# SPECIES RESPONSE TO RAPID ENVIRONMENTAL CHANGE IN A SUBARCTIC POND

by

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# Abstract

Unprecedented rates of anthropogenic environmental change have resulted in dramatic decreases in biodiversity worldwide. In order to persist during changes in both the abiotic and biotic environment resulting from anthropogenic stressors such as climate change and habitat degradation, populations must be able to respond or face extirpation. Predicted population-level responses to environmental change include i) range shifts as individuals disperse into more suitable regions, ii) phenotypic plasticity allowing for shifts in the mean phenotype of the population or iii) microevolution resulting from a genetic change within the population. The goal of this thesis is to assess how species within a community respond to a dramatic change in the environment.

This study used the sediment record of a Subarctic pond to investigate the impacts of a rapid increase in salinity on two species of the crustacean zooplankton *Daphnia*. One species, *Daphnia tenebrosa*, was unable to persist in the high salinity conditions and is believed to have been extirpated from the system. The other species, *Daphnia magna*, was tolerant of the new environmental conditions and was present throughout the sediment record. To investigate the changes in life history of *D. magna*, resting eggs from the sediment were hatched to compare iso-female lines from pre- and post-disturbance time periods. No differences were observed between the clone lines, suggesting that phenotypic plasticity allowed *D. magna* to persist despite the rapidly changing environmental conditions, and that microevolution in salinity tolerance may not have occurred in this population.

This study suggests that, in environments with moderate levels of post environmental change, pre-existing phenotypic plasticity may play a greater role than microevolution in species response to environmental changes. However, not all species from a community display the same response to environmental changes, as seen in this study with the extirpation of *D. tenebrosa*. To better understand how communities will be affected by future environmental change, further

investigations need to be made on what factors influence species response. Identifying species response may allow conservation efforts to focus on species that are unlikely to adapt to environmental change, and are most at risk.

# Co-Authorship

This thesis conforms to the Manuscript Format as outlined by the School of Graduate Studies and Research, with consolidated Acknowledgements and Literature Cited sections. Manuscripts resulting from this thesis are outlined below:

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# Table of Contents

# List of Figures

# List of Tables

# List of Abbreviations

AIC- Akaike Information Criterion

ANOVA – Analysis of variance

ARMA- Auto-Regressive Moving Average

GLS- Generalized Least Squares

µS- microSiemen, unit of electric conductance and electric admittance in the International System of Units

sd- Standard Deviation

SFR- Size at First Reproduction

TP – Total phosphorus

vB- von Bertalanffy model

WNP- Wapusk National Park

# Chapter 1

# General Introduction

## A Review of Environment Change & Species Responses

Earth's history is marked by natural environmental changes, such as Quaternary glacial / interglacial cycles over hundreds of thousands of years (Anderson et al. 2013) and contemporary interannual and multi-decadal shifts in climate produced by the North Atlantic Oscillation and the El Niño Southern-Oscillation (Stenseth et al. 2003). In addition to natural drivers of environmental change, human activity has been altering the planet since the advent of agriculture (Ruddiman et al. 2003). Global population increase results in an ever increasing demand for resources, and while advances in technology have been able to meet these demands, it has come at a significant cost to the environment (Foley et al. 2005).

Global change observed since the mid-20$^{th}$ century has occurred at an unprecedented rate (Vitousek 1994), and this rate is projected to continue to increase in future. Climate change due to the emission of greenhouse gases is expected to result in a 1.6 to 6.5°C average global temperature increase over the next 100 years (IPCC 2007a) and to dramatically alter current biodiversity patterns (Hughes 2000; Walther 2002; Parmesan 2006). Agriculture is currently, and is predicted to remain, the major driver of non-climatic environmental change due to loss of habitat, impact of pesticides, increase in salinity due to irrigation and nutrient loading in both terrestrial and aquatic environments (Tilman et al. 2001). Other global environmental stressors include changes in land use, the spread of invasive species and increased nitrogen deposition (Vitousek 1994; Sala et al. 2000; Dudgeon et al. 2006). In order to persist during past environment changes, contemporary species were either able to adapt to the new conditions or disperse into a more suitable environment (Ammann et al. 2000). However, contemporary environmental change is predicted to occur at a rate greater than any natural change in the last

two million years, and it is therefore unclear whether species' response will be able to match this rapid rate of change (Visser 2008). To better predict the biological consequences of current rapid environmental change, ecologists need to understand how species respond to abiotic and biotic changes within an ecosystem, and what responses are associated with different types of change.

To understand the distribution of species, ecologists have historically used Hutchinson's (1958) realized niche to define the abiotic and biotic factors that limit a species' distribution. As no environment is constant, ecologists became interested in how species respond when changes occur such that the realized niche is no longer present. Early studies suggested that range shifts were the primary mechanisms of persistence. If species were unable to disperse, community reassembly and species extirpation were viewed as other possible responses (Jackson and Overpeck 2000). These studies were later criticized for considering the species niche as "fixed", since they did not include on site adaptation as a possible response (Davis et al. 2005). Contemporary studies recognize three possible responses of populations to environmental change: i) they can disperse resulting in range shifts, ii) they can shift the mean phenotype of the population via phenotypic plasticity, or iii) a genetic change can occur within the population resulting in microevolution (Holt 1990; Davis et al. 2005; Gienapp et al. 2008).

Range shifts occur when a population is unable to tolerate new environmental conditions and individuals disperse into an environment to which they are better suited. Current research suggests that range shifts are most likely to occur when the severity of the change exceeds any possible plastic response (Gienapp et al. 2008), or when the rate of adaptation is slow, such as in species with longer generation times (Visser 2008). Numerous examples of range shifts have been documented in response to climate warming (Parmesan and Yohe 2003; Root et al. 2003), with the overwhelming trend of species moving polewards towards "cooler", higher latitudes. However, the power of range shifts to mitigate the effects of contemporary environmental change in the population is limited, especially when compared to climate changes experienced during the

late Quaternary (Davis and Shaw 2001). In order for a population to match its current environmental conditions it would be required to disperse 300-500km over the next century (Huntley et al. 1997) given current rates of climate change. However, historical dispersal rates are estimated to average only 20-40km/century (Davis and Shaw 2001). It has therefore been suggested that the rate of migration is likely insufficient compared to the rate of environmental change. Additionally, even if species were able to migrate at a sufficient rate, changes in land use have resulted in severe habitat fragmentation and thus few clear "corridors" exist for species to move from their current location to a more suitable region (Pimm and Raven 2000). It is therefore unlikely that range shifts alone will allow a species to persist in response to climate change (Davis and Shaw 2001). Range shifts also seem unlikely to be adequate to address other stressors such as biotic invasions or eutrophication events which tend to homogenize habitats, as opposed to climate change which causes a shift in the location of habitat types. Therefore, future shifts in response to environment change will likely involve range constrictions or be associated with adaptation in order to allow persistence in a new habitat.

Species that do not disperse effectively in response to environmental change will need to adapt or otherwise face a decline in abundance and possibly extirpation. One of the mechanisms of adaptation, phenotypic plasticity, occurs when a population experiences a shift in the mean phenotype (the observable traits or characteristics of an organism) but no change in the genetic structure (Bradshaw 1965). Plastic traits are the result of a single genotype's ability to express various phenotypes in response to the environment, allowing the mean trait value to shift with no genetic consequences. The other mechanism, microevolution, is a genetic shift in the population in response to altered abiotic or biotic conditions. As the new environment will exert different selection pressures, genotypes with higher fitness will increase in abundance resulting in a genetic shift within the population (Williams 1966). During the last decade the evolutionary responses of populations to environmental changes has been intensely investigated both theoretically (Chevin

et al. 2010) and empirically (Reale et al. 2003; Phillimore et al. 2012; van Buskirk et al. 2012) in an attempt understand species traits and habitat characteristics that favour one response over the other.

Phenotypic plasticity, either physiological or morphological, allows for the expression of differential traits of a single genotype in response to reliable environmental cues, thereby increasing an individual's lifetime fitness (Bradshaw 1965). Some of the best documented examples of phenotypic plasticity have been seen in response to climate change (Przybylo et al. 2000; Reale et al. 2003; Teplitsky et al. 2008; Ozgul et al. 2010; Phillimore et al. 2012). For example, Charmantier et al. (2008) documented a 14 day advancement in the breeding date of a British population of Great Tits, *Parus major*, over 47 years that was entirely attributed to phenotypic plasticity. In a meta-analysis of species from the Northern Hemisphere, Parmesan (2007) found an overwhelming trend for the advancement of spring phenology of approximately 2.3-2.8 days/decade. While not all of the observed advancement is necessarily attributed to phenotypic plasticity (Reale et al. 2003; van Buskirk et al. 2003), it is likely to have been significant. Phenotypic plasticity in response to other global stressors such as eutrophication and the introduction of exotic species is not well documented. However, it is a well-known response to predators, species interactions, and restrictions in resource availability (Miner et al. 2003). This suggests plasticity may play a key role in mitigating the impacts of land use change and invasive species. Plasticity is both limited in how it allows a genotype to respond to the environment, and costly (DeWitt et al. 1998). Maintenance and production of sensory and regulatory mechanisms is predicted to be energetically costly, however only weak empirical evidence has been observed to support the theory of plasticity costs (van Buskirk and Steiner 2009). Plastic responses are limited by environmental cues, and any lag between an environmental change and the production of the phenotype may result in a reduction in fitness. Additionally, plastic responses are often unable to develop extreme phenotypes, which can be a major disadvantage if the severity of an

environmental change is so great that it exceeds the range of possible phenotypes. In general, phenotypic plasticity is primarily viewed as a response to environmental variability which may allow species to persist after an initial change in the environment (Chevin and Lande 2010), however, it is unlikely to provide a long-term solution if the change is unidirectional and continuous.

Microevolution is often thought to be the likely long term response to environmental change (Davis and Shaw 2001; Reale et al. 2003; Berteaux 2004; Pulido and Berthold 2004; Davis et al. 2005; Thomas 2005). Genetic shifts in populations can occur in several different ways. The first, and most common, is that selection acts on pre-existing variation in the population. However, new genotypes can also be introduced into the population through mutation within the population, or through dispersal of genotypes from other populations. There is a much lower probability of microevolution by a novel mutation and requires a larger population size and greater number of generations, due to the improbability of a random beneficial mutation (Bell and Gonzalez 2009). Additionally, while dispersal increases the rate of microevolution at intermediate levels, high levels of dispersal can "swamp out" adaptive variation by introducing many maladapted genotypes (Bell and Gonzalez 2011).

Of all documented species level responses to climate change, few have been shown to be related to microevolution (Gienapp et al. 2008). However, clear microevolutionary responses have been observed in response to other environmental stressors, including changes in the bio-physical environment, changes in the host or food resource, a new predator community, or a new coexisting competitor (Thompson 1998; Reznick and Ghalambor 2001). Adaptations such as metal tolerance in plants (McNeilly and Bradshaw 1968) and toxin resistance in aquatic invertebrates (Hairston et al. 1999) suggest the potential of microevolution as a response to physio-chemical changes in the environment. While adaptive responses are expected in species with large population size and short generation times, it is unclear if microevolution will be an

important response for longer lived species that would require a much higher rate of change per generation in order to keep pace with the projected global change (Lynch and Lande 1993, Vander Wal et al. 2012).

Although the mechanisms of persistence are often presented as three discrete responses, they often co-occur in nature. Microevolution is prevalent along advancing range margins, due to high levels of dispersal (and therefore mixing of genotypes), which allows populations to match the new environment better (Davis and Shaw 2001). Investigations of changes in phenology have also revealed the presence of both microevolution and phenotypic plasticity (Reale 2003, Phillimore et al. 2012, van Buskirk et al. 2012) with varying levels of contribution to the overall observed change. Using detailed pedigree information of a Red Squirrel (*Tamiasciurus hudsonicus*) population in Alaska, Reale et al. (2003) were able to estimate the contribution of each response to the observed 18 day advancement in maturation date during the previous 10 years. This analysis revealed that 62% of the change was attributed to phenotypic plasticity, 13% to microevolution while 25% remained unknown. In comparison, in a study of spring arrival date in 27 bird populations over 45 years, Van Buskirk et al. (2012) estimated that the contribution of phenotypic plasticity was only 13-25%. These results suggest that different responses to environmental change are responsible for observed changes in discrete populations. Further investigation is needed to understand better how the different mechanisms of persistence interact and in what environments they may be expected to occur.

It is important to note that not all species will be able respond at a rate congruent with current rates of environmental change. If populations are unable to respond through range shifts, phenotypic plasticity or genetic changes, they will be extirpated (Pounds et al. 1999). Rates of biodiversity loss are already unprecedented (Vitousek 1994, Ricciardi and Rasmussen 1999, Sala et al. 2000) and are expected to remain high if environmental change continues at its current rate (Pimm and Raven 2000, Thomas 2005). Predictive theory that incorporates all three mechanisms

of response may help identify the species and populations most at risk as a result of current global change.

## *Daphnia* & Environmental Change

*Daphnia* is a genus of small crustacean zooplankton from the order Cladocera, and are commonly found in freshwater ecosystems. Species from this genera are usually transparent (although the development of melanic pigment has been observed), 0.2 and 5 mm in length, and are identifiable by their discus shape, singular compound eye and jerky swimming movement, resulting in their common name "water fleas" (Figure 1-1). This genus provides a key link between primary producers and secondary consumers, as daphniids are highly efficient grazers of algae (Porter 1977) and are readily consumed by fish and other planktivorous organisms. In ecosystems without vertebrate predators, daphniids are often the pinnacle of the aquatic food web and are critical in the cycling of nutrients back to the primary producers (Dobson and Frey 2001). Thus daphniids are one of the keystone species of aquatic ecosystems as their removal would impact all trophic levels.

Daphniids are generally sensitive to changes in the environment, and many species are considered "specialist" with narrow environmental tolerances. Abundance and species richness have been found to be impacted by changes in pH (Haines 1981), salinity (Hart 1991), food web dynamics (Gliwicz 2002), temperature (Wojtal-Frankiewicz 2012) and nutrients (Edmondson and Litt 1982). Given the sensitivity of this genus, daphniids are often used as a proxy for past environmental conditions by examining the presence of exoskeletal remains in the sediment record. Information on the presence and abundance of species can often provide a clear inference of the environmental conditions for a given time period. The key position daphniids hold in aquatic ecosystems, as well as their responsiveness to abiotic and biotic changes, makes this genus an excellent group to use in the investigation of environmental change.

## Resurrection Ecology

To study how populations respond to changes in their environment one must know the ancestral state of the population. To infer how populations respond to environmental change, reciprocal transplants and common garden experiments are used to compare populations under different selection pressures (Kawecki and Ebert 2004). While these studies provide information on how a population that has experienced a disturbance compares to one that has not, assumptions are made about a common starting point of the populations. In contrast egg banks, which are the result of the accumulation of seeds or resting cysts over time, provide an opportunity to directly compare genotypes pre- and post-disturbance to determine the response of a population to environmental change (Hairston 1996). These studies have the potential to inform how a species responds to a variety of environmental stressors in natural conditions.

Resurrection ecology is the term coined for the experimental comparison of genotypes or often iso-female clone lines that have been stimulated to emerge from resting egg banks, and has been described as the combination of experimental ecology and paleoecology (Kerfoot et al. 1999). This technique has been used to describe changes in populations due to stressors such as eutrophication (Hairston et al. 1999, 2001), predation (Cousyn et al. 2001, Kerfoot and Weider 2004) and metal contamination (Kerfoot et al. 1999). Although any organism that produces a resting stage can be used, aquatic invertebrates, particularly *Daphnia*, are most often used due to an established protocol for collection and hatching of resting eggs, as well as the ease of rearing and experimental manipulation. Additionally, daphniids have a relatively short generation time thus populations generally respond quickly, over a few years to a decade, to environmental stressors (Kerfoot et al. 1999, Hairston et al. 1999, 2001, Cousyn et al. 2001, Kerfoot and Weider 2004). Therefore shifts in life history traits should be evident in experimental testing.

*Daphnia* reproduce via cyclical parthenogenesis. For the majority of their lifespan female daphniids reproduce asexually, and only produce resting eggs after sexual reproduction which is generally initiated by periods of stress. Resting eggs are encapsulated in a desiccation resistant ephippium (Zaffagnini 1987), a modified part of the mothers carapace, deposited in the sediment and hatch almost exclusively in spring (Wolf and Carvalho 1989; Hairston et al. 2000). The cues regarding hatching appear not only to be species specific but also regionally dependent (Schwartz and Hebert 1987; Vandekerhove et al. 2005), and are believed to be primarily linked to photoperiod and temperature (Gyllstrom and Hansson 2003). Each spring a portion of ephippia do not hatch, but rather are incorporated into the lake's sediment record producing a chronologically structured record of genotypes present within the system (Brendonck and De Meester 2003).

The first major study using resurrection ecology techniques examined the impacts of historical eutrophication on the *Daphnia* population of the highly disturbed central European Lake Constance (Hairston et al. 1999). Eutrophication is associated with the proliferation of cyanobacteria which can be toxic to zooplankton. Hairston et al. (1999) found that the reduction in growth rate of daphniids as a result of the inclusion of cyanobacteria in the diet was less for genotypes from contemporary populations compared to ancestral populations. Daphniids have also shown behavioural and morphological responses to changes in predation pressures (Cousyn et al. 2001; Kerfoot and Weider 2004; Michels et al. 2007), metal contamination (Kerfoot et al. 1999), parasites (Decaestecker et al. 2007), and salinity (Barry et al. 2005). With advances in genetic technologies, studies focusing on changes in life history have been either replaced or supplemented by investigations of genetic changes within populations (Cousyn et al. 2001; Limburg and Weider 2002; Orsini et al. 2012).

The resting egg bank can also be used to examine community-level responses to environmental change. Ephippia not only provide a record of what species were present during a given period, but also reflect the relative abundance of those species (Jeppesen et al. 2003).

Therefore community dynamics can be tracked throughout the existing sediment record and used to look for invasions, extinctions as well as recolonizations in relation to known changes in the environment (Hairston et al. 1999; Duffy et al. 2000; Pollard et al. 2003; Mergeay et al. 2007; Brede et al. 2009). Additionally, since the egg bank integrates ephippia from throughout a season it often more accurately reflects the species richness of a system than standard one-time annual sampling (Brendonck and De Meester 2003; Vandekerkhove et al. 2007). In an examination of the egg bank from both a coastal and open water site in Lake Superior, Kerfoot et al. (2004) found that contemporary community assemblages did not reflect the historical species community composition. They suggested that anthropogenic activity, both logging and shipping traffic, was initially responsible for a shift in community composition. The later change in *Daphnia* composition was credited to the invasion of the exotic invertebrate predator *Bythotrephes*.

Resurrection ecology is currently one of the best methods to investigate how populations respond to environment change (Hairston 1996). However, gaps still exist that, if addressed, would further aid understanding and increase predictive capabilities for future change. A publication bias may also be present in the field, as all investigations have observed a marked change in the population (but see Hairston et al. 2001), suggesting that daphniids are remarkably adaptable organisms. Investigations into a greater range of environmental stressors is needed to determine if the currently observed trend in adaptability is widespread in *Daphnia* populations, or is the product of focused investigations of a few stressors (primarily eutrophication and predation) on a limited number of species, *D. pulicaria* and *D. magna*. Additionally, while investigations of both changes in species composition and changes within a species in response to environmental stressors are abundant, it is rare for both to be used in the same study. Examining how different species are impacted by the same environmental change may enhance our understanding of the mechanisms of species persistence. Identifying differences in species that

were, and were not, able to persist will further increase our ability to predict which species are likely to be most impacted by specific stressors.

## Salinization and its Impact on Aquatic Communities

Secondary salinization of freshwater ecosystems is the result of anthropogenic alterations of the landscape (Williams 1987), and is a growing cause of environmental change globally (Williams 2001; Kuashal 2005; Smol and Douglas 2007). The primary cause of salinization in arid and semi-arid regions is changes in land use due to agriculture (Hart et al. 1991). Native plants with long root systems are removed from the landscape and replaced with crops with much shorter roots. Without an extensive root system, a greater proportion of the precipitation enters the groundwater, raising the water table and mobilizing ions in the soil (Pannell and Ewing 2006). Increased salinity of groundwater, in turn, results in an increase in salinity of freshwater systems (Hart 1991). Globally, there are diverse drivers of increased salinity in freshwater systems including alteration of the water table due to irrigation (Williams 2001; Fazio and O'Farrell 2005), discharge of saline agriculture waters (Williams 2001; Arle and Wagner 2013) and deicers/road salts in North America (Kuashal et al. 2005; Ramakrisnna and Viraraghavan 2005; Likens and Buso 2010). It is expected that the number of water bodies impacted by salinization will continue to increase as human populations grow and land degradation continues.

Climate change is rapidly becoming a contributing cause of salinization in many regions, especially in shallow or closed basin systems (Evans and Prepas 1996; Smol and Douglas 2007). One region where this problem is most apparent is the Arctic. Rising temperatures result in increased evaporation, and if there are no concurrent increases in precipitation to offset this water loss we see an increase in aridity (Rouse et al. 1997; Rautio et al. 2011). In the Arctic, where the landscape is dominated by small, shallow ponds that are vulnerable to changes in the environment, higher evaporation:precipitation (E:P) ratios have resulted in dramatic increases in

the specific conductivity (Smol and Douglas 2007). Similar increases in salinity in larger closed basin systems in North America have also been attributed to changes in E:P ratios (Evans and Prepas 1996). While large, open freshwater systems are relatively well protected, this type of salinization may become more important in regions predicted to undergo serve drought.

Changes in salinity can have drastic effects on the structure of aquatic ecosystems (Williams 1998). Most freshwater species have low salinity tolerances, and communities start to experience adverse effects around 1g/L (≈1750µS/cm) (Hart et al. 1991). As salinity increases, species must invest more energy in osmoregulation resulting in decreases in both reproduction and recruitment (Hart et al. 2003). Over time, both species richness and abundance decrease and a previously diverse community is replaced with one or two tolerant species (Neilson et al. 2003; James et al. 2003). Not all taxonomic groups are equally impacted and while mature fish and macroinvertebrates appear to tolerate moderate increases in salinity, microinvertebrates, such as cladoceran and rotifer zooplankton, appear to be especially sensitive (Hart et al. 2003; Neilson et al. 2003).

All but one North American species of *Daphnia*, *D. salina*, are found in freshwater ecosystems and although they have different salinity tolerances, in general, increases in salinity have negative impacts on population growth. To study the impact of salinity, both acute and chronic trials can be used (OECD 1998, 2000). Acute trails usually are measured as the solute concentration which kills 50% of the population ($LC_{50}$), while chronic trials are used to study mortality and reproduction at a given solute concentration, which are then use to calculate intrinsic population growth rate.

The two species of interest for this study are *Daphnia tenebrosa* and *D. magna*. Very little information exists regarding the natural distribution or $LC_{50}$ of the arctic species *D. tenebrosa*. A survey of ponds on Ellesmere Island found *D. tenebrosa* was present in ponds with specific conductivities up to 1650µS/cm (Strecker et al. 2008). A four year survey of ponds in

Hudson Bay Lowlands found that *D. tenebrosa* occurred in ponds with specific conductivities ranging from 24 to 1500 µS/cm (Arnott, unpublished). In both surveys *D. tenebrosa* was not present in ponds with specific conductivities greater than 1650µS/cm, which suggests *D. tenebrosa* are most likely to be found in lower salinity ponds.

In contrast, an abundance of information is available regarding *D. magna's* salinity tolerance as it is commonly used as an indicator species in toxicity trails. $LC_{50}$ estimates for salinity of *D. magna* range from 0.49g/L (≈855µS/cm) (Semsari and Hait-Amar 2001) to 11.3g/L (≈19700µS/cm) (Schuytema et al. 1997) (Table 1.1), highlighting a problem in toxicity testing. Studies tend to use only a few genotypes (usually just one), often from the same area without taking into account differences their historical exposure to salt. So while toxicity tests may be useful in providing information about a specific system, one must take caution in applying the findings from one study to the whole species. Additional studies have found that intrinsic population growth rates for *D. magna* tend to decrease as salinity increases (Martinez-Jeronimo and Martinez-Jeronimo 2007; Goncalves 2007). However an investigation of the effects of salinity on a single *D. magna* genotype obtained from a rockpool adjacent to the ocean by Arner and Koivisto (1993) found the highest intrinsic population growth rate at 4 ‰ (≈7000µs/cm), which then decreased at higher salinity. The combined findings of these studies suggest that while it appears *D. magna* generally has a high salinity tolerance for cladoceran zooplankton, tolerance of a given genotype is dependent on the environment.

## Thesis Objectives

The goal of this thesis is to assess how species within a community respond to a dramatic change in the environment. First, egg bank records from an impacted and control site will be used to examine how community composition is altered by a dramatic environmental change. Second, life history experiments on iso-female clone lines from pre- and post-impact periods will be used

to assess the mechanism of response of a specific species. By combining species composition information from the eggbank and life history experiments, this study will help fill in gaps in the resurrection ecology literature about how different species from the same community respond to a discrete change in the environment. Furthermore this study will provide information on the response of species to a known anthropogenic stressor, salinization, in a region threated by global change.

## Study Site: Wapusk National Park & Snow Goose Impact

This study took place in Wapusk National Park (WNP), located on the west coast of Hudson Bay, approximately 45 km southeast of Churchill, Manitoba (Figure 1-2). The park is made of three primary ecozones, coastal fen, interior peatlands and spruce forest, and is part of Hudson-James Lowlands, one of the world's largest and most complex wetlands. Water bodies in this region are generally small and shallow, typically less than 1 hectare in area and rarely more than 1 m deep. Due to their small size ponds are very responsive to environmental change, such as change in precipitation or inputs in nutrients. Within the park there are over 11,000 lakes and ponds, two of which were selected as study sites, WNP-GG (15V 6502867 474424), a pond that has recently experienced dramatic environmental change, and WNP-X (15V 6399426 477552), a control site.

La Perouse Bay (15V 6509330 475606), on the northern border of the park, is a historical nesting site of the Lesser Snow Goose, *Chen caerulescens caerulescens*, and is currently the epicenter of habitat alteration being experienced in the park. Over fifty years of monitoring at the nesting site has documented exponential growth and expansion of goose populations (Cooke 1995). Population sizes throughout the 1960-70s were estimated to be around 2,500,000 individuals, however since that time populations have grown to over 22,500,000 individuals (Alisauskas et al. 2011). This dramatic increase was first noted by Ankney (1996)

who suggested the “explosion” was due to changes in agriculture practices in the species’ wintering grounds. Later reports confirmed that although the area of land being cultivated in the southern United States was constant during goose population growth, dramatic increases in agricultural productivity were resulting in large amounts of left over grain (corn, rice, wheat) being either spilled or left in the fields which was then consumed by the geese (Abraham et al. 2012). At the same time wildlife refuges were being established throughout the interior United States along the species migration route and often adjacent to high resource farmland. Therefore changes in farming and conservation practices resulted in a safer migration route lined with many resource rich stopovers (USFWS 2007) and a wintering ground with more food than the geese could consume (Krapu et al. 2004). Accordingly survival of geese during this time periods was very high, estimated to be between 83-89% (Alisauskas et al. 2011).

Geese have a dramatic impact on the landscape due to spring “grubbing”, which removes below ground plant biomass, and summer grazing, which removes the remaining above ground vegetation (Abraham and Jefferies 1997). Loss of vegetation results in increased evaporation from the soil, drawing inorganic salts from the underlying marine clays to the surface. The removal of the root system by geese further exaggerates this problem as the soil is unable to hold ions, which are washed into surrounding water bodies (Abraham et al. 2005). In a study of the invertebrate chironomids, Milakovic et al. (2001) attributed the differences in community assemblages in the goose-impacted region compared to those from elsewhere in the park to the high salinity of the goose ponds. Surveys of the park conducted between 2008 and 2010 also confirm that goose ponds have significantly greater specific conductivities than other ponds in the region (Figure 1-3). Additionally, total phosphorus, a proxy for primary productivity, has been found to be significantly higher in these ponds, likely due to nutrient input from feces (Manny et al. 1994).

Degradation of the original nesting site at La Perouse Bay and the rapid growth of Snow Geese populations have forced geese to move inland and utilize new freshwater habitats for nesting and feeding sites (Abraham et al. 2012). Primary nesting grounds have now expanded to sixteen times the original area, devastating the fragile Subarctic landscape (Cooke 1995). Study site WNP-GG, located approximately 6.5km south of La Perouse Bay, is one of many freshwater sites in the region that has experienced dramatic changes in abiotic conditions due to geese expansion.

This region was chosen to investigate how species respond to environmental change due to the dramatic and well documented nature of these recent changes. The magnitude of the increase in conductivity, from approximately 200-300µS/cm to 10,000-15,000 µS/cm, is so large and has occurred so rapidly that either an extirpation or adaptation response will have occurred in the population. Intense monitoring of the goose population indicates that these changes started to occur around 1990 (*pers. comm* Jon Sweetman, Park Biologist). The more than twenty years since the initial environmental changes to the undertaking of field work in this study could be enough time to detect a signal of microevolution if present in the population (Kerfoot et al. 1999; Hairston et al. 1999, 2001; Cousyn et al. 2001; Kerfoot and Weider 2004). Finally WNP-GG eggbank was rich in resting eggs. As the techniques of resurrection ecology, which often have a low hatching success rate, were planned to be used in this study an abundance of ephippia was required to complete the experiment.

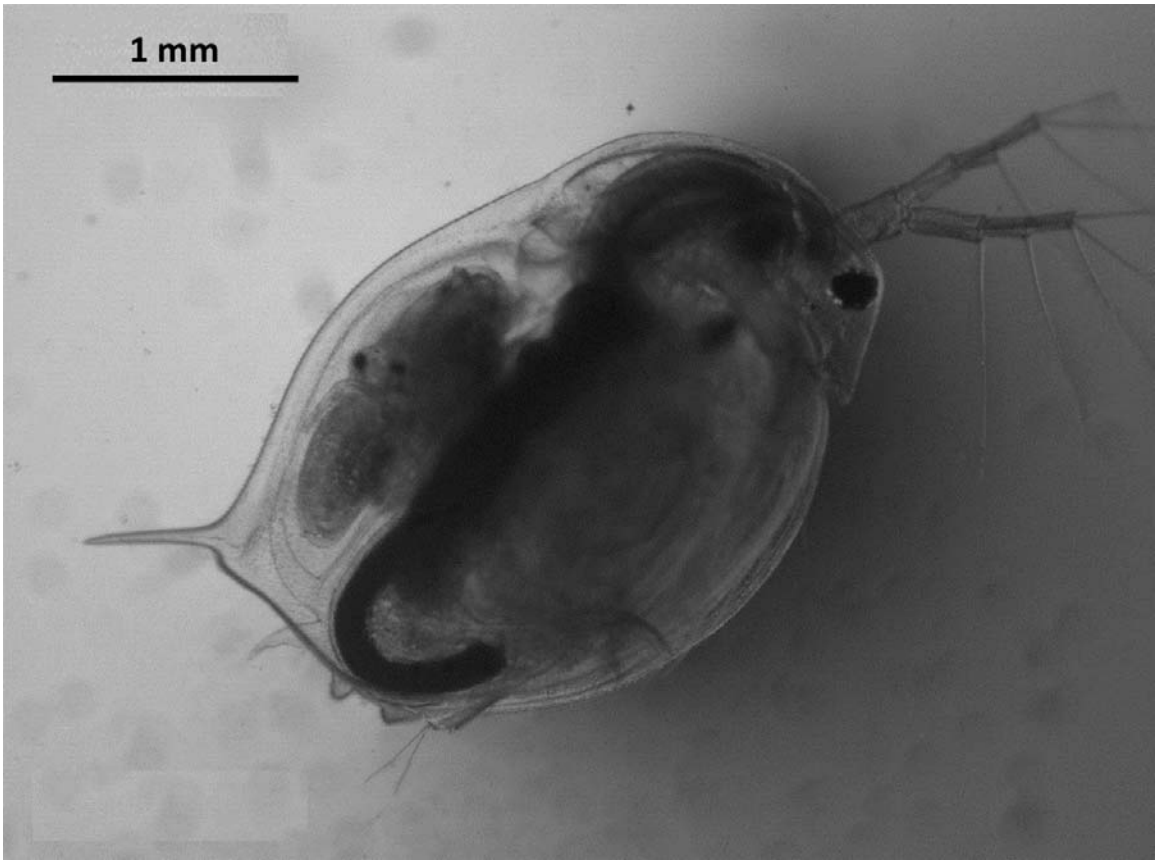


**Figure 1-1** A specimen of Daphnia magna, cultured in the Arnott lab, Queen's University. The clone line was developed by hatching resting eggs collected from WNP-GG in Wapusk National Park, MB. This adult female is carrying developing embryos.

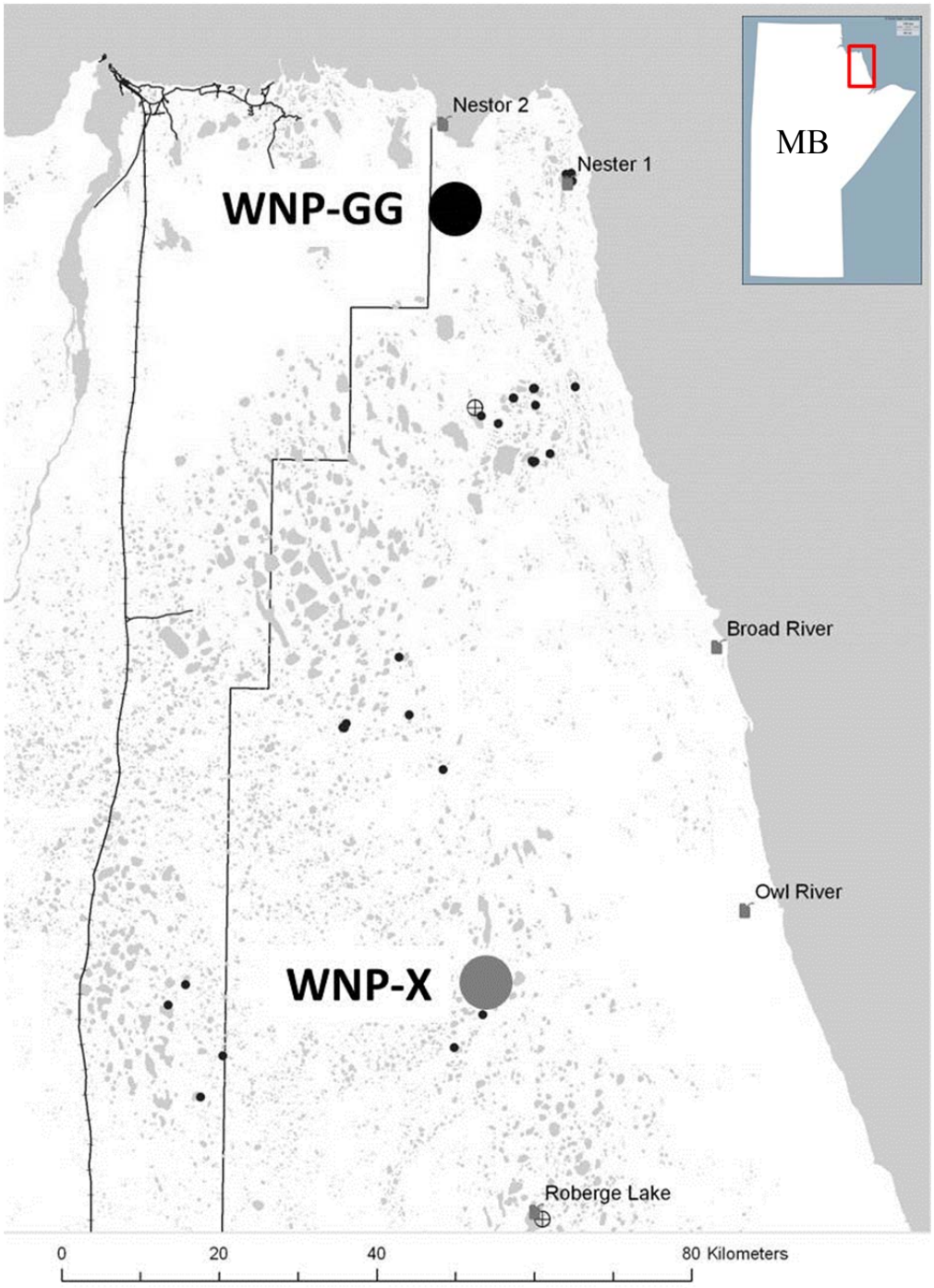


**Figure 1-2** Location of study sites, goose impacted WNP-GG and control site WNP-X in Wapusk National Park, Manitoba, Canada. The park boundary is marked by the solid verticle line. The hatched line repersents the railline between Thompson and Churchill, place names repersent fuel chaches and field stations within the park and small black dots repersent locations sampled in the 2008-2012 survey.

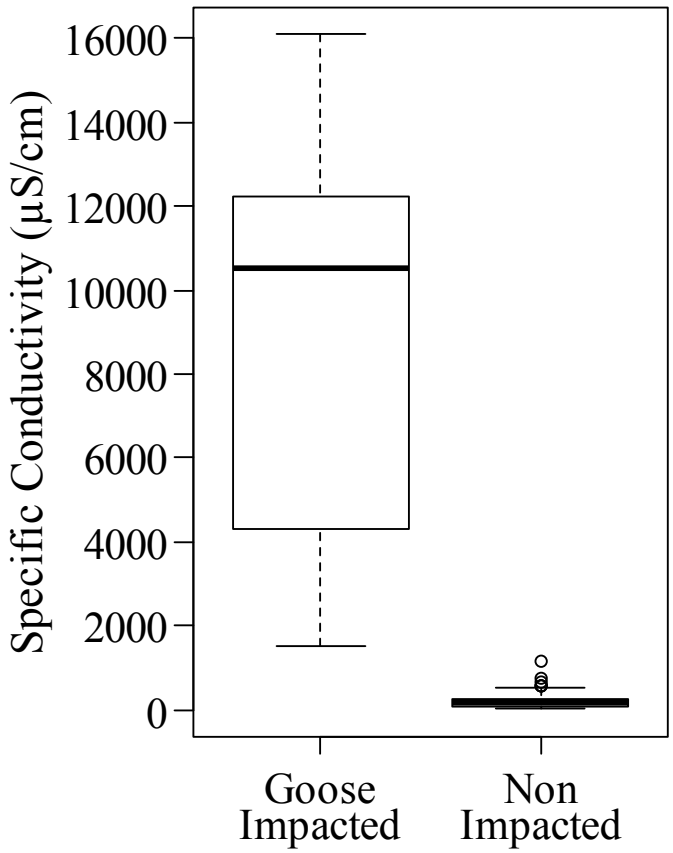


**Figure 1-3** Specific conductivity (µS/cm) of 3 goose-impacted and 30 non-impacted ponds in Wapusk National Park, MB sampled between 2010-2012. Six non-impacted ponds were not sampled in 2010, and three in 2012, as they had dried out due to high temperatures.

# Chapter 2

# Response of *Daphnia* to Rapid Environmental Change in a Subarctic Pond


## Abstract

Anthropogenic environmental change is currently occurring at an unprecedented rate globally. Populations must respond to these changes or face extirpation. Current predicted population-level responses include i) range shifts, ii) phenotypic plasticity or iii) microevolution. However, it is not well understood what factors influence how a population responds to changes in the environment. The goal of this study was to examine the impact of rapid unidirectional environment change on community composition and to determine the adaptation mechanism of persisting species. Sediment cores were taken from a Subarctic pond that experienced a dramatic salinization event approximately 15 years ago. *Daphnia* resting eggs were extracted from the sediment to examine changes in community composition and hatched to investigate differences in life history characteristics from pre- and post-disturbance iso-female clone lines. Two distinct responses were observed in the *Daphnia* community to the rapid environmental change. One species, *D. tenebrosa*, was unable to persist and was likely extirpated from the system. The other species, *D. magna*, was tolerant of the high salinity conditions and was present throughout the sediment record. No differences were observed in the acute or long term life history traits or growth metrics between *D. magna* clone lines from pre- and post-disturbance time periods. This indicates that microevolution in salinity tolerance likely did not occur in this population and that phenotypic plasticity allowed *D. magna* to persist despite the rapidly changing environmental conditions. This study suggests that in variable environments pre-existing phenotypic plasticity may play a greater role than microevolution in species response to environmental changes.

## Introduction

Unprecedented rates of anthropogenic environmental change over the last few decades have resulted in dramatic decreases in biodiversity worldwide (Vitousek 1994). Climate change is expected to result in a 1.6 to 6.5°C temperature increase over the next 100 years (IPCC 2007a). In addition, changes in land use, such as agriculture and urbanization, are leading to increased salinity and nutrient loading in both marine and freshwater systems (Tilman et al. 2001). Other global stressors, including the introduction of invasive species, hydrological modifications, industrial pollution, and nitrogen deposition are all expected to have substantial impacts on biodiversity (Sala et al. 2000; Dudgeon et al. 2006). Populations must respond to these anthropogenic changes or face extirpation. Thus, understanding the mechanisms that allow populations to persist in a rapidly changing environment will play a key role in predicting the future consequences of environmental change.

Predicted population-level responses to environmental change include i) range shifts as individuals disperse into more suitable regions, ii) phenotypic plasticity allowing for shifts in the mean phenotype of the population or iii) microevolution resulting from a genetic change within the population (Holt 1990; Davis et al. 2005; Gienapp et al. 2008). Currently, the role that each response will play in species persistence during periods of environmental change remains unclear. However, it is apparent that if a population does not disperse or adapt in response to the new environmental conditions, populations will likely face dramatic declines in abundance and possibly extirpation as other more tolerant species establish in the region (Brown et al. 1997, Ruokolainen et al. 2007).

Range shifts were traditionally viewed as the primary mechanism of response to changes in the environment (Jackson and Overpeck 2000; IPCC 2007b) and have been documented in many organisms in response to climate warming (Parmesan and Yohe 2003; Root et al. 2003). Grabherr et al. (1994) demonstrated that even moderate changes in climate induced upwards

elevation shifts in alpine vegetation. A similar move towards cooler climates was observed by Parmesan et al. (1999) in 22 of 35 non-migratory European butterfly populations as species ranges shifted north by 35–240km during the last century. The current rate of climate change is estimated to require populations to disperse 300-500km/century in order to match their current environmental conditions (Huntley et al. 1997). However, historical dispersal rate estimates average only 20-40km/century (Davis and Shaw 2001), with extreme examples of up to 150km/century (Ritchie and MacDonald 1986). While it therefore appears unlikely that, for many populations, dispersal will be able to match long term environmental changes, range shifts may still play an important role in mitigation if combined with other mechanisms of persistence (Davis and Shaw 2001). For other environmental stressors such as nutrient loading, range shifts are not likely to be as prevalent, since those stressors do not often result in adjacent habitats becoming more suitable in the manner that climate warming does. However, range shifts or contractions may still occur as populations disperse from unsuitable habitat.

Phenotypic plasticity, the ability of one genotype to express multiple phenotypes based on environmental cues, is commonly observed in response to climate change (Gienapp et al. 2008; Berg et al. 2010). Plastic responses, such as the advancement in breeding events in birds and flowering time in plants (Przybylo et al. 2000; Reale et al. 2003; Charmantier et el. 2008; Teplitsky et al. 2008; Nicotra et al. 2009; Phillimore et al. 2012), as well as increases in the body mass of mammals (Post et al. 1999; Ozgul et al. 2010), have all been observed in response to climate change. On the other hand, plastic responses to other forms of environmental change are less well documented. One example found that native soft shell clams were able to change their position in the sediment in response to a chemical cue released by the green crab *Carcinus maenas*, an invasive predator (Whitlow 2010). Overall, the role of phenotypic plasticity in contemporary global change remains largely unknown. As plastic responses usually only occur over a small gradient of environmental conditions they do not often lead to the expression of

extreme phenotypes (DeWitt et al. 1998). Therefore plasticity has been suggested as only a short term mechanism of persistence in response to long term unidirectional environmental change because there is not enough scope in the plasticity for the organism to persist (Lynch and Lande 1993).

Microevolution is the result of a genetic shift within a population. Such shifts may occur if i) a mutation occurs within the population resulting in a better suited genotype, ii) regional dispersal results in the establishment of a more adapted genotype from another population or iii) genetic variation is present within a population. Microevolution is advantageous for species as it allows for long term persistence in an altered environment (Davis and Shaw, 2001; Davis et al., 2005; Thomas, 2005). To date, the role of microevolution in mitigating the impacts of climate change appears to be small (Gienapp et al. 2008); however, this may be due to the difficulties of detecting relevant genetic changes in wild populations. In contrast, empirical examples of microevolution to other stressors are abundant in the literature (reviewed by Thompson 1998, Reznick and Ghalambour 2001). Microevolution has been observed in response to changes in the physico-chemical environment, such as the development of metal tolerance in plants (McNeilly and Bradshaw 1968), behavioral changes in daphniids in response to predation pressure (Cousyn et al. 2001) as well as changes in morphology in response to new host species in the soapberry bug (*Jadera haematoloma*) (Carroll et al. 1997).

Although extirpation has been documented in response to global change (Pimm and Raven 2000), it is becoming increasingly apparent that species are able to adapt and persist in altered environments (Thompson 1998). The prevalence of plastic responses to climate change, in contrast to the abundant examples of microevolution to other stressors, suggests that the type of environmental change experienced influences the type of species response. Investigations of the impacts of climate change have generally included a large number of species from one taxonomic group, such as Fitter and Fitter's (2002) study of changes in phenology for 385 British plant

species. On the other hand, studies examining stressors such as nutrient loading, acidification, and invasive species tend to focus on impacts to a single species (reviewed in Reznick and Ghalambor 2001). This suggests that further studies on the impact of environmental change are needed to determine if the pattern of differential species responses to specific stressors is widespread.

One way of studying how multiple species respond to change is to examine changes at the community level. While numerous studies have been conducted on the response of individual populations to environmental change, the impact on the community as a whole still remains unclear (Urban et al. 2012). Species' responses are expected to be influenced by past environmental conditions (Bell and Gonzalez 2011); therefore, species from the same region with similar life history characteristics may be expected to respond in a similar fashion. However, in an examination of the impact of acidification on zooplankton communities, Derry and Arnott (2007) found that only two of fourteen species in a historically acidified lake displayed an increase in acid tolerance. This suggests that responses to environmental change are species-dependent rather than regionally-dependent. To better understand how communities are impacted by environmental change the responses of multiple species from the same region must be examined.

The goal of this study was to examine the impact of rapid unidirectional environment change on community composition and to determine the adaptation mechanism of persisting species. Our study was conducted in Wapusk National Park (WNP) near La Pérouse Bay, northern Manitoba, Canada. This region provided an ideal study site as some water bodies have recently experienced a dramatic increase in salt ions (salinization), a common anthropogenic stressor in aquatic environments (Williams 2001, Kuashal et al. 2005, Smol and Douglas 2007). Additionally the landscape in WNP is densely covered by small shallow freshwater ponds, in which *Daphnia,* an invertebrate crustacean grazer, is common. Daphniids have previously been shown to be responsive to changes in the environment, including salinization (Barry et al. 2005;

Weider et al. 2010), and therefore make an ideal organism to study the impact of environmental change.

La Pérouse Bay (15V 6509330 475606) is a nesting site for Lesser Snow Geese, *Chen caerulescens caerulescens*, in WNP. Due to increases in agricultural productivity in the species' wintering grounds beginning in the 1970s (Abraham et al. 2005), the WNP population has experienced exponential growth over the last forty years (Cooke 1995, Figure 2-1). As populations grew, the nesting area expanded into freshwater sites previously outside of the geese's historical nesting range (Abraham et al. 2012). These sites were dramatically altered by goose "grubbing" which removes both the vegetative stalks and roots systems of plants thereby exposing the underlying sediments (Abraham and Jefferies 1997). The lack of vegetation increases evaporation and draws inorganic salts from the underlying marine clays to the surface, resulting in salinization of the soil (Srivastava and Jefferies 1995). With little remaining vegetation, the salt ions are washed into surrounding water bodies (Abraham et al. 2005) resulting in a dramatic increase in salinity of the aquatic environment over a short time period (Milakovic et al. 2001).

*Daphnia*, the focus organisms for this study, is a cyclic parthenogen that produces sexual resting eggs at the end of the season or when environmental conditions become unfavourable (Dobson and Frey 2001). Over time, resting eggs collect in the sediment creating a chronologically structured egg bank. Resurrection ecology is the experimental method of comparing genotypes that have been hatched from the egg bank (Kerfoot et al. 1999). As Daphniids have a relatively short generation time, populations generally respond quickly to environmental stressors; therefore, shifts in life history experienced in the population should be evident by experimental testing. This technique has previously been successful in detecting microevolution in *Daphnia* populations including behavioural and morphological responses to

changes in predation pressures (Cousyn et al. 2001, Kerfoot and Weider 2004, Michels et al. 2007), metal contamination (Kerfoot et al. 1999), and parasites (Decaestecker et al. 2007). In this study, we used the *Daphnia* resting egg bank to track changes in community composition over time by hatching eggs from pre- and post-disturbance time periods. We also examined differences in life history of the *Daphnia* species when exposed to increasing salinity, from 0g/L to 2g/L NaCl. We hypothesized that we would detect i) a shift in *Daphnia* species assemblage after the goose impact and ii) a change in both the acute and chronic life history responses of *Daphnia* species to salinity. We predicted that a community re-assembly event would occur due to dramatic changes in environmental conditions. Further, we expected to see a signal of microevolution in persisting *Daphnia* species as daphniids from other study populations have been previously shown to adapt to increases in salinity (Barry et al. 2005; Weider et al. 2010). A shift in the life history of persistent genotypes would be evidence for microevolution in this population in response to rapid environmental change.

## Materials and Methods

### *Study Site & Sample Collection*

In summer 2011, two ponds within Wapusk National Park, Canada, were sampled for water chemistry and sediment cores. WNP-GG (unofficial name, 15V 6502867 474424) is a small pond (0.6 ha, 0.5m deep) located approximately 6.5km south of La Pérouse Bay (Figure 2-2a). This site shows obvious physical signs of goose damage, including exposed soft sediments and dead willows up to 100m from the perimeter of the pond. Signs of goose presence were also evident, including feces, feathers and tracks (Figure 2-2b). WNP-X (unofficial name, 15V 6399426 477552) was chosen as a non-impacted control site (Figure 2-2a,c). This pond had similar physical conditions (0.7 ha, 0.4m deep) as WNP-GG, but was located further inland,

approximately 50 km from the coast. This pond was surrounded by sedges, grasses and abundant mosses and showed no signs of goose impact. From both ponds 4L water samples were collected from ~10 cm below the surface, the samples were then prepared in the lab for water chemistry analysis using the Environment Canada (1994) guidelines. Analysis for specific conductivity, ion concentration and phosphorus was performed at the National Water Research Institute in Burlington ON, Canada. Specific conductivity was used in this study as a proxy for salinity.

A 20cm core was collected in 2010 from WNP-GG for dating and diatom analysis using a 7.32 cm diameter maxi Glew (1989) gravity corer. Eight sediment cores were collected in 2011 from both WNP-GG (Aug 1) and WNP-X (July 31) and were immediately flown by helicopter to a field camp for processing. Cores were extruded at 0.5 cm intervals from 0-9cm using a Glew (1988) vertical extruder in order to encompass sediments deposited both before and after goose impact. Sections were stored in the dark at 4°C during transport and storage to prevent premature hatching of ephippia.

***Sample Processing***

The 2010 sediment core was dated using $^{210}$Pb to determine sedimentation rates. As a clear decay signal was present in the core it indicates that there has been no deep mixing of the sediments due to geese grubbing or other animal disturbances. A diatom analysis of the pond displayed an increase in both the diatom *Fragilaria pinnata,* as well as chlorophyll *a* pigments starting at 4.5 cm depth and continuing to the sediment surface, suggesting an increase in primary production through time. Both dating and diatom analysis were performed by the WATER Lab at University of Waterloo (L. Macdonald, unpublished). Based on the $^{210}$Pb dating, this increase started circa 1990, an estimate that aligns with Park staff estimates of goose presence in that region. Therefore, increased primary productivity is most likely the result of increased nutrients entering the system as a result of goose defecation in and around the pond. No core from WNP-X

was dated. The sedimentation rate of WNP-GG is believed to be greater than WNP-X due to increased primary productivity since it was goose impacted. By sampling to the same depth in WNP-X and WNP-GG the same time period should be encompassed by both cores, with the core from WNP-X likely extending further back in time.

Five cores each from WNP-GG and WNP-X were used for ephippia retrieval. Ephippia were extracted by passing a sediment sample through a series of stacked metal sieves (52, 110, 250 and 500 µm mesh size). Material retained on the 110 and 250 µm mesh from each layer was processed using a Leica M165-C dissecting scope at 16x magnification to isolate the ephippia from the remaining organic matter. Ephippia were enumerated and identified to species based on morphology (Hebert 1995, Figure 2-3). Ephippia isolated from each layer were combined in a 1.5mL microfuge tube and placed in the dark at 4°C until used for hatching. Each layer from each core was processed individually in order to retain a measure of variability between cores.

***Daphnia Species Composition Over Time***

Ephippia counts from WNP-GG (goose impacted) and WNP-X (not impacted) were used to analyse changes in *Daphnia* species composition overtime. In WNP-GG raw ephippia abundance was adjusted to ephippia/year using estimates from $^{210}$Pb dating. The ratio of *D. tenebrosa* : *D. magna* was used to investigate the impact of goose-disturbance on *Daphnia* composition within WNP-GG. In WNP-X abundance was analysed as only one species, *D. tenebrosa*, was found.

To evaluate changes in abundance of *D. tenebrosa* and *D. magna* over time, a three-way interaction model was used with the fixed effects of pond, layer and species in the R package *nlme* (Pinheiro et al. 2011). To account for large heteroscedasticity in variance, a varIdent variance structure was used in a generalized least squares (GLS) model allowing each layer within a pond to have a different variance (Zuur et al. 2009). Additionally, the lack of

independence due to temporal correlation between sediment layers was accounted for using an auto-regressive moving average (ARMA) model for the residuals (Zuur et al. 2009). The best ARMA model was fit using 3 auto-regressive parameters and was compared to eight other models with various numbers of auto-regressive and moving average parameters using Akaike Information Criterion (AIC) values. To investigate changes in ephippia abundance within a pond, the interaction between layer and species was analyzed separately for WNP-GG and WNP-X using the R package *multcomp* (Hothorn et al. 2008), using a GLS model with the same variance and correlation structure. To analyze changes in the relationship between the two species pre- and post-disturbance, a varIdent variance structure was used allowing each time period to have a different variance structure

***Hatching Protocol & Culturing Conditions***

Ephippia from the same pond and layer were pooled and deposited into 50mL of COMBO, an artificial culture medium designed to reflect lake water conditions (Kilham et al. 1998), in 100mL glass jars. In an attempt to replicate subarctic hatching conditions, incubator temperature was initially set to 13°C with a 12:12 light:dark photoperiod. After five days, temperature and daylight hours were increased to 20°C and 16L:8D to induce hatching. Ephippia were checked once daily for the first week, and twice each day for the following 3 weeks. With each check, the position of jars within the incubator was haphazardly re-assigned to minimize location effects on hatching. After one month, any remaining ephippia were deemed not viable and were placed into storage.

Each hatchling was placed into an individual 100 mL glass container filled with 50mL of COMBO. In total, 53 individuals hatched from the resting egg bank, all from WNP-GG. Of those, 28 (18 pre and 10 post impact) survived until reproduction and were used in experiments. All

hatched daphniids were of the species *D. magna*, with the exception of a single *D. tenebrosa* neonate from 2.5-3cm layer (1998-2003).

The 28 neonates were used to establish batch cultures for each iso-female clone line. All daphniids were cultured in COMBO (Kilham et al. 1998). Batch culture water was changed once per week and daphniids were fed with *Chlamydomonas rheinhardtii* at the following concentrations: *Daphnia* cultures with less than 5 individuals received 50,000 cells/mL every other day while cultures with more than 5 but less than 20 were fed 200,000 cells/mL. This higher amount for larger cultures was selected to maximize food availability as *D. magna* have large consumption rates (Porter et al. 1982). Cultures were stored in a single Percival I36LLVL incubator under a 16:8 light:dark photoperiod. Cultures were rearranged daily to minimize bias associated with location within the incubator. Daphniids were cultured for approximately 3 weeks to allow populations to reach a suitable size for use in experiments and to ensure that all experiments began with second generation individuals from each clone line.

***Acute Toxicity Trials***

48 hour acute toxicity trials were conducted to calculate LC50 values for all 28 iso-female lines following OECD (2000) guidelines. An analysis of the major ions in three goose impacted ponds showed a large increase in sodium (Na+) in comparison to control ponds (Table 2-1). We therefore used NaCl (Fisher Chemical 99% CAS 7647-14-5) to increase salinity. These trials were also used to determine the range of salinity to be used in subsequent life history trials. For each trial, a single neonate less than 24 hours old was placed in a 100mL jar containing 50mL of COMBO at one of eight salinity treatment levels (180, 2500, 3750, 5000, 6250, 500, 8750, 10000µS/cm). Jars were checked at 24 and 48 hours for mortality, and no food was provided during the trial. Three to five daphniids were run for each clone line at each treatment level; all daphniids from a core layer were grouped for analysis. A general linear model was fit to the

binomial (alive or dead) data using a probit distribution, using the *glm* function in the R base package (R Development Core Team 2012). $LC_{50}$ values were calculated by determining the value of the model at which 50% of the animals were dead. $LC_{50}$ from pre- and post-layers were compared using a one-way ANOVA. Assumptions were verified by observing plots of the fitted vs. residual values, histograms of the residual values, normal quantile plots and leverage plots (Pinheiro et al. 2011).

***Life History Trials***

Twelve iso-female clone lines were used for the life history experiments (8 pre-, 4 post-disturbance). Five treatment levels (180, 1000, 2500, 3125 and 3750µS/cm) were used for this experiment by adding NaCl (Fisher Chemical 99% CAS 7647-14-5) to COMBO until the desired treatment level was obtained, as measured by a WTW TetraCon 325 conductivity probe. Two treatments greater than 3750 µS/cm were initially included (5000 and 6250 µS/cm) in the life history trials. However, individuals did not survived to reproduction; therefore these treatment levels were not used for the final experiment.

To initiate trials, neonates were removed within 24 hours of emergence from the original batch culture and randomly distributed to a salinity treatment. All lineages were cultured under treatment conditions for at least two generations before experimentation to exclude maternal effects. All generations were cultured at 20°C in 50mL of treated media in a 16L:8D photoperiod, fed 50,000 *C. rheinhardtii* cells/day (~2 mg C/L), and water was changed once a week. The experimental individuals were monitored daily for mortality and neonate production, and were photographed every other day at 250x, using a Leica digital microscope camera to generate data on growth and body size. Trials were concluded after 21 days, with at least 15 individuals from each clone line having been exposed to each treatment level. In total 1707 individuals were followed over the course of this experiment. Trial start dates were staggered from September

2012 – May 2013 as handling time limited the number of individuals that could be processed during a trial.

For each clone line/salinity treatment, life history data was used to calculate i) days until mortality, ii) size at first reproduction, iii) total reproductive output, iv) average daily neonate production, v) time until first reproduction, as well as demographic information (intrinsic rates of natural increase, r and net reproductive rates, R0). The assumption of independence and uncorrelated residuals required for an ANOVA was violated in this experiment as multiple individuals from each clone line were exposed to each treatment (Zuur et al. 2009). Therefore, linear mixed models were used to determine if clone lines from pre- and post-disturbance time periods displayed different life history characteristic in response to increased salinity. Demographic data for each treatment were derived using the PopTools add-in for Microsoft Excel (http://www.cse.csiro.au/poptools). To estimate variance associated with these metrics, life table data were jackknifed (Meyer et al., 1986) and the resulting pseudo values for R0 and r were analyzed via a linear mixed model in the R package *nlme* (Pinheiro et al. 2011).

***Growth Analysis***

The von Bertalanffy growth model $L_t = L_\infty\left(1 - e^{-K(t-t_0)}\right)$ (Equation 1) was used to model each individual's growth trajectory through time. The non-linear model has three parameters: i) $L_\infty$, the maximum attainable length of an individual, ii) K, a growth coefficient, measures and is interpreted as the exponential rate of approach to the asymptotic size and iii) $t_0$, representing the time at which length was equal to zero. Generally, $t_0$ is viewed as an artifact of the model as there is no real point in time at which the individual has zero length (Schnute and Fournier 1980); therefore, $t_0$ will not be investigated in this analysis. Using the von Bertalanffy model ensures that all growth dynamics are incorporated into the model regardless of the longevity of an individual. This study analysed differences in both $L_\infty$ and K, as they are the two

biologically relevant parameters. For each individual, Equation 1 was fit to the raw morphologic data using the R package *nls* which determines the values for the three parameters of the model using least squares analysis. Starting values for the *nls* analysis were generated using the *vbStarts* function from the *FSA* package (Ogle 2013). Additionally, a traditional growth rate was calculated $Growth\ Rate = \frac{\ln(l_f) - \ln(l_i)}{\Delta t}$ (Equation 2) from the size at neonate emergence to the size at first reproduction birth. First reproduction was chosen as a final time point as it is generally viewed as the time at which energetic investment switches from growth to reproduction (Glazier and Calow 1992). Again, linear mixed models were used to investigate differences between pre- and post-disturbance clone lines.

***Model Selection***

In all linear mixed models a top-down approach (West *et al.* 2006) was used to define the best model. This process starts with the most complex model which is then simplified using likelihood comparisons until the model with the least terms, but still fitting the data as well as the complex model, is found (Zuur *et al.* 2009). The random effects for all models initially included the clone line and trial start date. These effects were used to account for differences between clone lines within a time period, as well as possible differences in food and media quality as not all trials were run simultaneously. To complete the model, the fixed effects were defined as the time period which the clone line was from, the salinity treatment level and the interaction. As outlined in Zuur et al. (2009), model comparison was first performed on the random effects structure using restricted maximum likelihood with all fixed effects included in the model. After the optimal random structure was determined, the p-values for the fixed effects were determined through likelihood comparisons using maximum likelihood methods. Again, assumptions were verified by examining plots of the fitted vs. residual values, histograms of the residual values, normal quantile plots and leverage plots (Pinheiro et al. 2011).

## Results

### Daphnia *Species Composition*

Ephippia abundance varied significantly across ponds, layers and species, as indicated by a significant three-way interaction (ANOVA, $F_{17,280} = 5.37$, $P < 0.0001$). In WNP-X raw ephippia abundance did not change over time (Table 2-2, Figure 2-5). For *D. magna* in WNP-GG ephippia abundance increased in the upper layers of the core (Table 2-2, Figure 2-4a). There was no change in the abundance of *D. tenebrosa* in WNP-GG (Table 2-2, Figure 2-4b).This change in composition of the *Daphnia* community is supported by the significant decrease in the ratio of *D. tenebrosa* to *D. magna* post-goose-disturbance (ANOVA, $F_{1,86}=9.91$, $p= 0.002$) (Figure 2-6). No *D. tenebrosa* were observed in the uppermost layer of WNP-GG, nor were they detected in any live zooplankton collections (Arnott, unpublished).

### *Acute Toxicity Trials*

*D. magna* $LC_{50}$ values for the twelve sediment layers ranged from 5174µS to 9920µS/cm (Table 2-3). The relatively high peak value for the 5.5-6cm sediment layer may be inflated due to small sample size. $LC_{50}$ values did not significantly differ between clone lines from pre- or post-goose-disturbance time periods in WNP-GG (ANOVA, $F_{1,10} = 0.61$, $P = 0.454$). Diagnostic plots of the residuals indicated that the observation from the 5.5-6 cm layer may have had undue influence on the model. To test this, the model was refit without the outlier (Quinn & Keough 2002). The same result was obtained.

### *Life History Trials*

Salinity was the only significant predictor for the life history characteristics: i) days until mortality, ii) total reproductive output, iii) average daily neonates production and iv) time until first reproduction (Table 2-4). During selection of the model, the random effect “trial start date”, which accounted for the staggered start dates of the trials, was determined to significantly

contribute to the variation in response. As trial start date was nested within clone line, both were retained as random effects in the model. Subsequent model comparisons aimed at determining the significance of the main effects of time period and salinity demonstrated that the best fit linear mixed model included only salinity (Table 2-4). Overall, all of the life history characteristics monitored for both pre- and post-disturbance clone lines were diminished at increased salinity (Figure 2-7 to 2-9).

In contrast, both time period and salinity were significant predictors of size at first reproduction (SFR), and the interaction term was not significant (Table 2-4). For both time periods, SFR decreased with increased salinity (Figure 2-10). The body size of pre-disturbance clone lines was larger at low conductivities. However, at the highest salinity treatment clone lines from both time periods displayed similar SFR as body size decreased at difference rates. As with other life history characteristics, both trial start date and clone line contributed to variation in response and were therefore retained in the models.

Intrinsic population growth rate (r) and net reproductive rate ($R_0$) were also significantly impacted by increased salinity; however, the response of clone lines from pre- and post-disturbance did not differ (Table 2-4). Demographic characteristics were calculated for each clone line using the life history parameters from each individual. A significant effect of clone line was detected and was therefore included as a random effect in the model. Overall, r and $R_0$ decreased at higher salinity, and again the response of clone lines did not differ between pre- and post-disturbance time periods

Clone line growth was analyzed using both growth rate until first reproduction and the output parameters, K and $L_\infty$, from the von Bertalanffy growth model. For growth rate until first reproduction and the maximum attainable length, only salinity was determined to be significant, with both response variables decreasing with increased salinity (Table 2-4). Growth rate and $L_\infty$,

decreased with increasing salinity for both pre- and post-disturbance clone lines. However, for the growth constant of the von Bertalanffy model, neither time period nor salinity was significant.

## Discussion

Two distinct responses to a dramatic increase in salinity were detected in the *Daphnia* community of WNP-GG. One species, *D. tenebrosa* did not persist in the new conditions of increased salinity and was likely extirpated from the system. The other species, *D. magna* was tolerant of the high salinity conditions and was present throughout the sediment record. This study provides evidence that phenotypic plasticity allowed *D. magna* to persist despite the rapidly changing environmental conditions, and that microevolution in salinity tolerance did not occur in this population.

### *Environmental Change in WNP-GG*

The expansion of snow geese nesting grounds inland from La Pérouse Bay resulted in a change of environmental conditions for impacted ponds such as WNP-GG. Goose impacted ponds have significantly higher salinity (8576 µS/cm sd=5692.0) than other ponds in the region (206.57µS/cm sd=187.9). The grubbing feeding of snow geese decimated the local vegetation and resulted in hypersalinization of the soils (Abraham and Jefferies 1997). Ions from the soil were washed into nearby waterbodies resulting in the observed increase in salinity. Goose feces are also known to increase nutrients in water bodies (Manny et al. 1994), which has also been observed in the goose ponds (mean total phosphorus = 151.2 µg/L sd=229.7, n=5) when compared to non-impacted ponds (mean total phosphorus = 23.2µg/L sd=16.6, n=29). Additionally, both zooplankton (Arnott, unpublished) and the aquatic invertebrate chironomid communities (Milakovic 2001) are different in goose impacted ponds when compared to other ponds on the landscape.

Unfortunately no goose ponds were monitored before 2010. However, a survey of 30 ponds in the regions allowed us to estimate what pre-impact conditions may have been like and clearly shows that the goose-impacted ponds are significantly different than other ponds on the landscape. Parks Canada data indicate that snow geese breeding populations expanded into the region surrounding WNP-GG around 1990. This estimate is supported by the increase in abundance of the eutrophic diatom *Fragilaria pinnata* and chl-*a* pigments in sediments from that time period. Goose presence was confirmed during the first study in 2010 with feathers, droppings, footprints and visual sites of geese surrounding the pond. This evidence confirms that WNP-GG is a goose impacted system and indicates that the pond has undergone a significant change in environmental conditions since goose arrival.

***Response of D. tenebrosa***

The ephippia record for WNP-GG displayed a shift in *Daphnia* species composition after the expansion of snow geese into the ponds catchment (Figure 2-6). While *D. magna* increased in abundance post-goose impact, there was no coinciding increase in *D. tenebrosa*, resulting in significantly different species ratios between the two time periods (Figure 2-4). No decrease in *D. tenebrosa* was observed from the non-impacted pond WNP-X, suggesting that the decrease in relative abundance in WNP-GG was not a species response to a different change in the region, such as climate warming (Figure 2-5). The absence of *D. tenebrosa* in the contemporary sediment layer and in water column zooplankton samples suggests that this species has recently been extirpated from the pond.

The disappearance of *D. tenebrosa* was likely due to the alteration of the habitat by geese. One explanation is that the increased salinity of the pond may have surpassed the physiological tolerance threshold of the species. Although no studies of the salinity tolerance of *D. tenebrosa* have been conducted, field surveys suggest tolerance is low. A five year survey of

33 sites in WNP found *D. tenebrosa* was not present in ponds with a specific conductivity greater than 1500 µS/cm (Arnott, unplublished), and Strecker et al. (2008) did not observe the species in ponds with conductivity greater than 1650 µS/cm on Ellesmere Island in the high Arctic. Additionally the acute $LC_{50}$ for the single *D. tenebrosa* hatched from WNP-GG was 3366 µS/cm (unpublished data). Although this value is higher than any pond where the species has been observed, it is significantly less than the $LC_{50}$ for all *D. magna* clone lines in this study (mean=6544 µS/cm sd=1217.4). This suggests that *D. tenebrosa* has a low salinity tolerance and which likely contributed to the observed extirpated from WNP-GG as salinity increased. However, *D. tenebrosa* ephippia are present the sediment record post-goose-impact. As the sediment record integrates ephippia produced throughout the ice-free season this may reflect temporal pulses of *D. tenebrosa.* These pulses may have occurred during brief periods of time when environmental conditions were suitable for their survival such as periods of low conductivity associated with spring snowmelt. Although we did not detect *D. tenebrosa* in any samples collected during the 2008-2012 survey, it is possible that *D. tenebrosa* may have briefly occupied the water column at other points of the season.

Alternatively, the decline in *D. tenebrosa* may be due to increased competition for resources (Roukolainen 2007). Intrinsic population growth rate for *D. magna* increases with increasing resource availability, even in high algal concentration environments (2 mg Carbon/L, Porter et al. 1983) such as those produced by the rapid influx of nutrients from goose feces. In contrast, many daphniids suffer when food is extremely abundant due to the energy required for grooming feeding filters (Dobson and Frey 2001). While no studies have been performed on *D. tenebrosa,* its size suggests it may have a lower fitness at high food concentrations, as seen in other small bodied species (Bukovinszky et al. 2012). The possible energy expenditure of *D. tenebrosa* and the ability of *D. magna* to thrive in high algae environments may have given *D.*

*magna* a competitive advantage. However, it is most likely that the decrease and eventual extirpation of *D. tenebrosa* was a result of both physiological stress and increased competition.

***Response of D. manga***

This study provides empirical evidence of phenotypic plasticity in response to a change in the physical environment rather than microevolution. While phenotypic plasticity has widely been acknowledged as an important mechanism for dealing with environmental change (Berg et al. 2010), microevolution in salinity tolerance has been observed in past studies where habitats have experienced unidirectional shifts in salinity (Barry et al. 2005, Weider et al. 2010). It was therefore surprising that no differences were observed in the $LC_{50}$ values (Table 2-3), life history traits or growth metrics between clone lines from pre- and post-goose-disturbance time periods (Table 2-4, Figures 2-7 to 2-9). The lack of differences between clone lines indicates that the change in environmental conditions since ca. 1990 has likely not resulted in a microevolutionary shift in response to increased salinity for the *D. magna* population in WNP-GG. If microevolution had occurred, we would expect the life history characteristics of the post-disturbance clone lines to be less negatively impacted by an increase in salinity. Instead, we found that both pre- and post-disturbance clone lines were negatively impacted in a similar way (Figures 2-7 to 2-9). It is possible that if clone lines were exposed to higher salinity treatments (>3750µS/cm) differences between pre- and post-disturbance clone lines may have been observed. However, higher treatment levels initially included in this experiment were not used as individuals did not survive to reproduction. The similar negative impact on life history observed for both pre- and post-disturbances clone lines between treatment levels instead suggest a plastic response to increases in salinity. Since there are no differences between clones from the two time periods, this would indicate that the observed plasticity must have been maintained throughout the history of the population.

The physiological mechanism resulting in salinity tolerance in *Daphnia* has been attributed to the up-regulate genes which produce osmoprotectants and ion transport proteins (Latta et al. 2012). The increased production of these proteins allows cells to cope with increased differences in molarity between the external and intracellular environment. It is possible that *D. magna* may have a greater abundance of osmoprotectants or ion transport proteins relative to other species, allowing for their persistence. Alternatively this *D. magna* population may display an adaptive plastic response in high salinity environments which results in the up-regulation of these genes. As this study did not investigate the abundance of either osmoprotectants or ion transport proteins it is unknown what is enabling *D. magna* to tolerate a large gradient in salinity. It is clear however that a similar plastic, although not necessarily adaptive, response in life history was observed in both pre- and post-disturbance clone lines.

The only observed difference between pre- and post-goose impact clones was size at first reproduction (SFR) (Table 2-4). While clones from both periods decreased in body size with increasing salinity, post-disturbance clone lines first reproduce at a smaller size (Figure 2-10). Why the SFR of post-impact clones is consistently smaller is puzzling. A possible explanation is that the smaller SFR was originally induced by a plastic response to the increase in salinity (Arner & Koivisto 1993). Over time, small SFR may have been selected for, and eventually became a genetically-determined canalized trait through genetic assimilation, resulting in a loss in plasticity (Waddington 1961). Reduction in SFR has previously been seen as a response to stressors such as temperature (Van Doorslaer et al. 2010) and salinity, and has been associated with a reduction in reproductive output (Arner & Koivisto 1993). In this study reproductive output is not different between periods at any treatment level, suggesting that while SFR may have been canalized, other life history traits have not. This ‘mosaic’ of plastic and non-plastic traits has been observed in other organisms (Carroll et al. 1997), and is likely the result of differential responses of traits to a new environment (Ghalambor et al. 2007). The possible canalization of SFR in response to

increased salinity appears to have no influence on the fitness of post-impact clones when compared to pre-impact clones in low salinity condition.

### *Phenotypic Plasticity and Microevolution in Response to Environmental Change*

Phenotypic plasticity may be present in this population due to the large temporal variability in conductivity, both interannually and within a season, that has been documented in this region (Figure 2-11). Phenotypic plasticity allows a genotype to have a broad tolerance to environmental variables (Bradshaw 1965, Sultan 1987, Schlichting & Pigliucci 1998) and could allow the species to persist despite such variability. The small size of WNP-GG makes it vulnerable to small changes in the environment (e.g., Smol and Douglas 2007). Periods of high temperatures and evaporation, or periods of intense rainfall, may have produced historical fluctuations in salinity in this small pond that are not typically experienced in larger systems. A five year survey of goose- and non-impacted ponds in WNP indicates that the specific conductivity of the ponds is quite variable (mean coefficient of variation 28.6 sd=25.0, Arnott, unpublished). Seasonal variation which was measured for three ponds from 2010-2012 was even greater (55.05 sd= 22.4, *pers. comm.* L. Macdonald, University of Waterloo). In contrast large temperate lakes only experienced a coefficient of variation of 6.1 (sd=1.8) for July sampling during the time periods between 2008 to 2012, and the seasonal variation was even lower (mean coefficient of variation 3.1 sd=1.9) (*pers. comm* Andrew Patterson, Ontario Ministry of the Environment). Previous variability in the pond, although not likely as dramatic as the increase observed after goose impact, may have selected for genotypes able to tolerate fluctuating salinity levels (Bradshaw 1965). The presence of genotypes with a high level of phenotypic plasticity is likely how this population persisted during the drastic recent change in the environment.

This study suggests that in variable environments pre-existing phenotypic plasticity may play a greater role than microevolution in species response to environmental changes. Ortells et

al. (2005) investigated the salinity tolerance of *Daphnia* genotypes from a lake which had been exposed to a dramatic increase in salinity after years of moderate changes in salinity due to marine storm surges. Genotypes present in the lake after the increase in salinity displayed broad salinity tolerance and the authors suggest that the previous moderate changes in the environment resulted in the production of tolerant genotypes. Examples of phenotypic plasticity in variable environments mitigating the impact of environmental change have been observed in other species such as the European Spruce Pine (*Pinus sylvestris*) (Richter et al. 2012). In that study, genotypes from variable climatic environments that express a plastic response to changes in precipitation were better able to adapt to the impacts of climate warming than species from more climatically stable populations.

While examples of microevolution in response to non-climatic stressors are abundant in the literature (review in Thompson 1998; Reznick and Ghalambor 2001), many of these studies have been conducted in otherwise stable environments. In situations of moderate environmental change pre-existing plasticity may allow populations to persist without microevolution. In cases of continuous directional change that eventually exceeds the range of phenotypic plasticity, the initial plastic response may allow the species to persist in the environment long enough for a microevolutionary shift to occur (Chevin and Lande 2010).

***Population Salinity Tolerance***

Intriguingly, the $LC_{50}$ for all clone lines, including the clones from the uppermost sediment layer which represent the most contemporary populations, are less than the 2010-2012 specific conductivity values for WNP-GG (Table 2-1). This may indicate that lab hatching and culturing conditions may not accurately reflect *in situ* pond conditions. However, an investigation comparing the measured field distribution (MFD) and lab $LC_{50}$ for salinity in aquatic taxa found that while the two measures are generally correlated, in 16% of macroinvertbrates and 21% of

fish species the MFD was greater than the lab $LC_{50}$ (Kefford et al. 2004). Therefore, the observed difference between field conductivity and lab $LC_{50}$ in the WNP-GG clones is not necessarily indicative of a failure to accurately represent the population. The artificial lakewater media, COMBO (Kilham et al 1998) used in this experiment may have not accurately recreated the pond environment of WNP-GG. This may have resulted in the low $LC_{50}$, as have been previously observed for other toxins when COMBO is compared to other artificial media (Samel et al. 1999). The lack of nutritional resources during the trial as compared to natural conditions may have also contributed to the difference between the measured $LC_{50}$ and the observed field specific conductivity of WNP-GG. Since the focus of this study is comparative between time periods the use of the WNP-GG clone lines is appropriate, despite the low $LC_{50}$.

The $LC_{50}$ of all 12 clone lines in this study are also lower than values reported in the literature, which typically range from approximately 9620 µS/cm (Martinez-Jeronimo and Martinez-Jeronimo 2007) to 12768 µS/cm (Do Hong et al. 2004). However, Jones (2012) found that *D. magna* from the WNP region had an average $LC_{50}$ of 4280-6010 µS/cm when the population originated from freshwater or brackish habitats, while the $LC_{50}$ of WNP-GG was 6544 µS/cm sd=1217.4. As $LC_{50}$ values from both studies are similar it suggests that the regional environment may influence salinity tolerance and indicates that comparison of genotypes from different regions may not be appropriate. Therefore, while the $LC_{50}$ values observed in this study are lower than found in previous studies they are similar to other populations from this region.

### *Using Resurrection Ecology to Examine Environmental Change and Potential Limitations*

We have used the resting egg bank in the sediments of Subarctic ponds to infer changes to *Daphnia* populations through time. Many studies have successfully used this approach to look at changes in species composition over time (Kerfoot et al. 1999, Mergeay 2007, 2011) and microevlutionary shifts within *Daphnia* populations (Hairston et al. 1999 and 2001, Cousyn et al.

2001, Michels et al. 2007, Kerfoot et al. 1999, Decaestecker et al. 2007). It has been argued that due to differences in life history, the egg bank may not accurately reflect species abundance (Janowski et al 2003). However, in a study of 135 lakes, Jeppesen et al. (2003) found that surface sediments did reflect the relative abundance of species present in the water column.

Some authors believe that the egg bank more accurately reflects the true species richness of a system than one-time annual sampling, as it integrates ephippia throughout the season (Brendonck and De Meester 2003, Vandekerkhove et al. 2007). Although it may be possible that *D. tenebrosa* was abundant in our study pond even though it was only present in low abundances in the ephippia record, the absence of the species both from the final layer of the sediment record and all zooplankton samples suggest that the species has been extirpated from the pond.

Questions may also be raised as to whether the clone lines hatched in our study reflected the genotypes present in the water column. Jankowski et al. (2003) demonstrated that the genetic composition from 1989-2000, estimated by electrophoresis of water column samples of the most abundant *Daphnia* species, was well represented by the ephippia hatchlings. This suggests that the *D. magna* clones used in this study likely reflect the genotypes present in the pond during the time period they originated.

### *Conclusions & Future Directions*

Many of the regions predicted to experience the most dramatic environmental changes, such as the alpine, Arctic and Subarctic (IPCC 2007b), are already highly variable environments. Species from these regions may already express plastic responses to changes in the environment and therefore are well positioned to persist during events of rapid environmental change (Lloyd 1984, Richter et al. 2012). Changes in population traits due to phenotypic plasticity in response to climate change have already been seen in some arctic and subarctic species, such as advancements in flowering, bird egg-laying and mammal maturation dates (Høye et al. 2007).

Not all species from a community display the same response to environmental changes. In this study the *D. tenebrosa* population from WNP-GG appears to have been extirpated while the *D. magna* persisted. Differential species responses have been observed to other environmental stresses such as pH (Derry and Arnott 2007) and metal toxicity (Bradshaw and McNeily 1991). In both these studies a small portion of the species in the community are able to adapt through microevolution to changes in the environment. However, this response was not widespread within the community.

To understand better how communities will be affected by future environmental change, further investigations need to be made on what factors influence species response. It is clear that both past environmental variability and species life history influences whether a species will respond through microevolution or phenotypic plasticity. However, the importance of these factors and how they interact is unclear; daphniids have been shown to respond both through microevolution (Barry et al. 2005, Weider et al. 2010) and phenotypic plasticity (Ortells et al. 2005, this study). By understanding how factors such as physiology, life history and environment influence species response, it may be possible to create predictive models to determine how different species within a region respond to environmental change. Identifying species response may allow conservation efforts to focus on species that are unlikely to adapt environmental change, and hence are most at risk.

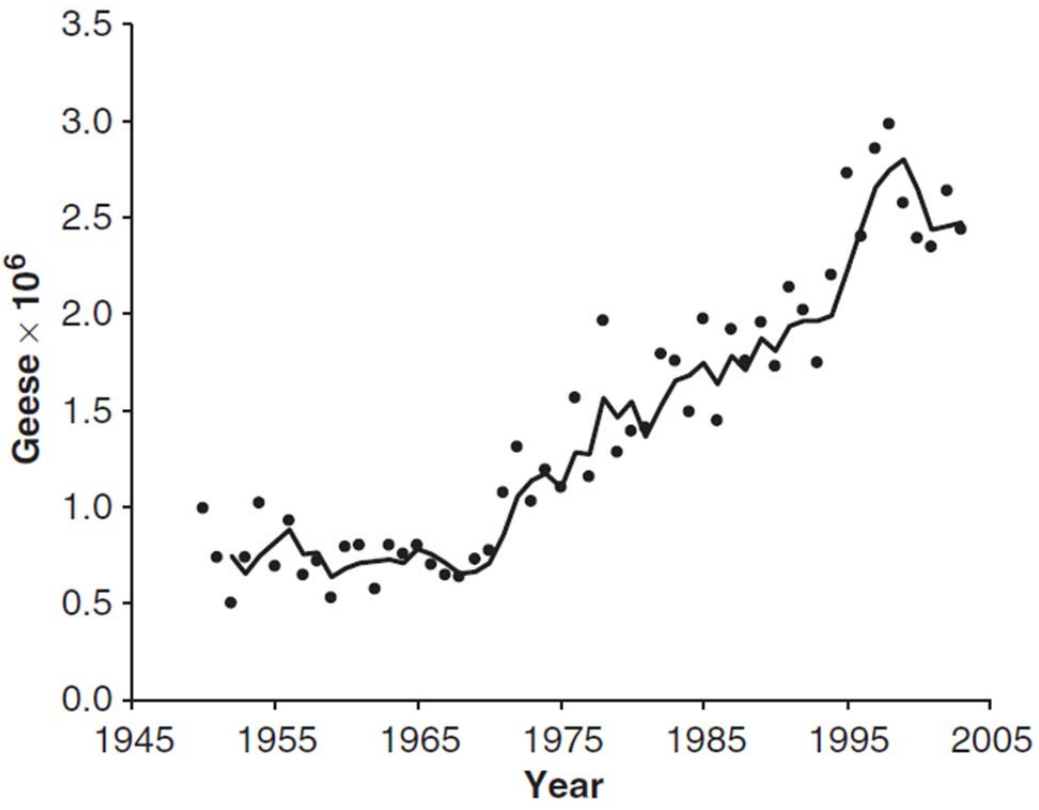


**Figure 2-1** Mid-Winter Index of Lesser Snow geese and Ross's geese (*Chen rossii*) in the Mid-Continent Population, 1950–2003. Solid line is based on a 3-year running average. Reproduced from Abraham et al. 2005.

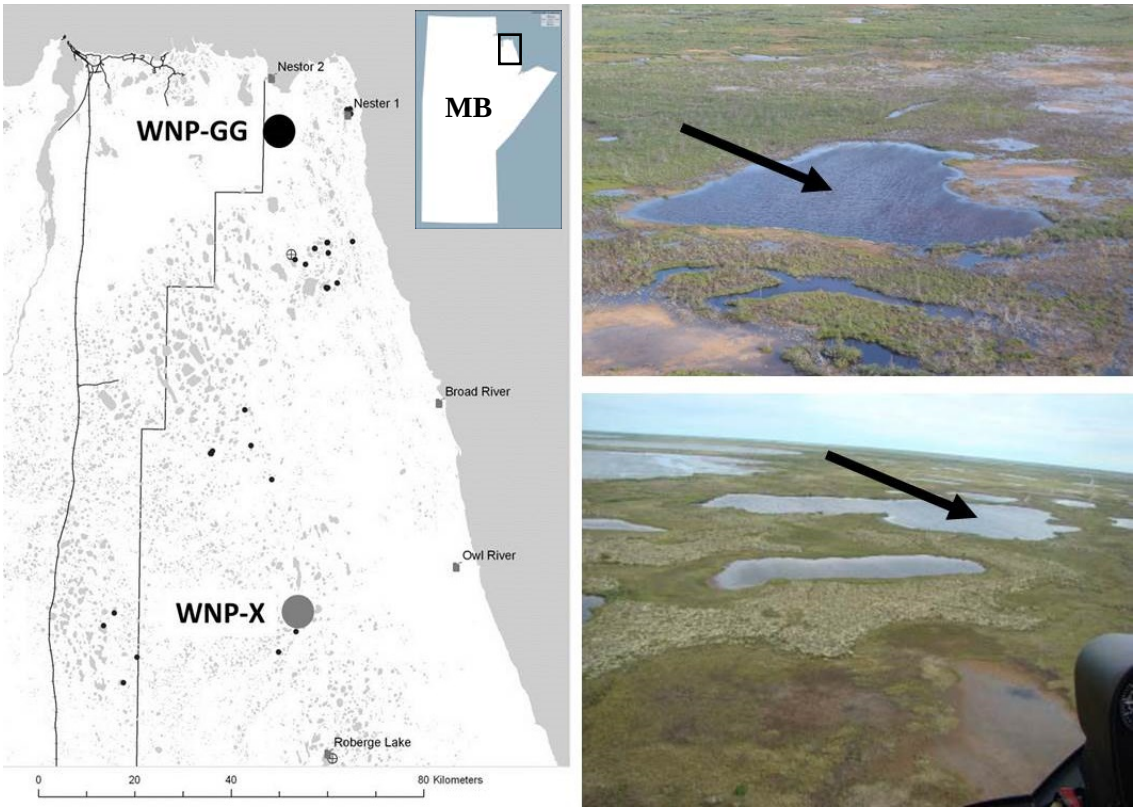


**Figure 2-2** A) Map of Wapusk National Park, MB with study site locations and aerial photos of (B) goose-impacted study site WNP-GG and (C) the non-impacted control site WNP-X. In A) the park boundary is marked by the solid verticle line. The hatched line repersents the railline between Thompson and Churchill, place names repersent fuel chaches and field stations within the park and small black dots repersent locations sampled in the 2008-2012 survey.

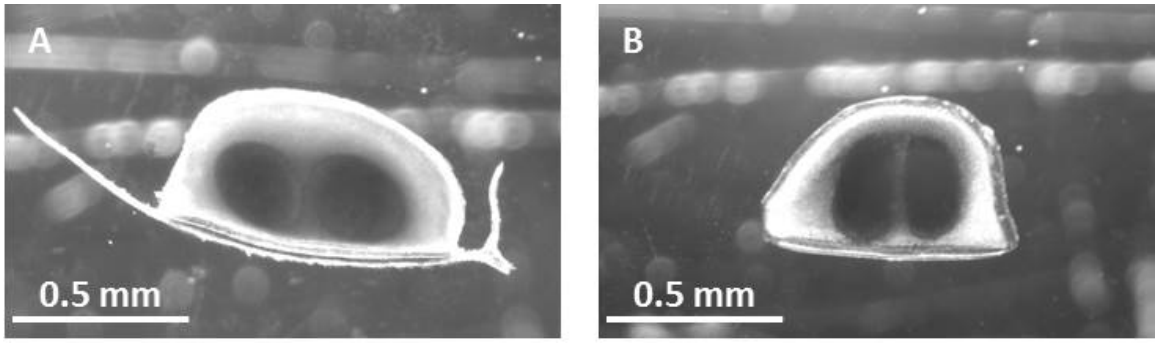


**Figure 2-3** Ephippium of (A) *D. magna*, identifiable by its large size, elongated shape and distinctive spine and (B) *D. tenebrosa*, identified by compact shape and no spine. Two resting eggs are encased in each ephippium.

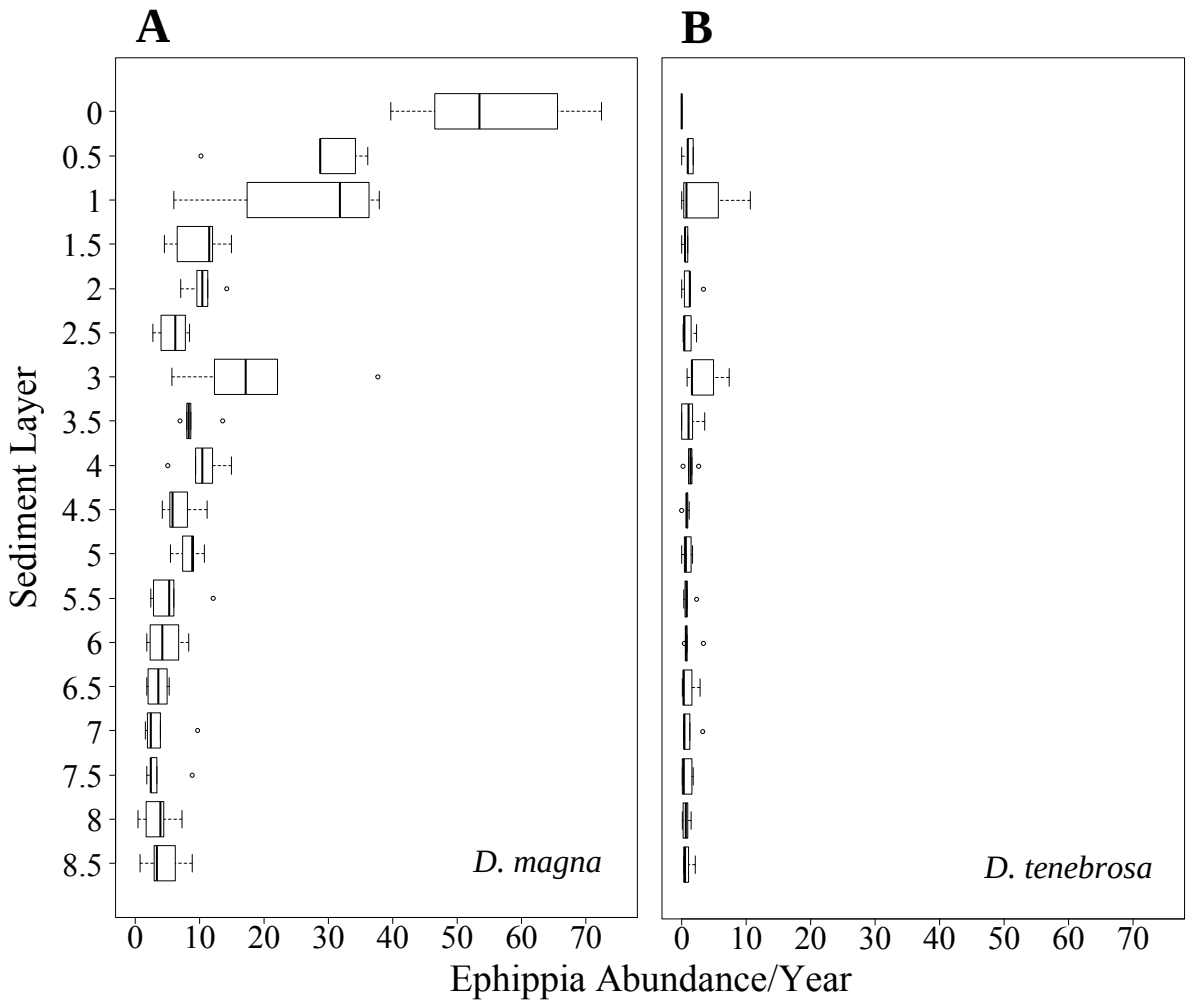


**Figure 2-4** Ephippia abundance adjusted per year by sediment layer for (A) *D. magna* and (B) *D. tenebrosa* in WNP-GG. Boxes repersent the variation in ephippia abundance between the 5 cores collected from WNP-GG. 0 cm represents the most recent sediment layer.

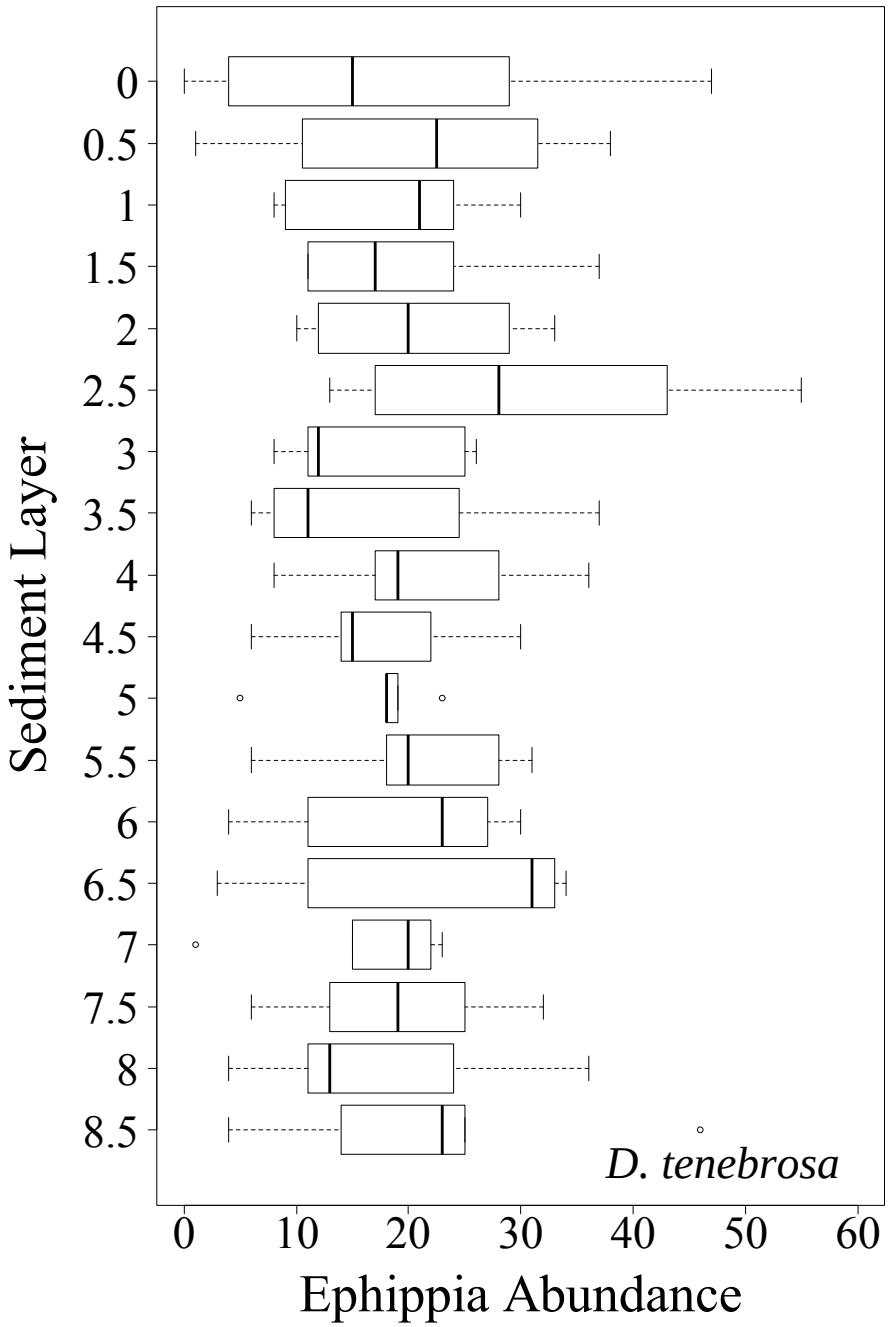


**Figure 2-5** Ephippia abundance by sediment layer for *D. tenebrosa* in WNP-X. Boxes repersent the variation in ephippia abundance between the 5 cores collected from WNP-X. 0 cm represents the most recent sediment layer.

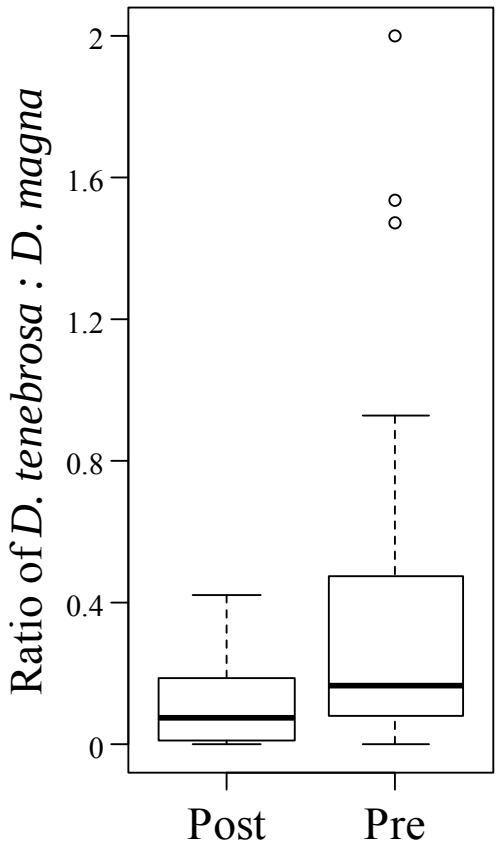


**Figure 2-6** Ratio of *D. tenebrosa* : *D. magna* ephippia in the 5 cores from WNP-GG during pre and post goose-impact time periods

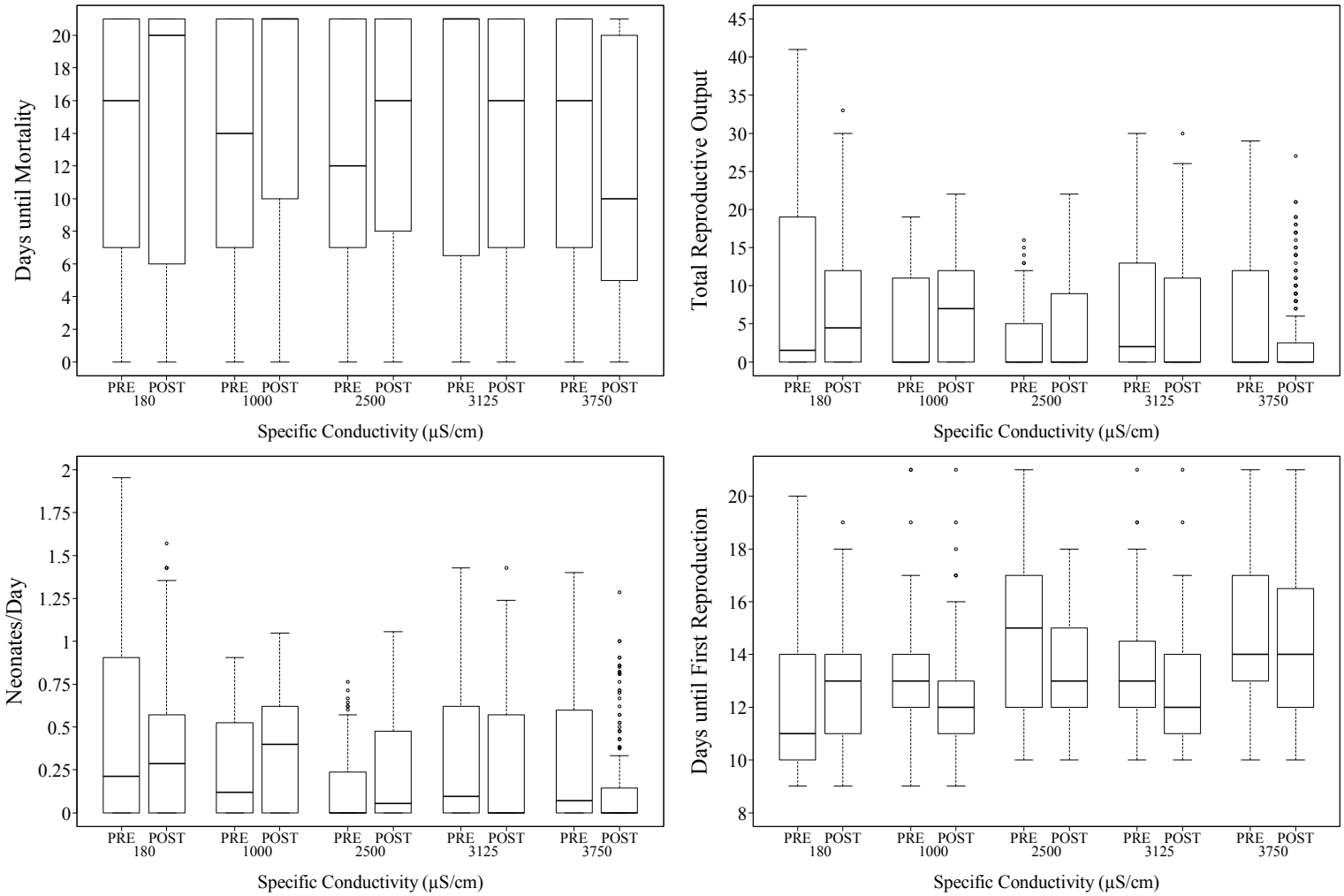


**Figure 2-7** Days until mortality, total reproductive output, neonates per day, and days until first reproduction of iso-female clone lines from pre- and post-goose-impact time periods in response to specific conductivity.

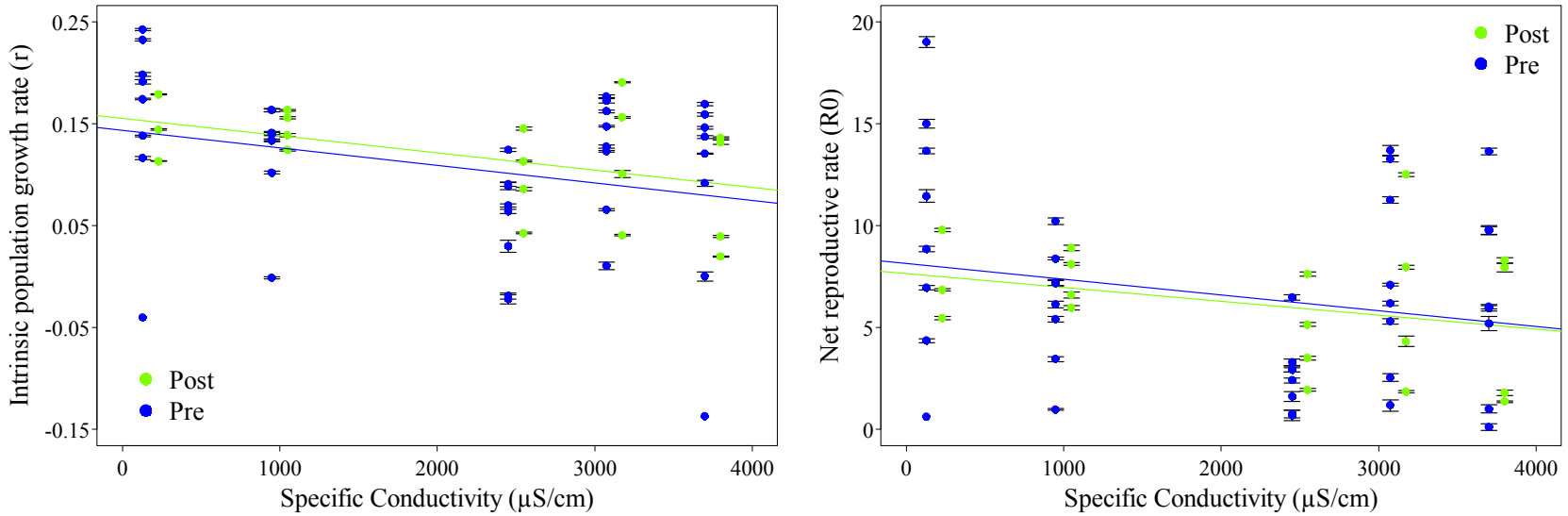


**Figure 2-8** Intrinsic population growth rate (r) and net reproductive rate ($R_0$) of iso-female clone lines from pre- (blue) and post- (green) impact time periods in response to specific conductivity. Solid lines represent the linear trend associated with each period. Error bars repersent the 95% confidence intervals calculated by jacknifing r and $R_0$ (Meyer et al., 1986).

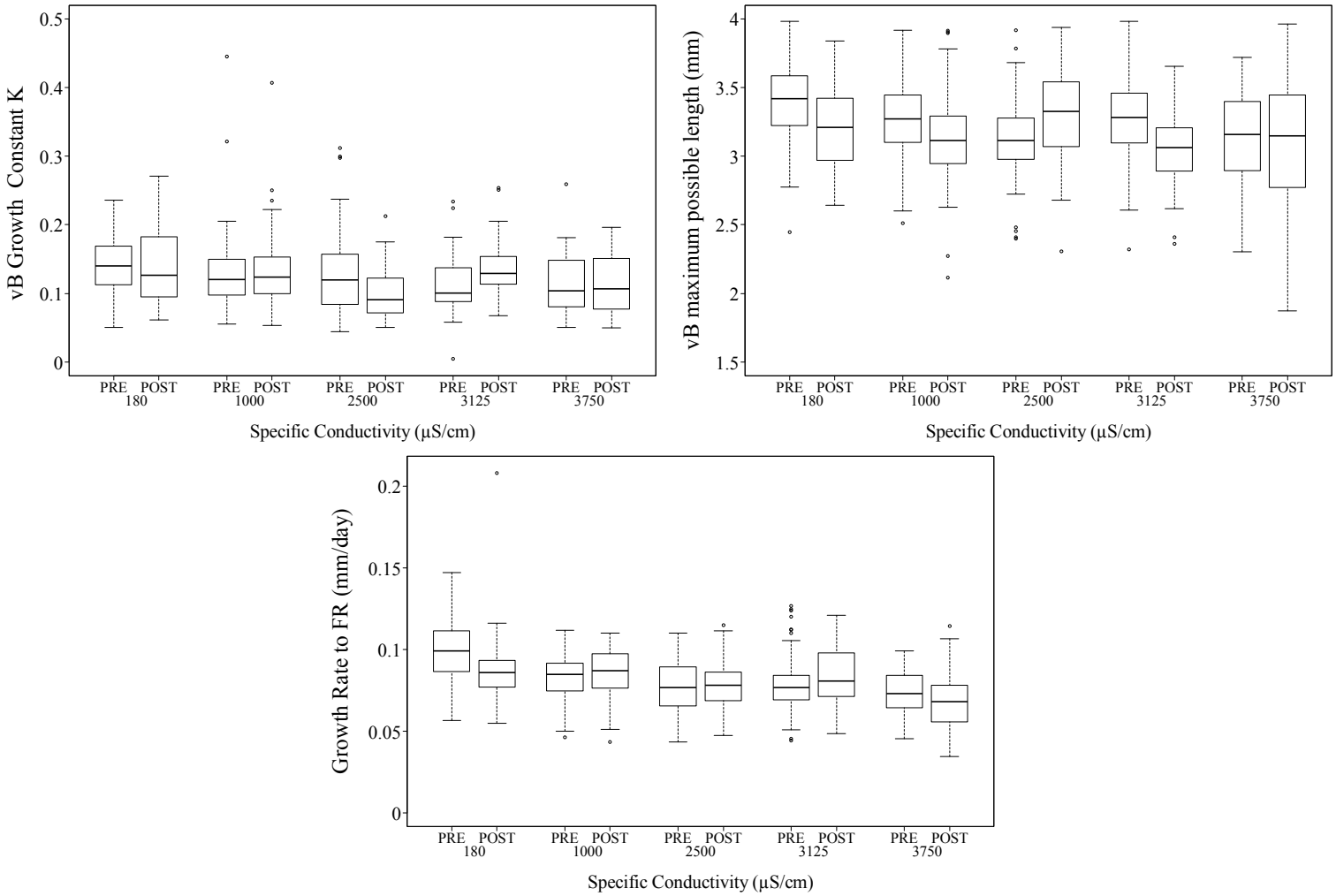


**Figure 2-9** von Bertalanffy growth constant K, von Bertalanffy $L_\infty$ and growth rate to first reproduction of iso-female clone lines from pre- and post-impact time periods in response to specific conductivity.

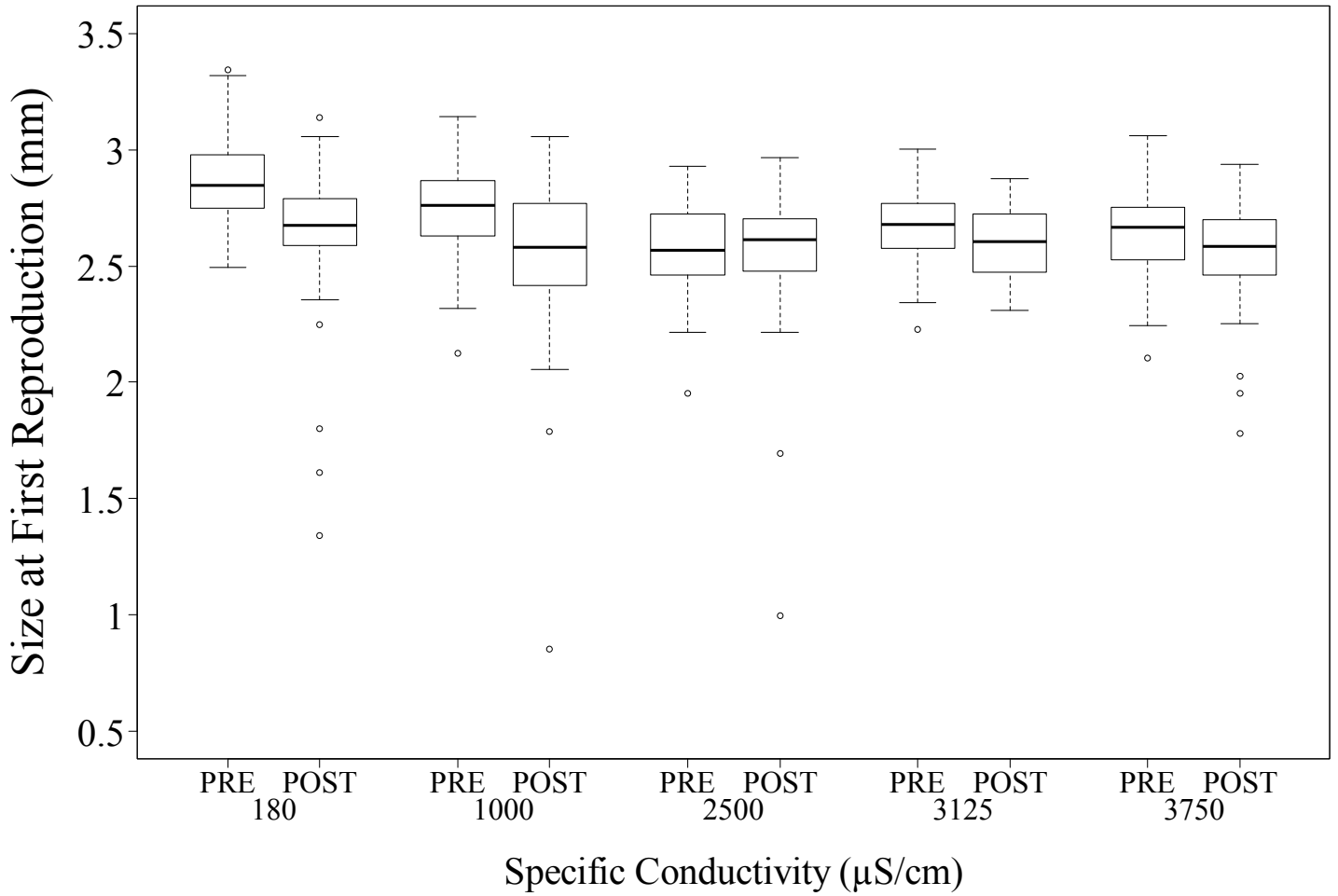


**Figure 2-10** Size at first reproduction of iso-female lines from pre- and post-impact time periods in response to specific conductivity.

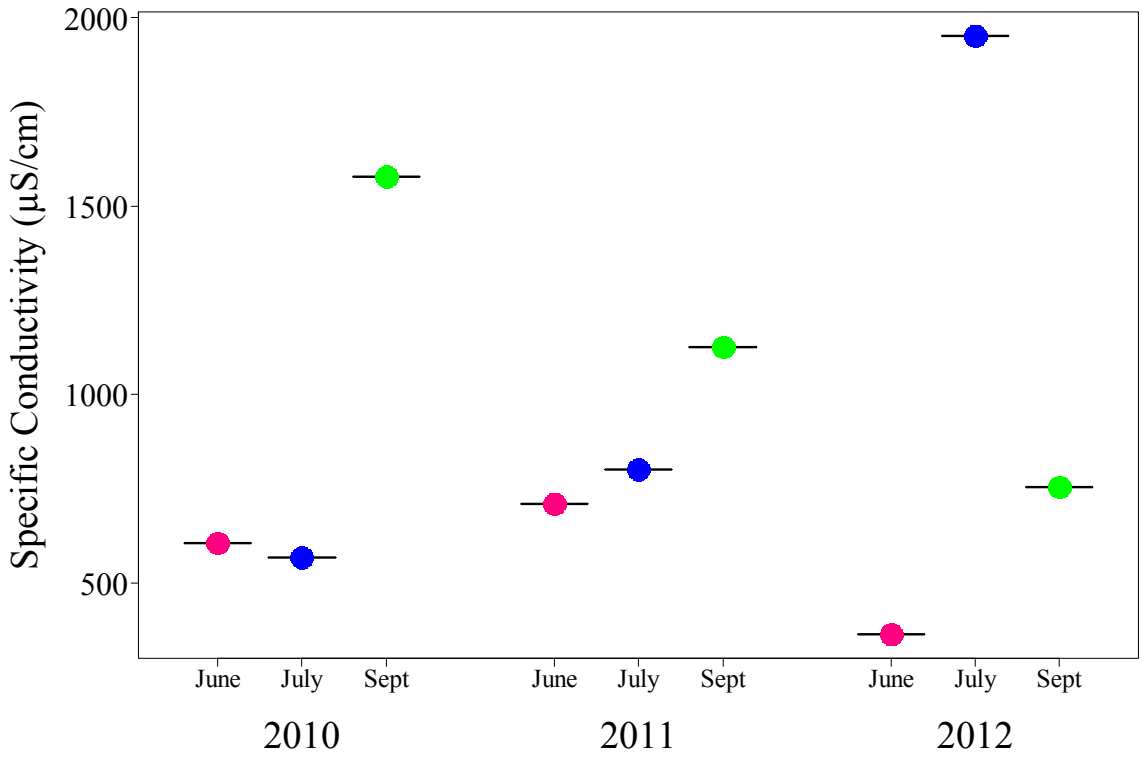


**Figure 2-11** Seasonal and inter-annual variation in specific conductivity of a goose-impacted pond WNP-FF. Data provided by Lauren McDonald, University of Waterloo. Pink points represent the specific conductivity measure for June, blue points represent July and green points represent September.

**Table 2-1** Ion concentration for study sites WNP-GG and WNP-X, and mean ion concentration and standard deviation for goose-impacted and non-impacted ponds in WNP. Specific Conductivity is presented in µS/cm and all major ions are presented in mg/L. Survey data was collected approximately during the last week of July 2010-2012.

| Pond | Year | n | Specific Conductivity | | $Cl^-$ | | $SO_4^{2-}$ | | $Ca^{2+}$ | | $Mg^{2+}$ | | $K^+$ | | $Na^+$ | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | mean | sd | mean | sd | mean | sd | mean | sd | mean | sd | mean | sd | mean | sd |
| **WNP-GG** | 2010 | | 11100 | | 3450.0 | | 434.0 | | 216.0 | | 185.0 | | 60.4 | | 502.0 | |
| | 2011 | | 10500 | | 3170.0 | | 430.0 | | 139.0 | | 197.0 | | 60.8 | | 1730.0 | |
| | 2012 | | 14000 | | 4310.0 | | 542.0 | | 232.0 | | 259.0 | | 85.9 | | 2340.0 | |
| **WNP-X** | 2010 | | 156 | | 7.1 | | 0.2 | | 23.7 | | 3.7 | | 0.2 | | 3.9 | |
| | 2011 | | 130 | | 5.2 | | 0.2 | | 19.4 | | 3.0 | | 0.1 | | 3.1 | |
| | 2012 | | 149 | | 6.1 | | 0.1 | | 23.2 | | 3.5 | | 0.2 | | 3.4 | |
| **Mean Goose** | 2010 | 3 | 8810 | 6068 | 2734.3 | 2001.9 | 359.7 | 271.2 | 136.8 | 74.4 | 171.7 | 136.4 | 40.5 | 27.3 | 960.3 | 1123.0 |
| **Impacted** | 2011 | 5 | 7924 | 3984 | 2384.6 | 1268.5 | 275.8 | 153.7 | 114.9 | 41.9 | 157.7 | 79.1 | 41.5 | 25.6 | 1329.4 | 673.7 |
| | 2012 | 3 | 10743 | 7532 | 3397.7 | 2544.3 | 426.7 | 321.8 | 176.6 | 92.5 | 231.3 | 177.1 | 53.0 | 40.5 | 1762.7 | 1277.9 |
| **Mean Non** | 2010 | 24 | 194 | 167 | 22.2 | 31.9 | 1.9 | 3.4 | 21.5 | 16.3 | 4.4 | 3.0 | 0.8 | 1.2 | 11.9 | 16.7 |
| **Impacted** | 2011 | 30 | 205 | 162 | 21.6 | 30.9 | 1.9 | 3.8 | 23.8 | 15.5 | 4.5 | 3.0 | 0.7 | 1.4 | 11.4 | 18.6 |
| | 2012 | 27 | 252 | 247 | 31.7 | 50.6 | 2.5 | 6.6 | 26.8 | 20.6 | 5.6 | 4.9 | 1.0 | 2.3 | 16.1 | 30.7 |

**Table 2-2** Results for two-way and one-way ANOVA testing the effect of layer and species on the ephippia abundance/year in goose-impacted WNP-GG and raw abundance in non-impacted WNP-X. Bold P-values are significant.

| Pond | Variable | df | F | P |
|---|---|---|---|---|
| WNP- GG | Layer | 17 | 39.2 | **<0.001** |
| | Species | 1 | 6.5 | **<0.001** |
| | Layer x Species | 17 | 16.1 | **<0.001** |
| | Error | 140 | | |
| WNP-X | Layer | 17 | 1.2 | 0.27 |
| | Error | 70 | | |

**Table 2-3** $LC_{50}$ values for *D. magna* from each sediment layer of WNP-GG. Clone Lines indicated the number of iso-female clone lines used in the $LC_{50}$ trials from each layer. 0 cm is the most recent layer.

| **Period** | **Layer** (cm) | **Clone Lines** | **Total *Daphnia*** | **$LC_{50}$** (µS/cm) |
|---|---|---|---|---|
| Post-Impact | 0-0.5 | 3 | 106 | 6657 |
| | 2-2.5 | 3 | 113 | 5799 |
| | 3.5-4 | 2 | 51 | 5934 |
| | 4-4.5 | 2 | 83 | 6209 |
| Pre-Impact | 4.5-5 | 2 | 43 | 5174 |
| | 5-5.5 | 4 | 124 | 6135 |
| | 5.5-6 | 1 | 35 | 9920 |
| | 6-6.5 | 5 | 175 | 6657 |
| | 7-7.5 | 3 | 87 | 6459 |
| | 7.5-8 | 3 | 96 | 5621 |
| | 8-8.5 | 2 | 68 | 6453 |
| | 8.5-9 | 1 | 35 | 7507 |

**Table 2-4** Likelihood values (L) and Akaike Information Criterion (AIC) values used during linear mixed model selection for the response variable i) Days until mortality (DM), ii) Total reproductive output (TRO), iii) Daily neonate production (DNP), iv) Days until first reproduction (DFR), v) Size at first reproduction (SFR), vi) intrinsic population growth rate (r), vii) Net reproductive rate ($R_0$), viii) the von Bertalanffy growth constant K (vB K), ix) the von Bertalanffy maximum possible length (vB $L_\infty$) and x) Growth rate until first reproduction (GR FR). The starting point in model selection was the Beyond Optimal Model (BOM). Significance is denoted with a * and indicates that the model missing the specified effect is significantly different from the BOM.

| **Response Variable** | **BOM** | **Random Effects** | | | | **Fixed Effects** | | | | | | | **Final Model** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | No Trial Start Date | | No Clone ID | | Full Model | No interaction | | No Period | | No Salinity | | |
| | AIC | L | AIC | L | AIC | AIC | L | AIC | L | AIC | L | AIC | |
| DM | 11160.2 | <0.001* | 11540.2 | --- | --- | 11133.4 | 0.16 | 11133.4 | 0.72 | 11131.5 | 0.03* | 11136.0 | Sal + (1\|ID/Start) |
| TRO | 11142.4 | <0.001* | 11740.7 | --- | --- | 11114.6 | 0.10 | 11115.4 | 0.88 | 11113.4 | <0.001* | 11142.0 | Sal + (1\|ID/Start) |
| DNP | 880.6 | <0.001* | 1446.8 | --- | --- | 828.7 | 0.06 | 830.3 | 0.86 | 828.3 | <0.001* | 855.6 | Sal + (1\|ID/Start) |
| DFR | 3564.1 | <0.001* | 3815.0 | --- | --- | 3529.8 | 1.00 | 3527.8 | 0.78 | 3525.9 | <0.001* | 3550.09 | Sal + (1\|ID/Start) |
| SFR | -183.2 | <0.001* | -133.7 | --- | --- | -236.4 | 0.06 | -234.9 | <0.001* | -211.32 | 0.01* | -230.06 | Per +Sal + (1\|ID/Start) |
| r | -93.0 | NA | NA | 0.89 | -94.9 | -148.7 | 0.52 | -153.5 | 0.76 | -155.4 | 0.01* | -148.0 | Sal + (1\|ID) |
| $R_0$ | 307.9 | NA | NA | 0.94 | 305.9 | 335.6 | 0.49 | 331.0 | 0.66 | 329.2 | 0.01* | 337.157 | Sal + (1\|ID) |
| vB K | -2119.4 | <0.001* | -1966.3 | --- | --- | -2182.975 | 0.65 | -2184.8 | 0.28 | -2185.6 | 0.26 | -2185.5 | 1 + (1\|ID/Start) |
| vB $L_\infty$ | 885.1 | <0.001* | 1603.0 | --- | --- | 843.9 | 0.22 | 843.4 | 0.40 | 842.1 | 0.01* | 849.0 | Sal + (1\|ID/Start) |
| GR FR | -4374.5 | <0.001* | -4131.8 | --- | --- | -4449.0 | 0.93 | -4451.0 | 0.42 | -4452.3 | <0.001* | -4405.4 | Sal + (1\|ID/Start) |

# Chapter 3

# General Discussion

Species impacted by rapid environmental change must respond to new conditions or face declines in abundance and possible extirpation. Three responses — i) range shifts, ii) phenotypic plasticity and iii) microevolution — can enable species persistence after an abiotic or biotic change in the environment (Holt 1990; Davis et al 2005). Past climate changes associated with glacial / interglacial cycles resulted in dramatic range shifts (Jackson and Overpeck 2000), and range shifts are currently being observed in the poleward movement of species distributions (Parmesan and Yohe 2003; Root et al. 2003). Another common response to climate change is shifts in breeding phenology due to phenotypic plasticity (Parmesan 2007). While plastic responses to non-climate stressors are less well documented, they have been suggested as a crucial mechanism of survival during periods of environmental change (Berg et al. 2010). Finally microevolution, which results in a shift in the genetic make-up of the population, is often seen in response to rapid anthropogenic physico-chemical changes in the environmental (Reznick and Ghalambor 2001).

These three discrete responses to change have been well defined; however it remains difficult to predict how a species will response to a given stressor (Chevin et al. 2010). Past research has demonstrated that both differential (Derry and Arnott 2007) and consistent (Phillimore et al. 2012) species responses can be observed within a community. The response of multiple species to a single stressor needs to be further examined to understand how the community as a whole is impacted by changes in the environment (Berg et al. 2010, Urban et al 2011).

The goal of this thesis was to further understand how species within a community respond to environmental change by examining the impact of a dramatic increase in conductivity

in a small Subarctic pond (WNP-GG) that occurred as a result of habitat degradation caused by expanding goose populations. The first objective of the study was to investigate how species composition was impacted by goose-induced environmental changes using the pond's resting egg bank. A significant change in the ratio of the two species present was observed post goose impact. *D. magna* increased in abundance after impact while *D. tenebrosa* abundances remained low, and eventually extirpated in the most recent time interval. This difference in species response may be due to the conductivity levels surpassing the physiological tolerance of *D. tenebrosa*. Alternatively, extirpation may have been the result of increased competition pressure of *D. magna* post goose-impact.

The second objective of the study was to investigate the response mechanism of *D. magna* that allowed the population to persist despite the dramatic change in environmental conditions. A strong signal of microevolution to increased conductivity tolerance was expected given the findings of past studies that had shown shifts in salinity tolerance in two other *Daphnia* species — *D. thomansi* (Barry et al. 2005) and *D. pulex* (Weider and Hebert 2010). This study found that while all iso-female clone lines were negatively impacted by increased salinity, there were no differences in life history, population growth or individual growth metrics between pre- and post-disturbance genotypes. The lack of differentiation between genotypes indicates that the change in the environment at WNP-GG has not resulted in microevolution in conductivity tolerance for the *D. magna* population. Instead, it suggests that the response to increased conductivity is a phenotypically plastic trait that has been maintained throughout the history of the population. The plasticity of this population likely developed in response to the highly variable environmental conditions of the region, particularly at seasonal and interannual scales.

The absence of a microevolutionary response in salinity tolerance for this population is interesting given that numerous examples of rapid microevolution have been documented in response to anthropogenic change since the late 20$^{th}$ century (reviewed in Thompson 1998).

Additionally the majority of studies using resurrection ecology techniques have documented signals of microevolution (Kerfoot et al. 1999; Cousyn et al. 2001; Limburg & Weider 2002; Kerfoot and Weider 2004; Barry et al 2005; Michels et al. 2007; Decaestecker et al. 2007; Orsini et al. 2012), suggesting that microevolution is the primary mechanism of response to environmental change within the *Daphnia* genus. However, this study found that a plastic response to increased salinity was present throughout the history of the *D. magna* population from WNP-GG. This suggests that phenotypic plasticity can also be responsible for mitigation of environmental change within this genus, as seen in other species in response to climate change (Berg et al. 2010).

This study provides empirical support for past findings that community response to environmental change is species specific, and emphasizes the importance of examining the impacts of environmental change at the community level. This suggests that although past environmental conditions play a role in how species are impacted by change (Bell and Gonzalez 2011), there are other controlling factors such as physiological constraints and species interaction (Berg et al. 2010) that also influence species response. Therefore, communities as a whole cannot be expected to display a uniform response to ecosystem changes. By broadening the scope of the investigation to the community level we gain better understanding of the factors that influence species response.

### *Future Directions*

There is still much to learn about how communities and their composite species respond to changes in the environment. The unprecedented environmental changes being experienced globally due to anthropogenic activity, including climate change and habitat degradation, have resulted in dramatic losses in biodiversity, making the need to understand the impacts of global change ever more urgent. To increase the power of predictive models, multispecies interaction

must be included. This requires further empirical investigation of how multiple species within a community respond to environmental change, including species interactions during various stages of impact.

Resurrection ecology provides a powerful tool to investigate multispecies response to environmental change. The egg bank of a disturbed system allows one to track how species composition changes over time in response to a stressor. Life history experiments can be conducted on genotypes of multiple species from pre- and post-disturbance time periods to examine specific species response to a stressor. Finally, competition trials could be conducted between species among pre- and post-disturbance genotypes to determine how species interactions are influencing the observed response to environmental change. Lab trials could later be expanded to in-lake mesocosms experiments to examine the impacts of the stressor on community composition compared to control conditions in pre- and post-disturbance communities.

Further investigations are also needed on how past environmental conditions influence species response. Past resurrection ecology studies that documented signals of microevolution examined primarily focused on populations from large temperate lakes, while the population from this study was from a small Subarctic pond. This suggests that populations from a system that experiences more stable environmental conditions (the large lakes) may respond differently than populations from a more variable system. These differences need to be understood in order to better predict species response. Again resurrection ecology provides an effective approach to compare changes in community composition and mechanisms of persistence. A focal stressor would need to be found in both a variable and stable system, which would then allow for comparison of species response. Studies of populations from non-temperate large freshwater ecosystems will help determine if the response of microevolution is widespread or whether phenotypic plasticity is the dominant response mechanism.

Empirical studies of the response of multiple species from a diversity of ecosystems will allow for factors such as species interaction and past environmental variance to be included in predictive models. Increased predictive capabilities may help identify "high risk" species in regions predicted to undergo significant environmental change, possibly influencing conservation priorities. As ecologists better understand how species respond to environmental changes it will help inform policies aimed at managing the risks of global change.

***Summary***

1. Community level response to environmental changes in WNP-GG due to goose impact is species specific**.** *D. magna* was able to persist during the period of rapid increase in pond conductivity while *D. tenebrosa* was eventually extirpated. Differential response in species may be due to the salinity levels in the study site surpassing the physiological tolerance of *D. tenebrosa* and/or may have been the result of increased competition pressure of *D. magna* post goose-impact.

2. No signal of microevolution for increased salinity tolerance was observed in the post-disturbance *D. magna* population. Iso-female clone lines from both periods were similarly negatively impacted by increases in salinity. The lack of differentiation between periods suggests that the response to increased conductivity is a phenotypically plastic trait that has been maintained throughout the history of the population.

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# Appendices

**Appendix 1**- Wapusk National Park Pond Survey

**Table A-1** Goose impact status (N represents not impacted, Y represents goose-impacted), geographical coordinates in Universal Transverse Mercator (UTM) coordinates, area, total phosphorus and specific conductivity of ponds from the 2008-2011 Wapusk National Park pond survey. Years when a pond dried and therefore was not able to be sampled are represented by NA. Phosphorus values obtained for the total crustacean zooplankton community (McCauley 1984; Dumont *et al.* 1975, Girard & Reid 1990, Anderson & Hesson 1991 and Hesson & Lyche 1991) were then added to the P values from the water chemistry analysis to obtain total phosphorus (TP) for each pond.

| **Pond** | **Goose** | **UTM (zone 15)** | | **Area** | **TP (µg/L)** | **Specific Conductivity (µS/cm)** | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **Impacted** | Northing | Easting | (Ha) | 2011 | 2008 | 2009 | 2010 | 2011 | 2012 | mean | sd |
| WNP-A | N | 6474874 | 481745 | 0.19 | 23.3 | NA | 215 | NA | 227 | NA | 221.0 | 8.5 |
| WNP-AA | N | 6397823 | 437937 | 229.98 | 24.2 | 114 | 77.2 | 128 | 111 | 117 | 109.4 | 19.1 |
| WNP-B | N | 6474856 | 481816 | 0.12 | 16.2 | NA | 226 | NA | 236 | NA | 231.0 | 7.1 |
| WNP-BB | N | 6400434 | 440175 | 0.27 | 34.2 | 35.8 | 22.6 | 28.6 | 25.5 | 27.8 | 28.1 | 4.9 |
| WNP-C | N | 6473945 | 484547 | 3.77 | 15.6 | NA | 229 | 278 | 297 | 398 | 300.5 | 71.0 |
| WNP-CC | N | 6503372 | 489097 | 16.29 | 18.6 | 308 | 262 | 238 | 257 | 278 | 268.6 | 26.2 |
| WNP-D | N | 6476124 | 484294 | 0.51 | 18.3 | NA | 209 | NA | 280 | 426 | 305.0 | 110.6 |
| WNP-DD | N | 6503260 | 488462 | 1.04 | 12.9 | 237 | 198 | 186 | 219 | 219 | 211.8 | 20.0 |
| WNP-E | N | 6476095 | 484465 | 0.41 | 16.8 | NA | 227 | NA | 276 | 593 | 365.3 | 198.7 |

| Pond | Goose | UTM (zone 15) | | Area) | TP (µg/L) | Specific Conductivity(µS/cm) | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Impacted | Northing | Easting | (Ha) | 2011 | 2008 | 2009 | 2010 | 2011 | 2012 | mean | sd |
| WNP-EE | Y | 6508698 | 472846 | 0.06 | 561.2 | NA | NA | 13400 | 6500 | 16100 | 12000.0 | 4950.8 |
| WNP-F | N | 6476066 | 484336 | 0.43 | 20.8 | NA | 243 | NA | 296 | NA | 269.5 | 37.5 |
| WNP-FF | Y | 6501940 | 471766 | 0.09 | 30.2 | NA | NA | 1930 | 1520 | 2130 | 1860.0 | 311.0 |
| WNP-G | N | 6466843 | 484514 | 0.16 | 14.1 | 193 | 139 | 178 | 162 | 234 | 181.2 | 35.7 |
| WNP-GG | Y | 6502867 | 474424 | 0.60 | 63.2 | NA | NA | 11100 | 10500 | 14000 | 11866.7 | 1871.7 |
| WNP-H | N | 6466742 | 484309 | 0.09 | 18.7 | 174 | 101 | 237 | 205 | 301 | 203.6 | 74.2 |
| WNP-I | N | 6466928 | 484150 | 0.84 | NA | 254 | 224 | 125 | 242 | 246 | 218.2 | 53.2 |
| WNP-II | Y | 6507461 | 482664 | 3.75 | 32.9 | NA | NA | NA | 10400 | NA | 10400.0 | |
| WNP-J | N | 6471644 | 479834 | 4.67 | 13.3 | 384 | 272 | 469 | 428 | 489 | 408.4 | 86.2 |
| WNP-K | N | 6467801 | 486429 | 5.05 | 43.6 | 730 | 481 | 655 | 531 | 588 | 597.0 | 98.7 |
| WNP-KK | Y | 6501309 | 471815 | 0.471 | 70.9 | NA | NA | NA | 10700 | NA | 10700.0 | |
| WNP-L | N | 6502263 | 488726 | 120.66 | 14.3 | 195 | 169 | 174 | 172 | 183 | 178.6 | 10.5 |
| WNP-M | N | 6502398 | 489218 | 44.85 | 14.8 | 888 | 694 | 583 | 775 | 1170 | 822.0 | 224.3 |
| WNP-N | N | 6433487 | 460575 | 0.07 | 21.5 | 35.9 | 25.3 | 33.5 | 73.8 | 36.1 | 40.9 | 18.9 |
| WNP-O | N | 6433030 | 460113 | 1.22 | 30.5 | 34.3 | 24.7 | 32 | 29.5 | 36.7 | 31.4 | 4.6 |
| WNP-P | N | 6432961 | 460287 | 0.75 | 33.1 | 43 | 28.9 | 42.2 | 38.8 | 57.1 | 42.0 | 10.1 |
| WNP-Q | N | 6472606 | 477668 | 241.41 | 11.8 | 196 | 165 | 208 | 199 | 204 | 194.4 | 17.1 |
| WNP-R | N | 6476288 | 489585 | 2.76 | 13.4 | 327 | 250 | 232 | 276 | 335 | 284.0 | 45.8 |
| WNP-S | N | 6427697 | 47843 | 0.05 | 13.4 | 56.3 | 37.2 | NA | 38.3 | 98.9 | 57.7 | 28.8 |
| WNP-T | N | 6343458 | 468544 | 1.05 | 11.2 | 157 | 139 | 124 | 123 | 122 | 133.0 | 15.1 |
| WNP-U | N | 6441917 | 467253 | 9.27 | 12.3 | 242 | 197 | 269 | 236 | 242 | 237.2 | 25.9 |
| WNP-V | N | 6392437 | 474243 | 1.99 | 13.2 | 181 | 95.8 | 141 | 96.9 | 115 | 125.9 | 35.8 |
| WNP-W | N | 6396616 | 477847 | 7.91 | 35.6 | 28.5 | 24.8 | 28.5 | 26.3 | 24.7 | 26.6 | 1.9 |
| WNP-X | N | 6399426 | 477552 | 0.70 | 18.6 | 142 | 118 | 156 | 130 | 149 | 139.0 | 15.2 |
| WNP-Y | N | 6391360 | 444888 | 0.77 | 94.8 | 23.8 | 16.7 | 18.4 | 19.5 | 16.6 | 19.0 | 2.9 |
| WNP-Z | N | 6386174 | 442080 | 109.52 | 42.4 | 79.3 | 93.2 | 102 | 118 | 106 | 99.7 | 14.5 |

**Appendix 1 References**

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**Appendix 2** Ephippia Abundance by Core

**Table A-2** Raw ephippia abundance for *D. magna* (D.m) and *D. tenebrosa* (D.t) by core in WNP-GG and WNP-X. Blank values means sediment layer was not counted.

| | WNP- GG | | | | | | | | | | WNP-X | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Layer | Core 1 | | Core 2 | | Core 3 | | Core 4 | | Core 5 | | Core 1 | Core 2 | Core 3 | Core 4 | Core 5 |
| (cm) | D. m | D. t | D. m | D. t | D. m | D. t | D. m | D. t | D. m | D. t | D. t | D. t | D. t | D. t | D. t |
| 0-0.5 | 38 | 0 | 27 | 0 | 31 | 0 | 42 | 0 | 23 | 0 | 0 | 47 | 15 | 29 | 4 |
| 0.5-1 | 31 | 1 | 11 | 2 | 31 | 1 | 39 | 2 | 37 | 0 | 1 | 38 | 25 | 20 | |
| 1-1.5 | 50 | 14 | 8 | 1 | 46 | 0 | 38 | 1 | | | 8 | 30 | 21 | 24 | 9 |
| 1.5-2 | 30 | 2 | 9 | 1 | 23 | 0 | 24 | 2 | 13 | 1 | 17 | 24 | 37 | 11 | 11 |
| 2-2.5 | 27 | 8 | 25 | 3 | 34 | 3 | 17 | 0 | 23 | 1 | 12 | 20 | 33 | 10 | 29 |
| 2.5-3 | 36 | 12 | 14 | 3 | 43 | 1 | | | 27 | 2 | 55 | 13 | 43 | 17 | 28 |
| 3-3.5 | 46 | 9 | 15 | 6 | 7 | 2 | 27 | 2 | 21 | 1 | 12 | 11 | 25 | 8 | 26 |
| 3.5-4 | 31 | 13 | 29 | 4 | 49 | 0 | 30 | 6 | 25 | 0 | 10 | 6 | 37 | 12 | |
| 4-4.5 | 35 | 10 | 19 | 5 | 56 | 4 | 45 | 6 | 39 | 1 | 36 | 8 | 28 | 19 | 17 |
| 4.5-5 | 29 | 5 | 21 | 4 | 41 | 6 | 56 | 4 | 27 | 0 | 22 | 6 | 30 | 15 | 14 |
| 5-5.5 | 35 | 8 | 26 | 3 | 42 | 7 | 43 | 2 | 51 | 0 | 19 | 5 | 18 | 18 | 23 |
| 5.5-6 | 14 | 13 | 30 | 5 | 34 | 2 | 16 | 3 | 69 | 4 | 31 | 6 | 20 | 18 | 28 |
| 6-6.5 | 17 | 25 | 13 | 3 | 50 | 5 | 31 | 5 | 61 | 6 | 27 | 4 | 23 | 11 | 30 |
| 6.5-7 | 15 | 23 | 16 | 2 | 43 | 1 | 29 | 13 | 40 | 3 | 33 | 3 | 34 | 11 | 31 |
| 7-7.5 | 10 | 20 | 12 | 2 | 24 | 8 | 15 | 3 | 60 | 2 | 22 | 1 | 23 | 15 | 20 |
| 7.5-8 | 21 | 11 | 11 | 1 | 15 | 1 | 15 | 10 | 55 | 2 | 32 | 6 | 19 | 13 | 25 |
| 8-8.5 | 32 | 11 | 3 | 2 | 28 | 5 | 12 | 7 | 52 | 1 | 24 | 4 | 13 | 11 | 36 |
| 8.5-9 | 17 | 12 | 4 | 2 | 35 | 3 | 19 | 2 | 50 | 6 | 23 | 4 | 25 | 14 | 46 |

**Appendix 3** Life history trials *Daphnia* totals

**Table A-3** Number of individuals from a specified iso-female clone line run at each treatment level.

| | **Specific Conductivity (µS/cm)** | | | | |
|---|---|---|---|---|---|
| Clone line | 180 | 1000 | 2500 | 3125 | 3750 |
| 0-0.5 H3 | 6 | 19 | 53 | 16 | 16 |
| 2-2.5 H2 | 46 | 25 | 34 | 24 | 65 |
| 2-2.5 H3 | 57 | 53 | 28 | 26 | 25 |
| 4.4.5 H1 | 59 | 32 | 44 | 60 | 84 |
| 5-5.5 H3 | 20 | 17 | 11 | 32 | 14 |
| 5-5.5 H4 | 18 | 48 | 23 | 16 | 18 |
| 5.5-6 H2 | 16 | 23 | 15 | 19 | 19 |
| 6-6.5 H3 | 52 | 15 | 18 | 17 | 14 |
| 6-6.5 H4 | 47 | 15 | 21 | 15 | 16 |
| 7.5-8 H3 | 24 | 25 | 80 | 16 | 38 |
| 8-8.5 H1 | 34 | 16 | 20 | 22 | 16 |
| 8-8.5 H2 | 21 | 26 | 16 | 24 | 18 |