, because male function in *Sagittaria latifolia* appears to have greater nitrogen demands than female function. Thus, female function resulted in greater direct costs (biomass) and males incurred observably higher direct (physiological) and indirect costs (future growth and survival).

The timing of which costs are measured is also important, as it appears male function requires larger allocations of N early in the season, whereas female function requires larger biomass allocations at the end of the season. These findings suggest that the costs of reproduction are not static features of plants and can be significantly influenced by the environmental availability of nutrients. This study demonstrates that allocations to male function under limited nitrogen conditions can potentially lead to decreased fecundity and end-of-life occurrences.

Chapter 3: General Discussion

In this study, I have undertaken a comprehensive examination of the costs of reproduction using *Sagittaria latifolia* as an experimental model, exploring both short-term (direct) and long-term (indirect) consequences across varying resource availability. This study stands out as one of the first to analyze the costs of reproduction over different time scales and across a gradient of resource availability, specifically focusing on nitrogen and biomass as multiple resource currencies (Dorken and Van Drunen, 2012).

The results demonstrate that the costs of reproduction, both physiological/direct and indirect, are intricately linked to the environmental availability of nutrients, particularly nitrogen (Obeso, 2002). Intact males under limited nutrient conditions exhibited pronounced physiological impairments and compromised future growth, whereas high-nutrient conditions mitigated these observed costs. Thus, males suffered greater direct and indirect costs of reproduction compared to females, particularly in nutrient-poor conditions. The sexually dimorphic costs of reproduction reveal intriguing patterns, emphasizing the importance of resource availability in shaping reproductive outcomes. This study aligns with a growing body of literature emphasizing the influence of the environment on reproductive outcomes. Few studies have employed a gradient of resource availability to elucidate the impact on reproductive allocations (Muller *et al*., 2000). My findings indicate that nutrient-starved versus nutrient-rich conditions significantly alter reproductive costs, contributing valuable insights into the nuanced relationship between environmental factors and plant reproductive strategies.

The results of this study provide robust evidence that supports the hypothesis that the costs of reproduction depend on the resource environment (Reznick, 1985). They also advance our understanding of the sexually dimorphic implications of reproductive allocations (Obeso,

2002). While many studies focus on short-term (direct) costs, this research highlights the importance of considering long-term (indirect) consequences (Obeso, 2002; Miller *et al.*, 2012). Indirect costs, expressed as trade-offs against corm production, growth stunting, and reduced chlorophyll content, underscore the significance of repeated measurements over multiple growth periods. This contributes to a more holistic understanding of the effects of life-history trade-offs on population demography. By measuring reproductive costs across a resource-availability gradient, my results indicate that the allocation requirements for male function may be substantially greater than for female function in certain conditions.

Future studies should consider an approach that fully accounts for the allocation of nitrogen (and potentially phosphorus) in plants, including CNS (Carbon, Nitrogen and Sulfur) analysis of flowers. Male flower buds are small, and the CNS analysis techniques used here require biomass amounts equal to much more than one male *Sagittaria latifolia* flower bud. Female flower buds and mature fruits (receptacles) contain more mass and would have been easier to measure using the CNS protocols used in this study. By measuring the allocation of nutrients to flower buds we may be able to better understand the true nutrient demand for flower production among the sexes. Additionally, measuring indirect costs in natural populations is extremely challenging, due to the fact that it is hard to control for plant age and size. It may be worth investigating the role of nutrient cycling within populations to examine variation in nutrient availability across sites and at different times in the growing season to determine the extent to which the patterns found here are likely to apply to natural populations of *S. latifolia*.

In conclusion, this study advances our understanding of the costs of reproduction by examining both short-term and long-term consequences of reproductive allocations by analyzing multiple resource currencies and their demands across male and female functions. The most

recent literature review (Obeso, 2002) suggested that our understanding of the costs of reproduction would be benefited by linking plant physiology to demography. My findings support this and emphasize the dynamic nature of reproductive costs, influenced significantly by the environmental availability of nutrients. As future research endeavors unfold, it is imperative to continue measuring costs over multiple growth periods and under varying environmental conditions to best measure factors influencing the reproductive strategies of plants (Seger and Eckart, 1996; Dorken and Van Drunen, 2012).

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Appendix

Appendix 1: Results from the photosynthesis data:

Table 1A: Summary statistics from the linear regression model (LM) for the photosynthesis data. Model selected after an information criterion simplification.

Predictors	*Estimates*	*CI**	*P*
(Intercept)	9.75	7.78 – 11.72	**<0.001**
Treatment_Level [M]	3.17	0.39 – 5.94	**0.025**
Treatment_Level [H]	5.04	2.48 – 7.59	**<0.001**
Flower_Rem [N]	2.22	0.55 – 3.88	**0.009**
Time [During]	-0.48	-3.20 – 2.24	0.73
Time [After]	2.37	-0.36 – 5.09	0.088
Sex [M]	-0.22	-2.50 – 2.06	0.85
Treatment_Level [M] *Flower_Rem [N]	-1.92	-3.72 – -0.12	**0.036**
Treatment_Level [H] * Flower_Rem [N]	-2.14	-3.99 – -0.29	**0.024**
Treatment_Level [M] * Time [During]	-2.81	-6.48 – 0.86	0.133
Treatment_Level [H] * Time [During]	-3.78	-7.26 – -0.30	**0.033**
Treatment_Level [M] * Time [After]	0.21	-3.46 – 3.88	0.911
Treatment_Level [H] * Time [After]	-2.46	-5.94 – 1.02	0.166
Flower_Rem [N] * Time[During]	-0.7	-2.51 – 1.12	0.451
Flower_Rem [N] * Time[After]	2.12	0.30 – 3.93	**0.022**
Treatment_Level [M] * Sex[M]	-0.23	-3.44 – 2.98	0.888
Treatment_Level [H] * Sex[M]	1.44	-1.72 – 4.60	0.373
Time [During] * Sex [M]	-0.7	-3.95 – 2.54	0.671
Time [After] * Sex [M]	-4.04	-7.29 – -0.79	**0.015**
(Treatment_Level [M] Time [During]) Sex [M]	2	-2.58 – 6.57	0.391
(Treatment_Level [H] Time [During]) Sex [M]	-0.05	-4.56 – 4.45	0.981
(Treatment_Level [M] Time [After]) Sex [M]	0.82	-3.76 – 5.39	0.726
(Treatment_Level [H] Time [After]) Sex [M]	5.44	0.94 – 9.95	**0.018**

*CI is defined as the 95% confidence interval
**R^2 describes variance from just fixed variables, while adjusted R^2 describes proportion of variance from variables.

Table 1B: An information criterion (AIC) model simplification was employed to select a linear model that best fits the photosynthesis data. Source population was therefore excluded as a fixed effect.

Net_PS ~ Treatment_Level + Flower_Rem + Time + Sex + Treatment_Level:Flower_Rem + Treatment_Level:Time + Flower_Rem:Time + Treatment_Level:Sex + Time:Sex + Treatment_Level:Time:Sex				
Predictors	**Df**	**SS**	**RSS**	**AIC**
(Model)			24490	2926.2
+Treatment_Level:Flower_Rem:Time	4	193.49	24297	2927.4
+Flower_Rem:Sex	1	4.21	24486	2928.1
-Treatment_Level:Flower_Rem	2	185.41	24676	2928.7
-Treatment_Level:Time:Sex	4	337.86	24828	2930
+Source_Population	6	189.9	24300	2931.5
-Flower_Rem:Time	2	290.41	24781	2932.4

Table 1C: The type III SS ANOVA test results from the photosynthesis model:

Predictors	**SS**	**Df**	***F* Value**	**Pr(>*F*)**
(Intercept)	2767.3	1	94.58	**< 2.2e-16**
Treatment_Level	440	2	7.52	**0.001**
Flower_Rem	199.9	1	6.83	**0.009**
Time	138.1	2	2.36	0.095
Sex	1	1	0.04	0.850
Treatment_Level:Flower_Rem	185.4	2	3.17	**0.043**
Treatment_Level:Time	198.7	4	1.70	0.148
Flower_Rem:Time	290.4	2	4.96	**0.007**
Treatment_Level:Sex	37.5	2	0.64	0.527
Time:Sex	197	2	3.37	**0.035**
Treatment_Level:Time:Sex	337.9	4	2.89	**0.022**
Residuals	24490.2	837		

Table 1D: Summary of Tukey's post-hoc test of pairwise comparisons of photosynthetic rates cross treatment levels, flower removal, sex, and time.

#	**Contrast**	**Estimate**	**Standard Error (SE)**	**Degrees of freedom (df)**	***t*-ratio**	***P***
1	F L Before - M L Before	0.219	1.16	837	0.1886	1.0000

2	F L Before - F M Before	-2.205	1.31	837	-1.6814	0.9697
3	F L Before - M M Before	-1.755	1.12	837	-1.5628	0.9854
4	F L Before - F H Before	-3.966	1.24	837	-3.1895	0.1249
5	F L Before - M H Before	-5.184	1.18	837	-4.3888	**0.0017**
6	F L Before - F L During	0.827	1.31	837	0.6297	1.0000
7	F L Before - M L During	1.750	1.16	837	1.5045	0.9902
8	F L Before - F M During	1.435	1.32	837	1.0871	0.9998
9	F L Before - M M During	0.590	1.13	837	0.5243	1.0000
10	F L Before - F H During	0.640	1.25	837	0.5111	1.0000
11	F L Before - M H During	0.182	1.19	837	0.1525	1.0000
12	F L Before - F L After	-3.426	1.31	837	-2.6102	0.4471
13	F L Before - M L After	0.832	1.16	837	0.7156	1.0000
14	F L Before - F M After	-5.839	1.32	837	-4.4250	**0.0015**
15	F L Before - M M After	-2.167	1.13	837	-1.9245	0.9020
16	F L Before - F H After	-4.932	1.25	837	-3.9376	**0.0108**
17	F L Before - M H After	-7.552	1.19	837	-6.3399	**0.0000**
18	M L Before - F M Before	-2.424	1.17	837	-2.0655	0.8353
19	M L Before - M M Before	-1.974	0.97	837	-2.0410	0.8484
20	M L Before - F H Before	-4.185	1.11	837	-3.7751	**0.0197**
21	M L Before - M H Before	-5.403	1.04	837	-5.2116	**0.0000**
22	M L Before - F L During	0.608	1.18	837	0.5144	1.0000
23	M L Before - M L During	1.531	1.01	837	1.5205	0.9891
24	M L Before - F M During	1.216	1.19	837	1.0204	0.9999

25	M L Before - M M During	0.372	0.97	837	0.3837	1.0000
26	M L Before - F H During	0.421	1.11	837	0.3793	1.0000
27	M L Before - M H During	-0.037	1.04	837	-0.0357	1.0000
28	M L Before - F L After	-3.645	1.18	837	-3.0855	0.1640
29	M L Before - M L After	0.613	1.01	837	0.6093	1.0000
30	M L Before - F M After	-6.058	1.19	837	-5.0848	**0.0001**
31	M L Before - M M After	-2.386	0.97	837	-2.4640	0.5588
32	M L Before - F H After	-5.151	1.11	837	-4.6371	**0.0006**
33	M L Before - M H After	-7.771	1.04	837	-7.4570	**0.0000**
34	F M Before - M M Before	0.450	1.15	837	0.3924	1.0000
35	F M Before - F H Before	-1.762	1.27	837	-1.3895	0.9960
36	F M Before - M H Before	-2.979	1.20	837	-2.4725	0.5522
37	F M Before - F L During	3.031	1.33	837	2.2799	0.6979
38	F M Before - M L During	3.954	1.18	837	3.3384	0.0820
39	F M Before - F M During	3.639	1.33	837	2.7336	0.3587
40	F M Before - M M During	2.795	1.15	837	2.4352	0.5811
41	F M Before - F H During	2.845	1.27	837	2.2450	0.7226
42	F M Before - M H During	2.386	1.21	837	1.9759	0.8801
43	F M Before - F L After	-1.221	1.33	837	-0.9186	1.0000
44	F M Before - M L After	3.037	1.18	837	2.5639	0.4819
45	F M Before - F M After	-3.634	1.33	837	-2.7299	0.3612
46	F M Before - M M After	0.038	1.15	837	0.0329	1.0000
47	F M Before - F H After	-2.728	1.27	837	-2.1523	0.7842

48	F M Before - M H After	-5.347	1.21	837	-4.4275	**0.0015**
49	M M Before - F H Before	-2.212	1.06	837	-2.0773	0.8288
50	M M Before - M H Before	-3.429	0.99	837	-3.4592	0.0568
51	M M Before - F L During	2.581	1.15	837	2.2536	0.7165
52	M M Before - M L During	3.504	0.97	837	3.6167	**0.0341**
53	M M Before - F M During	3.189	1.15	837	2.7614	0.3400
54	M M Before - M M During	2.345	0.93	837	2.5351	0.5040
55	M M Before - F H During	2.395	1.08	837	2.2248	0.7366
56	M M Before - M H During	1.936	1.00	837	1.9283	0.9005
57	M M Before - F L After	-1.671	1.15	837	-1.4592	0.9930
58	M M Before - M L After	2.587	0.97	837	2.6699	0.4033
59	M M Before - F M After	-4.084	1.15	837	-3.5364	**0.0444**
60	M M Before - M M After	-0.412	0.93	837	-0.4457	1.0000
61	M M Before - F H After	-3.178	1.08	837	-2.9517	0.2267
62	M M Before - M H After	-5.797	1.00	837	-5.7729	**0.0000**
63	F H Before - M H Before	-1.218	1.12	837	-1.0891	0.9998
64	F H Before - F L During	4.793	1.26	837	3.7905	**0.0187**
65	F H Before - M L During	5.716	1.11	837	5.1715	**0.0000**
66	F H Before - F M During	5.401	1.27	837	4.2558	**0.0031**
67	F H Before - M M During	4.557	1.07	837	4.2587	**0.0030**
68	F H Before - F H During	4.607	1.20	837	3.8524	**0.0149**
69	F H Before - M H During	4.148	1.13	837	3.6601	**0.0295**
70	F H Before - F L After	0.540	1.26	837	0.4273	1.0000

71	F H Before - M L After	4.799	1.11	837	4.3415	**0.0021**
72	F H Before - F M After	-1.873	1.27	837	-1.4757	0.9921
73	F H Before - M M After	1.799	1.07	837	1.6816	0.9697
74	F H Before - F H After	-0.966	1.20	837	-0.8078	1.0000
75	F H Before - M H After	-3.586	1.13	837	-3.1638	0.1338
76	M H Before - F L During	6.010	1.20	837	4.9963	**0.0001**
77	M H Before - M L During	6.934	1.04	837	6.6984	**0.0000**
78	M H Before - F M During	6.618	1.21	837	5.4759	**0.0000**
79	M H Before - M M During	5.774	1.00	837	5.7952	**0.0000**
80	M H Before - F H During	5.824	1.13	837	5.1437	**0.0000**
81	M H Before - M H During	5.366	1.07	837	5.0355	**0.0001**
82	M H Before - F L After	1.758	1.20	837	1.4612	0.9929
83	M H Before - M L After	6.016	1.04	837	5.8121	**0.0000**
84	M H Before - F M After	-0.655	1.21	837	-0.5421	1.0000
85	M H Before - M M After	3.017	1.00	837	3.0278	0.1893
86	M H Before - F H After	0.252	1.13	837	0.2222	1.0000
87	M H Before - M H After	-2.368	1.07	837	-2.2224	0.7382
88	F L During - M L During	0.923	1.19	837	0.7788	1.0000
89	F L During - F M During	0.608	1.34	837	0.4539	1.0000
90	F L During - M M During	-0.236	1.15	837	-0.2057	1.0000
91	F L During - F H During	-0.186	1.27	837	-0.1465	1.0000
92	F L During - M H During	-0.645	1.21	837	-0.5323	1.0000
93	F L During - F L After	-4.253	1.33	837	-3.1933	0.1236

94	F L During - M L After	0.006	1.18	837	0.0048	1.0000
95	F L During - F M After	-6.666	1.34	837	-4.9795	**0.0001**
96	F L During - M M After	-2.994	1.15	837	-2.6069	0.4495
97	F L During - F H After	-5.759	1.27	837	-4.5249	**0.0010**
98	F L During - M H After	-8.378	1.21	837	-6.9113	**0.0000**
99	M L During - F M During	-0.315	1.19	837	-0.2654	1.0000
100	M L During - M M During	-1.159	0.97	837	-1.1895	0.9994
101	M L During - F H During	-1.109	1.12	837	-0.9889	1.0000
102	M L During - M H During	-1.568	1.05	837	-1.4916	0.9911
103	M L During - F L After	-5.176	1.18	837	-4.3695	**0.0019**
104	M L During - M L After	-0.917	1.01	837	-0.9077	1.0000
105	M L During - F M After	-7.589	1.19	837	-6.3533	**0.0000**
106	M L During - M M After	-3.917	0.97	837	-4.0285	**0.0077**
107	M L During - F H After	-6.682	1.11	837	-5.9958	**0.0000**
108	M L During - M H After	-9.302	1.05	837	-8.8939	**0.0000**
109	F M During - M M During	-0.844	1.16	837	-0.7272	1.0000
110	F M During - F H During	-0.794	1.29	837	-0.6177	1.0000
111	F M During - M H During	-1.253	1.22	837	-1.0235	0.9999
112	F M During - F L After	-4.861	1.34	837	-3.6311	**0.0325**
113	F M During - M L After	-0.602	1.19	837	-0.5043	1.0000
114	F M During - F M After	-7.274	1.34	837	-5.4254	**0.0000**
115	F M During - M M After	-3.602	1.16	837	-3.1099	0.1541
116	F M During - F H After	-6.367	1.28	837	-4.9861	**0.0001**

117	F M During - M H After	-8.986	1.22	837	-7.3793	**0.0000**
118	M M During - F H During	0.050	1.08	837	0.0463	1.0000
119	M M During - M H During	-0.409	1.01	837	-0.4065	1.0000
120	M M During - F L After	-4.016	1.15	837	-3.4977	**0.0503**
121	M M During - M L After	0.242	0.97	837	0.2488	1.0000
122	M M During - F M After	-6.429	1.16	837	-5.5517	**0.0000**
123	M M During - M M After	-2.757	0.93	837	-2.9695	0.2176
124	M M During - F H After	-5.523	1.08	837	-5.1146	**0.0001**
125	M M During - M H After	-8.142	1.01	837	-8.0806	**0.0000**
126	F H During - M H During	-0.459	1.14	837	-0.4039	1.0000
127	F H During - F L After	-4.066	1.27	837	-3.1950	0.1230
128	F H During - M L After	0.192	1.11	837	0.1723	1.0000
129	F H During - F M After	-6.479	1.28	837	-5.0742	**0.0001**
130	F H During - M M After	-2.807	1.08	837	-2.5999	0.4548
131	F H During - F H After	-5.573	1.20	837	-4.6292	**0.0006**
132	F H During - M H After	-8.192	1.14	837	-7.1737	**0.0000**
133	M H During - F L After	-3.608	1.21	837	-2.9760	0.2143
134	M H During - M L After	0.651	1.05	837	0.6221	1.0000
135	M H During - F M After	-6.021	1.22	837	-4.9440	**0.0001**
136	M H During - M M After	-2.349	1.01	837	-2.3309	0.6605
137	M H During - F H After	-5.114	1.14	837	-4.4781	**0.0012**
138	M H During - M H After	-7.734	1.08	837	-7.1881	**0.0000**
139	F L After - M L After	4.258	1.19	837	3.5930	**0.0369**

140	F L After - F M After	-2.413	1.34	837	-1.8015	0.9432
141	F L After - M M After	1.259	1.15	837	1.0967	0.9998
142	F L After - F H After	-1.506	1.27	837	-1.1842	0.9994
143	F L After - M H After	-4.126	1.21	837	-3.4050	0.0672
144	M L After - F M After	-6.671	1.19	837	-5.6197	**0.0000**
145	M L After - M M After	-2.999	0.97	837	-3.0774	0.1674
146	M L After - F H After	-5.765	1.12	837	-5.1384	**0.0001**
147	M L After - M H After	-8.384	1.05	837	-7.9757	**0.0000**
148	F M After - M M After	3.672	1.16	837	3.1632	0.1340
149	F M After - F H After	0.907	1.29	837	0.7052	1.0000
150	F M After - M H After	-1.713	1.22	837	-1.3991	0.9957
151	M M After - F H After	-2.765	1.08	837	-2.5676	0.4791
152	M M After - M H After	-5.385	1.01	837	-5.3550	**0.0000**
153	F H After - M H After	-2.620	1.14	837	-2.3071	0.6780

Appendix 2: Results from the nitrogen content in corm dataset.

Table1 2A: Summary statistics from the fixed effects linear regression (LM) model for the total nitrogen stored in corms of all plants.

Predictors	*Estimates*	*CI*	*p*
(Intercept)	4.16	3.79 – 4.53	**<0.001**
Treatment_Level [Medium]	0.19	-0.32 – 0.70	0.470
Treatment_Level [High]	1.30	0.86 – 1.74	**<0.001**
Flower_Rem [N]	0.10	-0.39 – 0.58	0.695
Sex [M]	-0.12	-0.58 – 0.33	0.598
Treatment_Level [Medium] * Flower_Rem [N]	0.03	-0.66 – 0.71	0.936
Treatment_Level [High] * Flower_Rem [N]	-0.72	-1.39 – -0.06	**0.033**
Treatment_Level [Medium] * Sex [M]	0.18	-0.46 – 0.82	0.579
Treatment_Level [High] * Sex [M]	0.23	-0.35 – 0.82	0.435
Flower_Rem [N] * Sex [M]	0.18	-0.44 – 0.80	0.571
(Treatment_Level [Medium] * Flower_Rem [N]) * Sex [M]	-0.11	-0.97 – 0.75	0.801

(Treatment_Level [High] * Flower_Rem [N]) * Sex [M]	0.37	-0.49 – 1.23	0.396

Random Effects

σ^2	0.52
$\tau_{00\ Source\_Population}$	0.04
ICC	0.07
$N_{\ Source\_Population}$	7
Observations	298
Marginal $\boldsymbol{R^2}$ / Conditional $\boldsymbol{R^2}$	0.338 / 0.384

Table 2B: The type III SS ANOVA test results from the fixed effects regression model for the nitrogen content in corms data.

	Chisq	**Df**	**Pr(>Chisq)**
(Intercept)	492.96	1	<2.2e-16 ***
Treatment_Level	40.6088	2	1.52e-09 ***
Flower_Rem	0.1537	1	0.69507
Sex	0.2786	1	0.59762
Treatment_Level:Flower_Rem	6.4866	2	0.03904
Treatment_Level:Sex	0.6315	2	0.72925
Flower_Rem:Sex	0.3202	1	0.57147
Treatment_Level:Flower_Rem:Sex	1.3764	2	0.50247

Table 2C: Summary of Tukey's post-hoc test of pairwise comparisons of total nitrogen stored in corms across nutrient treatment levels, flower removal treatments, sex, and time.

Contrast	**Estimates**	**Standard Error (SE)**	**Degrees of freedom (df)**	***t*-ratio**	***P***
(F Low I - M Low I)	2.12247	0.222	280	9.557	**<.0001**
(F Low I - F Medium I)	0.3064	0.252	280	1.217	0.2745
(F Low I - M Medium I)	0.42061	0.191	280	2.199	**0.044**
(F Low I - F High I)	-0.6382	0.205	280	-3.11	**0.0047**
(F Low I - M High I)	-0.76436	0.191	280	-3.998	**0.0002**
(F Low I - F Low N)	0.53452	0.221	280	2.42	**0.0267**
(F Low I - M Low N)	0.46056	0.18	280	2.552	**0.019**
(F Low I - F Medium N)	0.31825	0.198	280	1.606	0.1389
(F Low I - M Medium N)	0.19659	0.183	280	1.073	0.3351
(F Low I - F High N)	-0.15429	0.207	280	-0.746	0.5021
(F Low I - M High N)	-0.69237	0.189	280	-3.668	**0.0007**
(M Low I - F Medium I)	-1.3653	0.246	280	-5.54	**<.0001**
(M Low I - M Medium I)	-1.25109	0.184	280	-6.804	**<.0001**
(M Low I - F High I)	-2.30991	0.198	280	-11.654	**<.0001**
(M Low I - M High I)	-2.43606	0.183	280	-13.276	**<.0001**
(M Low I - F Low N)	-1.13718	0.215	280	-5.289	**<.0001**
(M Low I - M Low N)	-1.21114	0.176	280	-6.864	**<.0001**
(M Low I - F Medium N)	-1.35345	0.194	280	-6.988	**<.0001**

(M Low I - M Medium N)	-1.47511	0.177	280	-8.332	**<.0001**
(M Low I - F High N)	-1.82599	0.202	280	-9.031	**<.0001**
(M Low I - M High N)	-2.36407	0.184	280	-12.848	**<.0001**
(F Medium I - M Medium I)	0.56498	0.215	280	2.63	**0.0161**
(F Medium I - F High I)	-0.49384	0.224	280	-2.205	**0.044**
(F Medium I - M High I)	-0.61999	0.212	280	-2.929	**0.0074**
(F Medium I - F Low N)	0.67889	0.241	280	2.817	**0.0101**
(F Medium I - M Low N)	0.60492	0.205	280	2.944	**0.0072**
(F Medium I - F Medium N)	0.46262	0.219	280	2.115	0.0518
(F Medium I - M Medium N)	0.34096	0.206	280	1.657	0.1313
(F Medium I - F High N)	-0.00993	0.228	280	-0.044	0.9653
(F Medium I - M High N)	-0.548	0.213	280	-2.577	**0.0182**
(M Medium I - F High I)	-0.60805	0.186	280	-3.272	**0.0028**
(M Medium I - M High I)	-0.7342	0.17	280	-4.316	**0.0001**
(M Medium I - F Low N)	0.56468	0.204	280	2.77	**0.0113**
(M Medium I - M Low N)	0.49071	0.162	280	3.038	**0.0057**
(M Medium I - F Medium N)	0.3484	0.181	280	1.925	0.0793
(M Medium I - M Medium N)	0.22675	0.162	280	1.398	0.2032
(M Medium I - F High N)	-0.12414	0.19	280	-0.654	0.556
(M Medium I - M High N)	-0.66221	0.171	280	-3.878	**0.0003**

(F High I - M High I)	0.32462	0.186	280	1.749	0.1119
(F High I - F Low N)	1.62349	0.219	280	7.415	**<.0001**
(F High I - M Low N)	1.54953	0.18	280	8.602	**<.0001**
(F High I - F Medium N)	1.40722	0.195	280	7.218	**<.0001**
(F High I - M Medium N)	1.28556	0.18	280	7.16	**<.0001**
(F High I - F High N)	0.93468	0.205	280	4.567	**<.0001**
(F High I - M High N)	0.39661	0.186	280	2.133	0.0507
(M High I - F Low N)	1.74964	0.21	280	8.316	**<.0001**
(M High I - M Low N)	1.67568	0.171	280	9.818	**<.0001**
(M High I - F Medium N)	1.53337	0.188	280	8.158	**<.0001**
(M High I - M Medium N)	1.41171	0.17	280	8.29	**<.0001**
(M High I - F High N)	1.06083	0.197	280	5.374	**<.0001**
(M High I - M High N)	0.52276	0.178	280	2.945	**0.0072**
(F Low N - M Low N)	0.3768	0.21	280	1.798	0.1028
(F Low N - F Medium N)	0.2345	0.225	280	1.042	0.3453
(F Low N - M Medium N)	0.11284	0.21	280	0.536	0.6304
(F Low N - F High N)	-0.23805	0.233	280	-1.024	0.3492
(F Low N - M High N)	-0.77612	0.217	280	-3.581	**0.001**
(M Low N - F Medium N)	0.30846	0.187	280	1.648	0.1313

(M Low N - M Medium N)	0.1868	0.171	280	1.09	0.332
(M Low N - F High N)	-0.16408	0.197	280	-0.832	0.4543
(M Low N - M High N)	-0.70216	0.18	280	-3.911	**0.0003**
(F Medium N - M Medium N)	0.32911	0.2	280	1.643	0.1313
(F Medium N - F High N)	-0.02178	0.221	280	-0.098	0.9358
(F Medium N - M High N)	-0.55985	0.206	280	-2.712	**0.013**
(M Medium N - F High N)	0.09988	0.209	280	0.478	0.6629
(M Medium N - M High N)	-0.43819	0.191	280	-2.29	**0.0366**
(F High N - M High N)	-0.08731	0.237	280	-0.369	0.7346

Appendix 3: Results from the plant survival experiment; total chlorophyll in leaf tissues after 75-days.

Table 3A: Summary statistics from the linear regression model (LM) for the total chlorophyll content in leaf tissues after 75-days survival experiment.

Predictors	*Estimates*	*CI*	*p*
(Intercept)	14.58	10.62 – 18.55	**<0.001**
Treatment_Level [Medium]	6.19	0.26 – 12.12	**0.041**
Treatment_Level [High]	11.10	6.11 – 16.09	**<0.001**
Flower_Rem [N]	3.89	-1.58 – 9.36	0.164
Sex [M]	-4.46	-9.55 – 0.63	0.086
Treatment_Level [Medium] * Flower_Rem [N]	-6.23	-14.06 – 1.60	0.119

Treatment_Level [High] * Flower_Rem [N]	-2.55	-9.93 – 4.84	0.500
Treatment_Level [Medium] * Sex [M]	2.40	-4.86 – 9.67	0.517
Treatment_Level [High] * Sex [M]	8.41	1.87 – 14.95	**0.012**
Flower_Rem [N] * Sex [M]	4.80	-2.12 – 11.71	0.174
(Treatment_Level [Medium] * Flower_Rem [N]) * Sex [M]	-3.60	-13.33 – 6.13	0.469
(Treatment_Level [High] * Flower_Rem [N]) * Sex [M]	-4.24	-13.81 – 5.33	0.386
Random Effects			
σ^2	61.97		
$\tau_{00\ Source\_Population}$	1.13		
ICC	0.02		
$N_{\ Source\_Population}$	7		
Observations	278		
Marginal R^2 / Conditional R^2	0.363 / 0.375		

Table 3B: An information criterion (AIC) model simplification was employed to select a linear model that best fits the chlorophyll data. Source population was excluded as a fixed effect.

Total_Chloro~Treatment_Level + Flower_Rem + Sex + Treatment_Level:Flower_Rem + Treatment_Level:Sex

Predictors	**Df**	**SS**	**RSS**	**AIC**

(Model)			16857	1159.2
Treatment_Level:Sex	2	470.17	17328	1162.8
Treatment_Level:Flower_Rem	2	764.55	17622	1167.5

Table 3C: The type III SS ANOVA test results from the total chlorophyll content in leaves model.

	Chisq	**Df**	**Pr(>Chisq)**
(Intercept)	51.9714	1	5.632e-13 ***
Treatment_Level	19.1053	2	7.101e-05 ***
Flower_Rem	1.9391	1	0.16377
Sex	2.9526	1	0.08574
Treatment_Level:Flower_Rem	2.4542	2	0.29314
Treatment_Level:Sex	7.1216	2	0.02842 *
Flower_Rem:Sex	1.8454	1	0.17432
Treatment_Level:Flower_Rem:Sex	0.8587	2	0.65094

Table 3D: Summary of Tukey's post-hoc test of pairwise comparisons of chlorophyll content in leaves after 75-day survival experiment, with tracked treatment levels, flower removal, and sex.

Contrast	**Estimates**	**Standard Error (SE)**	**Degrees of Freedom (Df)**	***t*-ratio**	***P***
Low I F – Medium I F	-7.3053	2.39	269	-3.061	0.0973
Low I F – High I F	-12.5333	2.16	269	-5.815	**<.0001**
Low I F – Low N F	-6.7104	1.71	269	-3.931	**0.006**
Low I F – Medium N F	-5.7578	2.22	269	-2.588	0.2915
Low I F – High N F	-14.2273	2.29	269	-6.201	**<.0001**
Low I F – Low I M	1.7984	1.76	269	1.022	0.9971
Low I F – Medium I M	-5.7192	2.05	269	-2.785	0.1924

Low I F – High I M	-16.4797	2.07	269	-7.974	**<.0001**
Low I F – Low N M	-4.9119	2.31	269	-2.125	0.6056
Low I F – Medium N M	-4.1717	2.14	269	-1.951	0.7256
Low I F – High N M	-18.1737	2.23	269	-8.158	**<.0001**
Medium I F – High I F	-5.2279	2.23	269	-2.346	0.4469
Medium I F – Low N F	0.595	2.39	269	0.249	1
Medium I F – Medium N F	1.5476	1.66	269	0.935	0.9987
Medium I F – High N F	-6.922	2.36	269	-2.929	0.1368
Medium I F – Low I M	9.1038	2.28	269	3.99	**0.0048**
Medium I F – Medium I M	1.5861	1.74	269	0.913	0.999
Medium I F – High I M	-9.1744	2.14	269	-4.281	**0.0015**
Medium I F – Low N M	2.3934	2.13	269	1.123	0.9934
Medium I F – Medium N M	3.1336	2.62	269	1.196	0.9889
Medium I F – High N M	-10.8684	2.3	269	-4.728	**0.0002**
High I F – Low N F	5.8229	2.16	269	2.702	0.2311
High I F – Medium N F	6.7755	2.05	269	3.299	**0.0498**
High I F – High N F	-1.694	1.68	269	-1.008	0.9974
High I F – Low I M	14.3317	2.04	269	7.03	**<.0001**
High I F – Medium I M	6.814	1.87	269	3.648	**0.0163**
High I F – High I M	-3.9464	1.65	269	-2.397	0.4119
High I F – Low N M	7.6214	1.87	269	4.079	**0.0034**
High I F – Medium N M	8.3616	1.96	269	4.266	**0.0016**
High I F – High N M	-5.6405	2.37	269	-2.382	0.4217
Low N F – Medium N F	0.9526	2.22	269	0.428	1
Low N F – High N F	-7.5169	2.29	269	-3.276	0.0532
Low N F – Low I M	8.5088	2.58	269	3.294	0.0505
Low N F – Medium I M	0.9911	2.05	269	0.483	1
Low N F – High I M	-9.7694	2.07	269	-4.727	**0.0002**
Low N F – Low N M	1.7984	1.76	269	1.022	0.9971
Low N F – Medium N M	2.5387	2.14	269	1.187	0.9896
Low N F – High N M	-11.4634	2.23	269	-5.146	**<.0001**
Medium N F – High N F	-8.4695	2.2	269	-3.85	**0.008**
Medium N F – Low I M	7.5562	2.11	269	3.578	**0.0206**
Medium N F – Medium I M	0.0385	2.16	269	0.018	1
Medium N F – High I M	-10.722	1.96	269	-5.468	**<.0001**
Medium N F – Low N M	0.8459	1.95	269	0.434	1
Medium N F – Medium N M	1.5861	1.74	269	0.913	0.999
Medium N F – High N M	-12.416	2.13	269	-5.829	**<.0001**
High N F – Low I M	16.0257	2.19	269	7.333	**<.0001**
High N F – Medium I M	8.5081	2.03	269	4.198	**0.0021**
High N F – High I M	-2.2524	2.34	269	-0.964	0.9983

High N F – Low N M	9.3154	2.03	269	4.594	**0.0004**
High N F – Medium N M	10.0556	2.11	269	4.761	**0.0002**
High N F – High N M	-3.9464	1.65	269	-2.397	0.4119
Low I M – Medium I M	-7.5177	1.93	269	-3.893	0.0068
Low I M – High I M	-18.2782	1.94	269	-9.399	**<.0001**
Low I M – Low N M	-6.7104	1.71	269	-3.931	**0.006**
Low I M – Medium N M	-5.9701	2.02	269	-2.955	0.1282
Low I M – High N M	-19.9722	2.12	269	-9.442	**<.0001**
Medium I M – High I M	-10.7605	1.76	269	-6.097	**<.0001**
Medium I M – Low N M	0.8073	1.75	269	0.461	1
Medium I M – Medium N M	1.5476	1.66	269	0.935	0.9987
Medium I M – High N M	-12.4545	1.95	269	-6.384	**<.0001**
High I M – Low N M	11.5678	1.77	269	6.551	**<.0001**
High I M – Medium N M	12.308	1.86	269	6.609	**<.0001**
High I M – High N M	-1.694	1.68	269	-1.008	0.9974
Low N M – Medium N M	0.7402	1.85	269	0.4	1
Low N M – High N M	-13.2618	1.95	269	-6.794	**<.0001**
Medium N M – High N M	-14.0021	2.04	269	-6.865	**<.0001**

Appendix 4: Results from the plant midvein length after 75-day survival experiment.

Table 4A: Summary statistics from the linear regression model (LM) for the plant midvein length after 75-day survival experiment.

Predictors	*Estimates*	*CI*	*p*
(Intercept)	4.75	4.01 – 5.49	**<0.001**
Treatment_Level [Medium]	1.28	0.15 – 2.40	**0.026**

Treatment_Level [High]	2.66	1.71 – 3.60	**<0.001**
Flower_Rem [N]	-0.26	-1.30 – 0.78	0.628
Sex [M]	-1.37	-2.34 – -0.40	**0.006**
Treatment_Level [Medium] * Flower_Rem [N]	-0.41	-1.90 – 1.08	0.587
Treatment_Level [High] * Flower_Rem [N]	0.06	-1.35 – 1.46	0.937
Treatment_Level [Medium] * Sex [M]	0.45	-0.93 – 1.83	0.520
Treatment_Level [High] * Sex [M]	-0.31	-1.55 – 0.94	0.629
Flower_Rem [N] * Sex [M]	1.80	0.49 – 3.11	**0.007**
(Treatment_Level [Medium] * Flower_Rem [N]) * Sex [M]	-1.39	-3.24 – 0.46	0.141
(Treatment_Level [High] * Flower_Rem [N]) * Sex [M]	-0.27	-2.09 – 1.55	0.769
Observations	278		
R^2 / R^2 adjusted	0.367 / 0.340		

Table 4B: An information criterion (AIC) model simplification was employed to select a linear model that best fits the midvein length data. Source population was therefore excluded as a fixed effect.

Midvein_Length ~ Treatment_Level + Flower_Rem + Sex + Treatment_Level:Flower_Rem + Flower_Rem:Sex

Predictors	**Df**	**SS**	**RSS**	**AIC**
(Model)			602.2	230.89
Treatment_Level:Flower_Rem	2	24.372	626.57	237.92

Flower_Rem:Sex	1	26.945	629.15	241.06

Table 4C: The type III SS ANOVA test results from the midvein length model.

	Sum Sq	Df	F value	Pr(>F)
(Intercept)	361.00	1	161.6555	< 2.2e-16 ***
Treatment_Level	69.92	2	15.6561	3.732e-07 ***
Flower_Rem	0.53	1	0.2352	0.628069
Sex	17.4	1	7.7906	0.005632 **
Treatment_Level:Flower_Rem	1.06	2	0.2374	0.788838
Treatment_Level:Sex	3.14	2	0.7033	0.495847
Flower_Rem:Sex	16.25	1	7.2747	0.007440 **
Treatment_Level:Flower_Rem:Sex	5.52	2	1.2351	0.292461
Residuals	594.02	266		

Table 4D: Summary of Tukey's post-hoc test of pairwise comparisons of midvein lengths across treatment levels, flower removal, and sex.

Comparisons	**Estimates**	**Standard Error (SE)**	**Degrees of Freedom (Df)**	***t*-ratio**	***P***
Low I F - Medium I F	-1.4288	0.583	260	-2.45	0.3767
Low I F - High I F	-2.8543	0.494	260	-5.781	**<.0001**
Low I F - Low N F	0.1945	0.536	260	0.363	1

Low I F - Medium N F	-0.7556	0.51	260	-1.481	0.9446
Low I F - High N F	-2.5594	0.536	260	-4.778	**0.0002**
Low I F - Low I M	1.27	0.499	260	2.546	0.3168
Low I F - Medium I M	-0.4693	0.459	260	-1.022	0.9971
Low I F - High I M	-1.1316	0.47	260	-2.407	0.4054
Low I F - Low N M	-0.2462	0.458	260	-0.538	1
Low I F - Medium N M	-0.2016	0.48	260	-0.42	1
Low I F - High N M	-2.4538	0.512	260	-4.79	**0.0002**
Medium I F - High I F	-1.4255	0.53	260	-2.688	0.2382
Medium I F - Low N F	1.6234	0.579	260	2.806	0.1838
Medium I F - Medium N F	0.6732	0.546	260	1.234	0.9857
Medium I F - High N F	-1.1306	0.575	260	-1.965	0.7162
Medium I F - Low I M	2.6988	0.544	260	4.962	**0.0001**
Medium I F - Medium I M	0.9595	0.506	260	1.896	0.7607
Medium I F - High I M	0.2973	0.511	260	0.582	1
Medium I F - Low N M	1.1826	0.507	260	2.334	0.4556
Medium I F - Medium N M	1.2273	0.524	260	2.344	0.4486
Medium I F - High N M	-1.0249	0.557	260	-1.842	0.7933
High I F - Low N F	3.0489	0.487	260	6.265	**<.0001**
High I F - Medium N F	2.0987	0.448	260	4.685	**0.0003**
High I F - High N F	0.2949	0.484	260	0.61	1
High I F - Low I M	4.1244	0.444	260	9.294	**<.0001**

High I F - Medium I M	2.385	0.399	260	5.977	**<.0001**
High I F - High I M	1.7228	0.401	260	4.298	**0.0014**
High I F - Low N M	2.6082	0.404	260	6.464	**<.0001**
High I F - Medium N M	2.6528	0.42	260	6.317	**<.0001**
High I F - High N M	0.4006	0.453	260	0.884	0.9992
Low N F - Medium N F	-0.9502	0.506	260	-1.879	0.7711
Low N F - High N F	-2.7539	0.534	260	-5.157	**<.0001**
Low N F - Low I M	1.0755	0.496	260	2.17	0.573
Low N F - Medium I M	-0.6639	0.456	260	-1.455	0.9511
Low N F - High I M	-1.3261	0.459	260	-2.886	0.152
Low N F - Low N M	-0.4407	0.457	260	-0.965	0.9983
Low N F - Medium N M	-0.3961	0.474	260	-0.835	0.9995
Low N F - High N M	-2.6483	0.507	260	-5.22	**<.0001**
Medium N F - High N F	-1.8037	0.499	260	-3.613	**0.0184**
Medium N F - Low I M	2.0257	0.467	260	4.334	**0.0012**
Medium N F - Medium I M	0.2863	0.425	260	0.674	0.9999
Medium N F - High I M	-0.3759	0.427	260	-0.881	0.9992
Medium N F - Low N M	0.5095	0.422	260	1.207	0.9881
Medium N F - Medium N M	0.5541	0.441	260	1.257	0.9834
Medium N F - High N M	-1.6981	0.477	260	-3.562	**0.0218**
High N F - Low I M	3.8294	0.497	260	7.7	**<.0001**
High N F - Medium I M	2.0901	0.458	260	4.567	**0.0005**

High N F - High I M	1.4278	0.462	260	3.088	0.0908
High N F - Low N M	2.3132	0.456	260	5.071	**<.0001**
High N F - Medium N M	2.3578	0.475	260	4.966	**0.0001**
High N F - High N M	0.1056	0.508	260	0.208	1
Low I M - Medium I M	-1.7394	0.41	260	-4.246	**0.0018**
Low I M - High I M	-2.4016	0.417	260	-5.752	**<.0001**
Low I M - Low N M	-1.5162	0.416	260	-3.646	**0.0165**
Low I M - Medium N M	-1.4716	0.435	260	-3.386	**0.0384**
Low I M - High N M	-3.7238	0.469	260	-7.942	**<.0001**
Medium I M - High I M	-0.6622	0.37	260	-1.79	0.8223
Medium I M - Low N M	0.2232	0.365	260	0.612	1
Medium I M - Medium N M	0.2678	0.388	260	0.69	0.9999
Medium I M - High N M	-1.9844	0.428	260	-4.641	**0.0003**
High I M - Low N M	0.8854	0.373	260	2.373	0.4284
High I M - Medium N M	0.93	0.391	260	2.376	0.4265
High I M - High N M	-1.3222	0.428	260	-3.088	0.0908
Low N M - Medium N M	0.0446	0.388	260	0.115	1
Low N M - High N M	-2.2076	0.43	260	-5.133	**<.0001**
Medium N M - High N M	-2.2522	0.446	260	-5.051	**0.0001**

Appendix 5: Results from the corm count data analyses.

Table 5A: The results from the GLM test for the corm count data.

Predictors	*Incidence Rate Ratios*	*CI*	*p*
(Intercept)	15.80	13.40 – 18.62	**<0.001**
Treatment_Level [Medium]	1.08	0.91 – 1.28	0.374
Treatment_Level [High]	1.64	1.43 – 1.89	**<0.001**
Flower_Rem [N]	0.99	0.84 – 1.17	0.912
Sex [M]	1.08	0.93 – 1.26	0.326
Treatment_Level [Medium] * Flower_Rem [N]	1.00	0.80 – 1.26	0.979
Treatment_Level [High] * Flower_Rem [N]	0.80	0.64 – 0.98	**0.035**
Treatment_Level [Medium] * Sex [M]	0.98	0.79 – 1.22	0.875
Treatment_Level [High] * Sex [M]	1.04	0.87 – 1.25	0.644
Flower_Rem [N] * Sex [M]	0.95	0.77 – 1.18	0.658
(Treatment_Level [Medium] * Flower_Rem [N]) * Sex [M]	1.10	0.82 – 1.46	0.535
(Treatment_Level [High] * Flower_Rem [N]) * Sex [M]	1.25	0.96 – 1.63	0.101
Random Effects			

σ^2	0.05
$\tau_{00\ Source\_Population}$	0.02
ICC	0.33
$N_{\ Source\_Population}$	7
Observations	300
Marginal R^2 / Conditional R^2	0.395 / 0.592

Table 5B: An information criterion (AIC) model simplification was employed to select a linear model that best fits the corm count data. Source population was included as a fixed effect.

Corm_Count ~ Treatment_Level + Flower_Rem + Sex + Source_Population + Treatment_Level:Flower_Rem + Treatment_Level:Sex

Predictors	**Df**	**Deviance**	**AIC**
(Model)		1294.7	2745.8
+Flower_Rem:Sex	1	1292.9	2745.9
-Treatment_Level:Sex	2	1299	2746
-Treatment_Level:Flower_Rem	2	1300.7	2747.7
-Source_Population	6	1475.2	2914.2

Table 5C: The type III SS ANOVA test results from the corm count data.

	Chisq	**Df**	**Pr(>Chisq)**
(Intercept)	1082.3603	1	< 2.2e-16 ***
Treatment_Level	64.55	2	9.596e-15 ***
Flower_Rem	0.0123	1	0.91171
Sex	0.9643	1	0.32610

Treatment_Level:Flower_Rem	6.6328	2	0.03628 *
Treatment_Level:Sex	0.5287	2	0.76770
Flower_Rem:Sex	0.1956	1	0.65830
Treatment_Level:Flower_Rem:Sex	2.8542	2	0.24001

Appendix 6: Results from the corm weight data:

Table 6A: Summary statistics from the linear regression model (LM) for the corm weight data. Model selected after an information criterion simplification.

Predictors	*Estimates*	*CI*	*p*
(Intercept)	16.05	6.27 – 25.83	**0.001**
Treatment_Level [Medium]	2.75	-11.61 – 17.10	0.708
Treatment_Level [High]	38.55	26.28 – 50.82	**<0.001**
Flower_Rem [N]	1.49	-12.10 – 15.08	0.829
Sex [M]	1.10	-11.68 – 13.87	0.866
Treatment_Level [Medium] * Flower_Rem [N]	-0.59	-19.86 – 18.68	0.952

Treatment_Level [High] * Flower_Rem [N]	-11.49	-30.08 – 7.11	0.226
Treatment_Level [Medium] * Sex [M]	1.01	-16.94 – 18.97	0.912
Treatment_Level [High] * Sex [M]	9.30	-7.13 – 25.73	0.267
Flower_Rem [N] * Sex [M]	0.41	-16.98 – 17.80	0.963
(Treatment_Level [Medium] * Flower_Rem [N]) * Sex [M]	1.81	-22.32 – 25.94	0.883
(Treatment_Level [High] * Flower_Rem [N]) * Sex [M]	5.45	-18.67 – 29.57	0.658
Random Effects			
σ^2	417.10		
$\tau_{00\ Source\_Population}$	10.53		
ICC	0.02		
$N_{\ Source\_Population}$	7		
Observations	300		
Marginal R^2 / Conditional R^2	0.447 / 0.461		

Table 6B: An information criterion (AIC) model simplification was employed to select a linear model that best fits the corm weight data. Source population was therefore included as a fixed effect. Flower removal was excluded.

Total_Corm_Weight_grams ~ Treatment_Level + Sex + Source_Population + Treatment_Level:Sex

Predictors	**Df**	**SS**	**RSS**	**AIC**
(Model)			118855	1818.6
-Source_Population	6	5062.9	123918	1819.1
-Treatment_Level:Sex	2	1832.2	120687	1819.2
+Flower_Rem	1	0.4	118854	1820.6

Table 6C: The type III SS ANOVA test results from the corm weight model.

	Chisq	**Df**	**Pr(>Chisq)**
(Intercept)	10.3492	1	0.001295 **
Treatment_Level	48.7115	2	2.645e-11 ***
Flower_Rem	0.0464	1	0.829492
Sex	0.0283	1	0.866400
Treatment_Level:Flower_Rem	1.9108	2	0.384664
Treatment_Level:Sex	1.5957	2	0.450289
Flower_Rem:Sex	0.0021	1	0.963238
Treatment_Level:Flower_Rem:Sex	0.2056	2	0.902327

Table 6D: Summary of Tukey's post-hoc test of pairwise comparisons of corm weights across treatment levels and sex.

Comparisons	**Estimate**	**SE**	**Df**	***t*-ratio**	***P***
Low F Medium F	-2.95	4.88	288	-0.604	0.9907
Low F - High F	-33.88	4.69	288	-7.218	**<.0001**
Low F - Low M	-1.55	4.34	288	-0.358	0.9992
Low F - Medium M	-4.81	4.25	288	-1.131	0.8681
Low F - High M	-46.37	4.42	288	-10.479	**<.0001**
Medium F - High F	-30.93	4.58	288	-6.755	**<.0001**

Medium F - Low M	1.39	4.31	288	0.323	0.9995
Medium F - Medium M	-1.86	4.22	288	-0.44	0.9979
Medium F - High M	-43.42	4.38	288	-9.92	**<.0001**
High F - Low M	32.32	4.12	288	7.843	**<.0001**
High F - Medium M	29.07	3.99	288	7.282	**<.0001**
High F - High M	-12.49	4.15	288	-3.013	**0.0333**
Low M - Medium M	-3.25	3.61	288	-0.9	0.9463
Low M - High M	-44.81	3.84	288	-11.672	**<.0001**
Medium M - High M	-41.56	3.7	288	-11.231	**<.0001**