The Influence of Toad Tadpoles on the Environment

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2024

A thesis submitted to McGill University in partial fulfillment of the requirements
of the degree of Doctor of Philosophy in Biology

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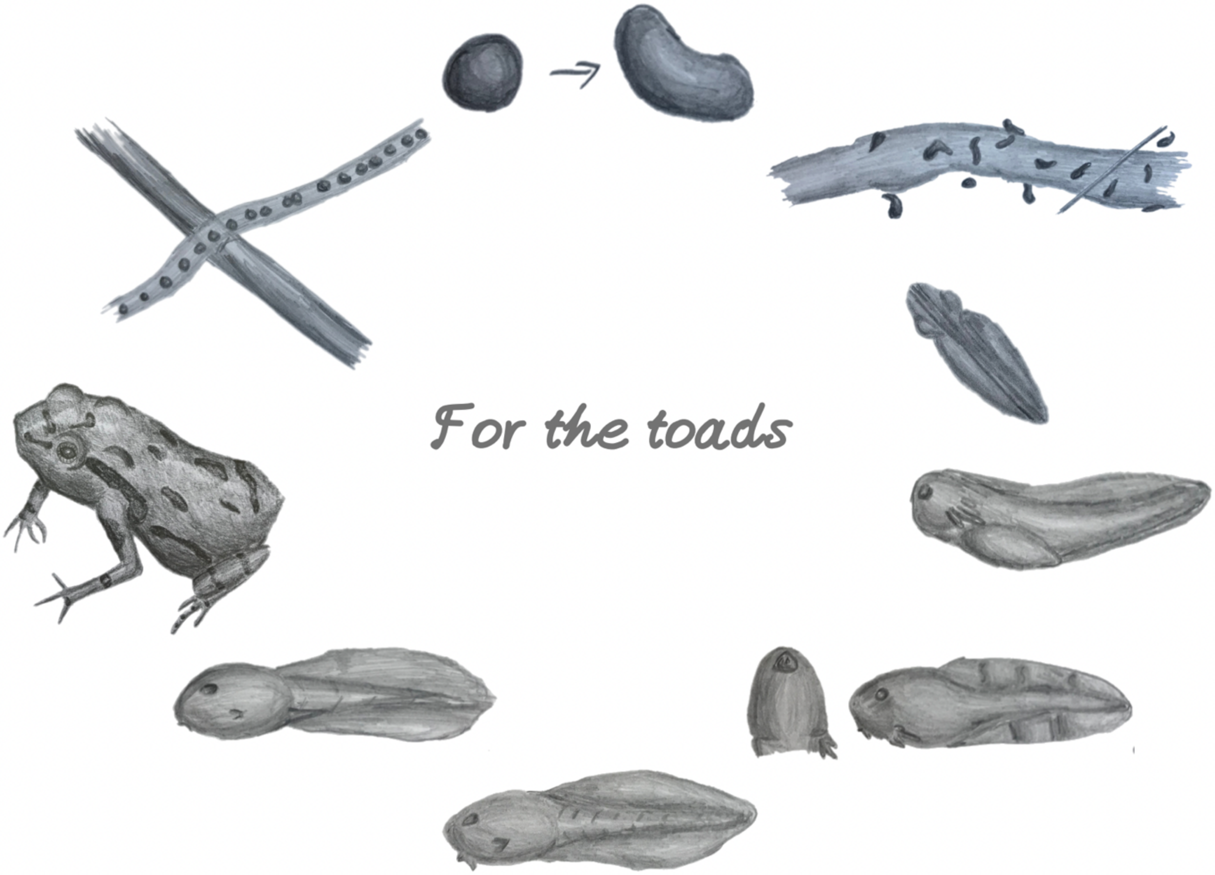

For the toads

Illustrations by Jessica Ford

Preface

Traditional Indigenous Territory Acknowledgement

As a scientist who works on the land and studies it, I have the responsibility to acknowledge and respect the lands where my research was conducted. McGill University is situated on the traditional territory of the Kanien'kehá:ka, which has served as a site of meeting and exchange among Indigenous peoples including the Haudenosaunee and Anishinaabeg. Long Point, Ontario, is situated on the traditional lands Mississauga and Haudenosaunee nations. As ecologists, we would not be able to do our work without these lands.

I acknowledge and thank the traditional stewards of the land where I completed this PhD.

Acknowledgements

This thesis is the direct result

Of everyone who cheered me on,

My own dedication,

And those who showed me I belong.

To all who said I couldn't do it,

You were wrong.

My time working on this PhD has been an adventure, and I have many people to thank for supporting me along the way.

I will begin by thanking those who have mentored me along this journey. I first thank my supervisor, Dr. David Green, for not only supporting my research and ideas, but also supporting my love of teaching and mentoring, and my desire to make the world of herpetology accessible through my outreach and equity, diversity, and inclusion work. I also thank my mentor Dr. Heather Gray, for her excellent advice and encouragement to be myself in science. I can't forget to thank Linda Paetow, for nurturing my love of working with animals and my curiosity about herps.

I thank my committee members, Bruce Pauli, Dr. Rene Gregory-Eaves, and Dr. Alison M. Derry for their guidance and support as my project grew and evolved. I thank Dr. Richard Wassersug for insightful discussion.

I also thank the Redpath Museum staff, in particular Ginette Dessureault, Annie Lussier, and Anthony Howell, for their endless support of me as a student and of my various initiatives in the museum. You all made working in the Redpath Museum a wonderful experience. I must thank Anthony Howell an additional time, for rescuing my samples on four separate occasions, once from a pandemic closure, once from my lab collapsing, once from asbestos, and once from a squirrel. Many of the samples included in this thesis would never have made it in without you.

On that note, I must thank the tight knit community of Redpath Museum students. It has been an absolute honour being your GARM President, and our conversations, coffee hours, and events made my days brighter. I must also thank all the members of STEMM Diversity, we have accomplished so much, and I am so proud of you all.

Through my initiatives, I have met some of my closest friends, Kirsten Crandall, Victoria Glynn, and Adithi Rao. You have all supported me as a leader and as a person, I've cherished working with you and being your friend just as much, you consistently impress me.

I thank the people who supported the student-led initiatives I founded, as well as all of the other outreach work I have done, especially Ingrid Birker, Jacky Farrell, and Megan Anne Phillips. You helped shape me into the science communicator I am today.

I also thank my amazing lab mates, current and past, for their incredible support; Jim Barnett, David O'Connor, Brandon Varela, Pablo Menendez, Eric Guerra-Grenier, Emilie Forget-Klien, Emma Lim, and especially Nathalie Jreidini and Victoria Tawa. Nat and Victoria, I couldn't have asked for better lab mates, field buddies, and friends.

Science doesn't happen alone, and I had an absolute army of undergrads helping me with this project both in the field and in the lab. This thesis would not have been possible without you all. I will do my best to name you all here: J. Benbahtane, A. Zerafa, E. Forget-Klein, D. Shaban,

T. Pulciano, A. Botsko, M. Martini, K. Kwaku, A. Brimacombe, C. Berruezo i Llacuna, S. Thivierge, J. Purdy, A. Malet, L. Meehan, J. Cuffaro, E. Jaeger, P. Harindranath, G. Rimok R. Tremblay, H. McCurdy-Adams, A. Pereira, G. Mango, A. Kemp, J. Wein, J. Wright, M. Gillis, R. Chen, E. Bussing Hudon, E. Savard, J. Osborne, J.Toth, N. Chow, N. Stafiej, N. Bonnefoy, O. Marotta, O. Wells, P. Lipczynski, K. Wilson, L. Visco, M. Sinkins, A. Wang, C. Huo, S. Quijada, and C. Livingston. A special shout out to my intern, Ali Farquhar, and research students Zoe Rabinovitch and Lilly Ragsadale, it was such a joy to watch you grow as scientists.

I acknowledge the doctoral scholarship from I received from Fonds de Recherche du Quebec en Nature et Technologie (FRQNT), as well as the additional funding support received from the Quebec Centre for Biodiversity Science (QCBS), and Environment and Climate Change Canada (ECCC). I also thank Long Point Provincial Park for their logistical support.

And finally, I thank my nearest and dearest. I thank my parents for encouraging my love of nature from a young age, and putting up with all the mud I’ve dragged home. I thank my sisters, Tori and Sarah, for cheering me on, cheering me up, and always listening to my frog facts, or at least pretending to. I thank my uncle, Chip, for listening to all my stories and celebrating my successes. I thank my dear friend, Klara Widrig, for fighting imposter syndrome at my side and having encouraged me to first speak with my PhD supervisor. And I thank my amazing and incredible fiancé, Lava, for being my number one fan, encouraging me to apply to all the awards and participate in all the outreach, and go for everything I desired, even when I wasn’t sure I could pull it off. I wouldn’t be here without you all cheering me on, supporting me, and going through this adventure at my side.

I thank my daughter, Pixie the turtle, for always validating my research ideas and being at my side this whole time, my son, Beans the snake, for trying his best, and the late Gucci the dog, for endless cuddles and making sure my R code worked.

Of course, and as always, I thank the toads.

Résumé

La fonction des écosystèmes peut être altérée par une perte de diversité biologique, en particulier dans les écosystèmes qui sont à l’origine peu diversifiés. Dans le cas échéant, la disparition d'une seule espèce peut avoir des effets profonds sur les communautés associées. Les larves d'amphibiens, en particulier les larves de crapauds, vivent souvent dans des étangs peu diversifiés et constituent souvent la biomasse de vertébrés principale dans les systèmes d'eau douce, en particulier en l'absence de poissons. Comme ces têtards représentent souvent le niveau trophique le plus élevé dans ces systèmes à faible diversité, leur disparition peut avoir des effets de cascade écologique sur les communautés trophiques inférieures et sur la fonction de l'écosystème. Alors que les effets des facteurs environnementaux sur les larves d'amphibiens ont été étudiés, on sait relativement peu de choses sur la manière dont les larves d'amphibiens affectent leur environnement. Pour mieux comprendre la dynamique des espèces dans les écosystèmes des petits étangs, j'ai reproduit une expérience en mésocosme extérieur évaluant l'effet des têtards de crapauds sur leur environnement pendant trois ans à Long Point en Ontario, en utilisant des têtards de crapauds de Fowler et de crapauds d'Amérique. J'ai évaluée les effets des têtards de crapauds sur les écosystèmes, l'impact du pâturage des têtards sur les communautés d'algues, les interactions entre les compétiteurs d'une année à l'autre et si l'impact des têtards de crapauds sur leurs écosystèmes est constant d'une année à l'autre. Mes résultats démontrent la relation dynamique que les têtards de crapauds entretiennent avec leur environnement, ainsi que la nécessité d'effectuer la même expérience à plusieurs reprises pour saisir toute l'ampleur de l'impact écologique d'une espèce.

Abstract

Ecosystem function can be altered by a loss of biological diversity, especially in ecosystems that had little diversity to begin with. In such cases, single species loss can have profound effects on associated communities. Amphibian larvae, especially toad larvae, often live in such low diversity ponds, and often comprise the main vertebrate biomass in freshwater systems, especially in cases where fish are not present. As these tadpoles often represent the highest trophic level in these low diversity systems, their loss may have cascading effects on lower trophic communities and ecosystem function. While the effects of environmental factors on amphibian larvae have been studied, comparatively little is known about how amphibian larvae affect their environment. To gain a better understanding of species dynamics in small pond ecosystems, I replicated an outdoor mesocosm experiment assessing the effect of toad tadpoles on their environment over 3 years, using Fowler's toad and American toad tadpoles in Long Point, Ontario. I assess the net effects of toad tadpoles on ecosystems, tadpole grazing impact on algal communities, competitor interactions across years, and whether the impact of toad tadpoles on their ecosystems was consistent across years. My results demonstrate the dynamic relationship that toad tadpoles have with their environment, as well as the need for more replicated field experiments to capture the full scope of a species' ecological impact.

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Contribution to Original Knowledge

In recent years, biodiversity loss, and individual species loss, has been of increased scientific and public interest. However, few studies assessing species loss, or indeed the impact of species being present or absent in an ecosystem, have focused on small, aquatic species. Few have focused on amphibians. Fewer still have focused on ephemeral species. Only a handful have focused on tadpole species, being that they are small, ephemeral, and only one life stage of a frog or toad. Tadpoles comprise the main form of vertebrate biomass in many of the systems in which they reside, even if they are only there for a few weeks. We do not know the ecological consequences of tadpole loss, or indeed the loss of most ephemeral animals, on ecosystems.

The majority of studies on tadpoles within their environment focus on how environmental factors affect the tadpoles. In my thesis, I turn this around, and focus on how tadpoles impact their environment. The limited amount of other studies that have assessed the impact of tadpoles on their environment have been with tropical stream species. My thesis focuses on temperate, pond breeding toads, a system which has not been assessed prior.

Additionally, mesocosm and field studies are very rarely replicated. As such, little is known about how ecosystems interact across different years, and how they may be impacted by year effects. My thesis involved replicating my mesocosm study in 3 different years, allowing us to assess how the ecological role of a species can shift with yearly conditions.

In Chapter 1, I assess the net effects of toad tadpoles on their environment using experimental mesocosms. These mesocosms mimic the shallow, sandy, low nutrient ponds present in Long Point, Ontario. I developed the methods necessary to raise Fowler's and American toad tadpoles in experimental mesocosms with minimal intervention, so as to assess their impact on the surrounding aquatic communities and abiotic factors. As such, this was the first time that we were able to assess the impact of Fowler's and American toad tadpoles on their ecosystem, and the main goal of this chapter was to determine if they had an affect at all. My coauthors and I show that these species affect nutrient concentration, algal biomass, and zooplankton diversity, warranting further investigations as to the ecological effect of these species as tadpoles.

In Chapter 2, I focus on one interaction between tadpoles and their environment – the impact of tadpole grazing on the algal community. To the best of our knowledge, this is the first time that the impact of any species of tadpoles on algal communities was assessed at the species level. We determined that Fowler's and American tadpoles, previously assumed to be functionally redundant, created different algal communities. In this study we also assessed if toad tadpoles left a legacy effect on their ecosystems after they underwent metamorphosis. Most studies involving tadpoles do not continue after metamorphosis, leaving a gap in the literature about the longer-term effects of tadpole presence. We showed that toad tadpoles indeed leave a legacy effect both on the abundance of algae and species composition of the algal community.

In Chapter 3, I determine how tadpole community composition affects the development of tadpoles through metamorphosis. I replicated this study in two years, something that is very rare with mesocosm studies. I demonstrate that, while being raised with American toad tadpoles always had a detrimental effect on Fowler's toadlets, this effect differed in different years. While it is generally considered that competition outcomes should be the same in seemingly the same conditions, this chapter shows that subtle changes in yearly conditions can change the outcome of species interactions.

In Chapter 4, I compare the net effects of toad tadpoles on their ecosystem across 3 different years. In addition to the novelty of replicating an outdoor mesocosm experiment, I showed that the impact of toad tadpoles differed between years, likely due to seasonally entrained chaos and year effects. Such differences are often attributed to statistical noise, but we argue that this a feature of these dynamic ecosystems, not a flaw. We suggest that these environmental differences when tadpoles are present between years, between species densities, and between species composition increase habitat heterogeneity, and are important parts of these fluctuating wetland systems.

Overall, my thesis demonstrates the novel findings that toad tadpoles are important ecosystem engineers in small pond systems, with dynamic ecological roles depending on yearly conditions, densities, and community composition. Additionally, my thesis shows the importance of replicating field experiments across years when assessing the ecological role of a species, as single-year studies may only capture part of the story.

Contribution of Authors

This thesis has been prepared in manuscript-based format. I developed the projects, methods, and objectives for each chapter of this thesis, with guidance from my supervisor. I conducted all the field work and lab work for all my thesis chapters with the assistance of field and lab volunteers. This is with the exception of the lab work to assess nutrient concentrations for Chapter 1 and Chapter 4, which was conducted by the GRIL lab at UdeM, and the identification of algal species for Chapter 2, which was conducted by an expert at UQAM. I analyzed all the data, produced the figures, and wrote the manuscripts that make up this thesis. Below are the author contributions for each chapter of the thesis, as well as the publication status of each chapter.

Chapter 1 | Status: In preparation for submission to Freshwater Biology

Authors: Jessica Ford, Alexandrea Farquhar, Alison Derry, and David Green

I developed the project and methods, conducted the field work, lab work, and statistical analyses, produced the figures, and wrote the manuscript. Alexandrea Farquhar helped immensely with lab work and data transcription. Alison Derry provided guidance on methods and tools to use. David Green provided supervisory guidance on the project and manuscript and provided comments on the manuscript.

Chapter 2 | Status: In preparation for submission to Freshwater Biology

Authors: Jessica Ford and David Green

I developed the project and methods, conducted the field work, lab work, and statistical analyses, produced the figures, and wrote the manuscript. David Green provided supervisory guidance on the project as well as the manuscript and provided comments on the manuscript.

Chapter 3 | Status: Published in Ecology and Evolution

Authors: Jessica Ford and David Green

I developed the project and methods, conducted the field work, lab work, and statistical analyses, produced the figures, and wrote the manuscript. David Green provided supervisory guidance on the project as well as the manuscript and provided comments on the manuscript.

Chapter 4 | Status: In preparation for submission to Oikos

Authors: Jessica Ford and David Green

I developed the project and methods, conducted the field work, lab work, and statistical analyses, produced the figures, and wrote the manuscript. David Green provided supervisory guidance on the project as well as the manuscript and provided comments on the manuscript.

General Introduction

Consumer Loss in Aquatic Ecosystems

Ecosystem function can be dramatically altered by a loss of biological diversity, particularly in ecosystems which already possess low biodiversity (Naeem et al, 1995). In such cases, single species loss can have profound effects on associated communities (Naeem et al, 1995). The loss of consumer communities in aquatic systems can have dramatic impacts on food web structure, species interactions, species composition, nutrient cycling, and primary production, all of which impact ecosystem function (Petchy et al, 2004; Thebault et al, 2007; Gruner et al, 2008).

Both top-down effects, such as predation, and bottom-up effects, such as nutrient cycling, are important in determining the structure of ecosystems, and both of these pathways are impacted by the presence of a top consumer (Petchy et al, 2004; Thebault et al, 2007; Gruner et al, 2008). As well, both direct and indirect effects influence each trophic level (Mittelbach, 2012). Direct effects are those caused by the consumer itself, usually through predation, but indirect effects vary widely and include density-mediated effects, trait-mediated effects, and trophic cascades (Mittelbach, 2012).

Aquatic ecosystems are particularly influenced by top-down control (Gruner et al, 2008), and algal (primary producer) and zooplankton (consumer) communities are expected to change following the loss of consumer species (Gruner et al, 2008). Algal communities are the base of aquatic food webs, and influence pond primary productivity and chemistry, resulting in cascading effects (Minshall, 1978). These pond communities are also heavily influenced by grazer communities (Kiffney and Richardson, 2001). The intermediate consumer community of zooplankton are known to plastically change in both their behaviors and their morphology when predators (Declerck and Weber, 2003; Scoville and Pfrender, 2010), kairomones (Dodson, 1989), or chemicals (Jansen et al, 2011) are present. Introduced species and changes in the environment can trigger changes to reproductive success and timing (Pijanowska and Stolpe, 1996), individual zooplankton size, the length of defensive spines, and elongated body parts in cladocerans (Dodson, 1989; Scoville and Pfrender, 2010), opening the door to examining trait-mediated effects. These measurable changes in algal and zooplankton communities make their responses suitable to study when examining the ecological changes associated with species loss.

A change in biodiversity can change food web length, which could result in a shift in ecosystem function (Mittelbach, 2012). Changes in food web length are expected to alter the response of communities, with different trophic levels responding in alternating ways (Oksanen et al, 1981). For example, in a food web with 2 trophic levels, one would expect to see an increase in the biomass of herbivores when primary productivity increases. In a food web with 3 trophic levels, however, herbivore biomass would not be expected to show a net change, as the additional herbivore biomass would be consumed by the predators in this food web, resulting instead in an increased predator biomass (Mittelbach, 2012). The removal of a consumer from a system would not only change food web length, but would also affect or eliminate the top-down and bottom-up influences that the consumer had exerted on associated communities.

Natural food webs are often complex, and to study these systems, researchers often simplify them to include only two trophic levels (Gruner et al, 2008). The addition of another trophic level to this setting would allow for more ecological relevance, applicability, and complexity. However, in too complex a system, it would be difficult to determine causality. Thus, a relatively simple system with low diversity that is still represented in nature offers significant promise as a study system.

Flipping the Narrative: Using Tadpoles to Study Consumer Loss

Amphibian larvae, especially North American toad larvae, often live in low diversity ponds where they comprise the main form of vertebrate biomass, especially in locations where fish are not present (Wood and Richardson, 2010). In particular, many North American toads preferentially breed in shallow, sandy, oligotrophic pools that have relatively simple food webs, making their tadpoles ideal and relevant for studying single species loss in an ecosystem. Although the effects of environmental factors on amphibian larvae have been studied extensively (Semlitsch and Boone, 2009), comparatively little is known about how these species affect their environment (Whiles et al, 2006; Arribas et al, 2015). As toad tadpoles often represent the highest trophic level in low diversity pond systems, their loss may have cascading effects on lower trophic communities such as algae and zooplankton, affecting ecosystem function (Petchy et al, 2004; Thebault et al, 2007).

While there is no consensus in the literature, amphibian larvae may have profound effects on periphyton and phytoplankton abundance (Whiles et al, 2006; Connelly et al, 2008, Wood and Richardson, 2010; Buck et al 2012), and zooplankton community composition (Hamilton et al, 2012; Buck et al 2012; Arribas et al, 2014). Moreover, the ecological functions of tadpole communities may not be replaced by functionally redundant consumers, such as invertebrate grazers (Whiles et al, 2013). Further, while generally considered to be herbivores, some tadpoles have shown trophic plasticity (Caut et al, 2013; Arribas et al, 2015), opportunistic omnivory, and carnivorous tendencies (Altig et al, 2007). Multiple tadpole species in a system may thus add a trophic level to the food web, as the additional tadpole species may act as a tadpole predator. The presence of a predator would also reduce the density of the prey, potentially leading to indirect, density-mediated effects.

Effects of Tadpoles in Aquatic Ecosystems

Amphibian populations are declining worldwide, and thus the question of what will happen when tadpoles disappear is not a theoretical one. Some areas have seen the disappearance of over 75% of their amphibian population (Whiles et al, 2006) and one third of amphibian species are now considered to be threatened (Stuart et al, 2004). Some studies argue that the loss of an anuran is akin to the loss of two species, as a primarily terrestrial, carnivorous adult frog or toad possesses a different role in an ecosystem than their mainly herbivorous aquatic larvae (Whiles et al, 2006). Although ephemeral, tadpoles make up a large proportion of the biomass in most ponds (Wood and Richardson, 2010). While the effects of many external factors on tadpoles have been studied extensively, few studies have focused on how tadpoles affect their immediate environment (Whiles et al, 2006; Arribas et al, 2015). This thesis hopes to provide insight into the ecological role of tadpoles, which may help determine the consequences of their possible disappearance (Altig et al, 2007; Arribas et al, 2015) – information that is of urgent importance within the context of a global decline of amphibians.

Tadpoles play a crucial role in regulating the aquatic communities that surround them, even acting as ecosystem engineers (Flecker et al, 1999, Altig et al, 2007; Colón-Gaud et al, 2010; Wood and Richardson 2010). In fishless pond systems, tadpoles often constitute the main form of vertebrate biomass (Seale, 1980). It has been suggested that tadpoles may have profound effects on sedimentation rates, periphyton and phytoplankton abundance, macroinvertebrate abundance, and zooplankton community composition (Flecker et al, 1992; Whiles et al, 2006; Connelly et al, 2008; Colón-Gaud et al, 2010; Wood and Richardson 2010; Hamilton et al, 2012; Buck et al 2012, Arribas et al, 2014). However, there is little consensus in the literature in regard to the net effect of tadpole presence or absence in an ecosystem, as this seems to vary between different species. For instance, certain studies have found that the presence of tadpoles decreases primary productivity (Kupferberg, 1997), other studies have shown the opposite (Kupferberg, 1997; Flecker et al, 1999; Wood and Richardson 2010). Notably, Kupferberg (1997) found that the presence of *Rana boylii* tadpoles increased primary productivity by 10%, whereas *Hyla regilla* tadpoles decreased this variable by 18% in a California river. These studies exemplify that the net effect of tadpoles in an ecosystem can vary depending on the species, as well as the importance of determining individual species effects on an ecosystem.

Within the algal community, tadpoles feed on both periphyton and phytoplankton, and seem to also influence marcophyte biomass (Seale, 1980; Arribas et al, 2014). Tadpoles preferentially eat periphyton while rasping or grazing, but will also consume phytoplankton while suspension feeding (Hamilton et al, 2012). However, direct consumption is not the only way in which tadpoles may influence primary producers. Arribas et al (2014) found that the presence of *Pelobates cultripes* tadpoles decreased macrophyte biomass, likely due to an increase in water turbidity. However, this study also found that when *Pelobates cultripes* (a strong competitor and voracious species) were removed from the system, the remaining tadpole community of *Hyla meridionalis, Pelophylax perezi, Discoglossus galganoi* and *Epidalea* *calamita* increased plant biomass, potentially due to increased nutrient content in the system (Arribas et al, 2014).

Tadpoles boost available nitrogen, an important and often limiting nutrient for plants, in aquatic systems by secreting ammonia as a waste product (Seale, 1980). This shift in nutrient flux could also contribute to an increase in phytoplankton and periphyton biomass, as these communities would uptake these resources (Seale, 1980, Rowland et al, 2017). Tadpoles also deposit nitrogen in their feces, but this nitrogen does not become immediately available in the system, as it must first be mineralized by bacteria (Ramamonjisoa and Natuhara, 2018). However, the more time the feces are present in the system, the more nutrients are leached from them, representing a possibly important latent source of nutrients for the aquatic system (Ramamonjisoa and Natuhara, 2018). It has been suggested that the nutrients secreted and excreted by tadpoles may influence detrivore communities in addition to primary producers by "favoring the activities of detrivorous microbes" (Iwai and Kagaya, 2007). The feeding activities of tadpoles may also help disperse the nutrients within small pond systems, as tadpoles actively stir the water and create small currents with their tails (Iwai and Kagaya, 2007).

As each level of the ecosystem is connected, the effects of tadpoles on nutrients and algae are related. Aside from direct predation, indirect nutrient stocking, and shifts in turbidity, tadpoles have also been found to shape their ecosystems through influencing competition between phytoplankton and periphyton (Liebold and Wilbur, 1992; Hamilton et al, 2012; Costa and Vonesh, 2013; Arribas et al, 2014; Rowland et al, 2017). These studies found that when periphyton biomass decreased, phytoplankton biomass increased, and vice versa, as these two species compete for nutrients. Therefore, when periphyton biomass is decreased, phytoplankton biomass can take advantage of the reduced competition for resources. Tadpoles can reduce periphyton biomass dramatically, as one study found that tadpoles reduced the periphyton biomass by 80% through grazing (Wood and Richardson, 2010).

Different species of tadpoles can also have differing effects on the relationship between phytoplankton and periphyton. Costa and Vonesh (2013) found that *Agalychnis callidryas* tadpoles reduce periphyton, and consequently increased phytoplankton, more intensely than *Dendropsophus ebraccatus* tadpoles in experimental mesocosms. This same study also found that the presence of *Agalychnis callidryas* tadpoles influenced the zooplankton community in the mesocosms, whereas *Dendropsophus ebraccatus* tadpoles did not (Costa and Vonesh, 2013). In this instance, *Agalychnis callidryas* tadpoles altered zooplankton community composition and individual size, and reduced the total abundance of zooplankton in the mesocosms (Costa and Vonesh, 2013).

There have been several other instances of tadpoles influencing the total abundance or community composition of zooplankton. Seale (1980) found a slight negative correlation between zooplankton abundance and tadpole presence, though this was highly variable. Arribas et al (2014) found that tadpoles reduced zooplankton diversity by 12% to 25%, and resulted in a community dominated by copepods, as opposed to a community dominated by cladocerans. Buck et al (2012), however, found that the presence of *Pseudacris regilla* and *Rana cascadae* had no effect on the zooplankton community.

A shift in zooplankton abundance, and thus their grazing pressure on phytoplankton, is expected to have a greater effect in low nutrient lakes (Sarnelle, 1992). This is relevant as tadpoles can often be found in nutrient limited environments (Iwai and Kagaya, 2007), therefore, their influence on zooplankton may have strong impacts on the surrounding community.

While a shift in primary productivity would influence zooplankton populations, there is also a possibility of direct predation on zooplankton by tadpoles (Hamilton et al, 2012). Tadpoles are widely considered to be purely herbivorous, but this idea has been challenged (Altig and Johnston, 1989; Whiles et al, 2006; Altig et al, 2007; Schiesari et al, 2009; Caut et al, 2012; Arribas et al, 2015; Rowland et al, 2017).

Tadpoles generally feed by rasping at a surface and creating a slurry in the surrounding water column, which they then consume through suspension feeding (Altig et al, 2007). The preferred food source for tadpoles seems to be periphyton (Kupferberg 1997; Ranvestel et al. 2004; Altig et al, 2007; Connelly et al. 2008; Wood and Richardson, 2010, Hamilton et al, 2012). Notably, periphyton is not solely plant matter, but consists of algae, cyanobacteria, detritus, microbes, and occasionally small aquatic animals (Altig et al, 2007). As well, should a wandering zooplankton find its way into the slurry being filtered by a tadpole, little would stop the tadpole from consuming this organism. It has been suggested that this more omnivorous diet was missed in traditional tadpole gut analyses, as this method would only capture what the tadpole last ate and not the full range of food sources from which it could be feeding (Ranvestel et al. 2004). The true trophic status of tadpoles is now becoming apparent using stable isotope techniques (Whiles et al 2006; Schiesari et al, 2009; Arribas et al, 2015).

It has been suggested that tadpoles demonstrate a fair level of trophic plasticity, shifting what and how they eat based on food availability and the presence of predators (Seale, 1980; Altig et al, 2007; Caut et al, 2012; Arribas et al, 2015). Tadpoles may also shift their diet and feeding strategies in the presence of strong competitors. Arribas et al (2015) found that in the presence of *Pelobates cultripes*, a large and voracious tadpole, other tadpoles in the community (*Epidalea calamita* (in almost negligible densities), *Discoglossus galganoi, Pelophylax perezi*, and *Hyla meridionalis*) were forced to eat detritus or phytoplankton, as *Pelobates cultripes* dominated other resources.

Tadpole Competition Influencing Ecosystem Effects

The tadpoles of pond-breeding anurans are important components of many small aquatic ecosystems (Hamilton et al, 2012; Buck et al, 2012; Arribas et al, 2014). Competition between closely related tadpole species can influence breeding site selection (Buxton and Sperry, 2017), the structure of tadpole communities (Faragher and Jaeger, 1998; Stein et al, 2017), and the success of larval development (Wilbur, 1987). Tadpoles within ponds may be at very high densities, and often share the same primary food source of periphyton (Connelly et al. 2008; Wood and Richardson, 2010; Hamilton et al., 2012), facilitating strong competition between species (Wilbur, 1980; Pechmann, 1995; Altwegg, 2003; Gazzola and Buskirk, 2014). Tadpoles are ephemeral, and the timing of adult breeding in ponds can change each year, altering competition dynamics (Alford and Wilbur, 1985; Lawler and Morin, 1993; Rudolf, 2018).

Furthermore, the ponds in which tadpoles live will change in response to weather conditions (Florencio et al, 2009; Reinhardt et al, 2015; Rudolf, 2018; Florencio, 2020), which can affect aquatic community structure and impact the outcomes of interspecies competition. Tadpoles, being the larval form of amphibians, do not have other forms of competition such as sexual competition for mates, which may complicate studies on resource competition. The effects of larval competition, and the resulting fitness of metamorphs, can be correlated with time to metamorphosis, survivorship through metamorphosis, and the weight of metamorphs as they emerge (Dash and Hota, 1980; Bardsley and Beebee, 2001; Stein et al, 2017). These metrics can be measured in a relatively straightforward manner, making the model system of tadpole communities in ponds valuable for studying competition and inter-annual variation (Bardsley and Beebee, 2001).

Ecosystem Effects and Interannual Variation in Ponds

Small aquatic ecosystems in which tadpoles live can be heavily affected by inter-annual variation in precipitation, as this can change hydroperiod and pond size, with ensuing effects on the interactions between inhabitants (Reinhardt et al, 2015). Ponds are dynamic and often ephemeral habitats that support high biodiversity and serve as refuge sites for many species (Reinhardt et al, 2015; Hill et al, 2021). Inter-annual variation in species composition has been observed in ponds even in successive years, affecting the phenology of phytoplankton community blooms and their interactions with keystone herbivores in the zooplankton community (Winder and Schindler, 2004). Temporal variation in climatic conditions can also change the establishment times of invertebrate communities in ponds and alter their interactions with larval amphibian communities, as well as how the larval amphibians interact with one another (Reinhardt et al, 2015). This change in larval amphibian communities can, in turn, have profound feedback effects on the surrounding algal and zooplankton communities (Hamilton et al, 2012; Buck et al 2012; Arribas et al, 2014). Understanding the impacts of inter-annual variation in relation to larval amphibian interactions, such as competition, is thus essential to comprehending the ecology of small aquatic ecosystems.

The Novelty of Toad Tadpoles

It should be noted that the majority of the studies focusing on how tadpoles influence their immediate environment focus on frog tadpoles, often in streams in tropical zones. Only Seale (1980) looked at the influence of a tadpole community that included a North American toad tadpole (*Anaxyrus americanus*) in a fishless pond. In her study, Seale noted that American toad tadpoles were only present in the periphery of the pond, and that their numbers quickly declined to zero while still in the early stages of development. This study, although very informative, consisted only of field observations and thus did not have proper controls. As well, the community that was observed by Seale had frog tadpoles in it as well, and thus it was not possible to differentiate the effect of the toad tadpoles from the influence of the frog tadpoles.

Only a few other studies included true toad tadpoles in their experiments (Ranvestel et al, 2004; Iwai and Kagaya, 2007; Connelly et al, 2008; Caut et al, 2012; Arribas et al, 2014; Arribas et al, 2015). These studies either only focused on one aspect of the potential impact the toad tadpoles had on the environment (Iwai and Kagaya, 2007), focused on stream dwelling, tropical tadpoles (Ranvestel et al, 2004; Connelly et al, 2008), or had such a high mortality of toad tadpoles that their effects on the environment were negligible (Caut et al, 2012; Arribas et al, 2014; Arribas et al, 2015). Further, none of these studies looked at the impact of toad tadpoles alone, but always in combination with frog tadpole species. As a result, while the influence of frog tadpoles in both tropical and temperate regions is partially understood, the impact of tadpoles from the entire family of Bufonidae is drastically understudied.

Study species – Fowler's and American Toads

The Fowler's toad (*Anaxyrus fowleri*) and American toad (*Anaxyrus americanus*) are both pond breeding, temperate toad species that have aquatic larvae. The Fowler's toad is considered endangered in Canada (COSEWIC 2010), and the population in Long Point, Ontario, has been monitored by Dr. David Green for 30 years. This population is particularly at risk due to habitat loss from development and the introduction of an invasive reed, *Phragmites australis* (Greenberg and Green, 2013). While the secondary compounds secreted by *Phragmites australis* did not directly affect tadpoles, the reduction of suitable breeding habitat due to the invasive reed has caused population declines (Greenberg and Green, 2013).

Information from this ongoing population survey includes data on individual toads, breeding efforts, and the number of tadpoles and toadlets found. Due to these extensive records and surveys, we know for certain that in the summer of 2017, for the first time in at least 30 years, the Fowler's toads did not breed in Long Point, Ontario. No male choruses were formed, no pairs were found in amplexus, no Fowler's toad tadpoles were seen, and no toadlets were found. This makes Long Point Ontario, and the Fowler's toads therein, relevant for the study of the influence of toad tadpoles on their immediate environments, and the possible consequences of their loss.

Long Point, Ontario is a sand spit on the Northern shore of Lake Erie. The Northern length of the spit is entirely marsh area, with the southern length consisting of a sandy beach. These two habitat types are separated by dunes, in which the toads hibernate. Upon emerging from hibernation, the toads feed and absorb water on the beach side of the dunes, and then proceed to breed in the marsh. In the marshy side the toads breed in shallow, sandy bottomed, oligotrophic ponds and pools. The vast majority of these systems are fishless, and many do not include other tadpole species, making these toads the main form of vertebrate biomass in these systems.

American toads sometimes breed in the same shallow, sandy, oligotrophic pools as the Fowler's toads, and sometimes in the still shallow but much larger ponds that also include fish and the tadpoles of various frog species. Both Fowler's and American toads share the same habitat and breeding pool preferences, and both breed at a similar time of year (with the American toads breeding a few weeks earlier). As such, the only species that the Fowler's toad is likely to share its breeding habitat with is the American toad. While the Fowler's and American toads do hybridize, the adult Fowler's toads and American toads rarely interact outside of the breeding season, with American toads dispersing shortly after their choruses have finished. As such, the tadpole life stage is the main point when Fowler's toads and American toads interact and possibly compete.

While Kupferberg (1997) and Luhring (2013) did study the different influences of two separate tadpole species, these two species were distantly related and notably different. And while Luhring (2013) did include both competition tanks and single species tanks in his study, he was unable to draw anything conclusive from it. To date, no one has studied the differing influences of two closely related species, nor the different impacts these species have when together or apart, which could show drastic differences considering the amount of trophic plasticity tadpoles have been shown to have (Seale, 1980; Altig et al, 2007; Caut et al, 2012; Arribas et al, 2015).

To date, there have been no other studies on the influence of tadpoles on the environment that include *Anaxyrus fowleri,* and only Seale (1980) and Luhring (2013) include *Anaxyrus americanus*. The most ecologically similar toad species that researchers have attempted to study the influence of is the natterjack toad, *Epidalea calamita*, which also breeds in sandy, shallow, oligotrophic ponds in a marsh alongside a beach. However, there has been difficulty successfully rearing these tadpoles in mesocosms without prohibitively high mortality (Caut et al, 2012; Arribas et al, 2014; Arribas et al, 2015). With the continual decline of the Long Point Fowler's toad population, it was important to determine how the environment was shaped by the toad's presence before their possible extirpation.

Study system

The ecosystem in which toad tadpoles live can be successfully replicated in mesocosms due to its simplicity. Mesocosms provide an important middle ground between lab experiments in microcosms and full ecosystem experiments in the field (Semlitsch and Boone, 2009). Mesocosms offer more control than a field experiment, allowing one to better determine causality, and provide more realism than a lab experiment, as mesocosms can support more natural and complex food webs (Wilbur, 1995). Toad tadpoles can be reared in relatively self-sufficient mesocosms that do not need additional food or frequent water changes. Using methods I developed in 2017 (Ford and Green, 2021), these mesocosms can remain relatively unperturbed, making it possible to study the response of the ecosystems within them.

There are some drawbacks to mesocosm studies. For instance, it is important to remember that mesocosms are still artificial settings, and thus the results of such experiments cannot be completely applied to natural ecosystems (Carpenter, 1996). As well, because mesocosms include a more complex ecosystem than lab experiments, mesocosms have more variation between replicates than would be expected in a laboratory setting (Skelly, 2002).

The first use of mesocosms for amphibian ecology was by Warren Brockelman, who used floating pens in a natural pond setting (Brockelman, 1969, Semlitsch and Boone, 2009). Container style mesocosms first came into use in 1977 with Henry Wilbur and Joe Travis (Wilbur and Travis 1984, Semlitsch and Boone, 2009). The first experiments used galvanized steel cattle watering tanks that were buried in the ground and were filled with pond water (Wilbur and Travis 1984). This technique was later improved upon by Peter Morin, in 1980 (Morin 1981, Semlitsch and Boone, 2009). Morin used above ground cattle watering tanks, and filled them with tap water that he then inoculated with plankton (Morin 1981). This technique proved to be a crucial advancement in the field of experimental amphibian biology (Semlitsch and Boone, 2009).

Many containers have been used as experimental mesocosms for raising tadpoles, including floating pens, artificial polyethylene ponds, kiddie wading pools and cattle watering tanks (Semlitsch and Boone, 2009). For their accessibility, for this experiment both kiddie wading pools and cattle watering tanks were considered. However, kiddie wading pools proved to be too flimsy for the amount of water they had to hold for the experiment, and thus cattle watering tanks were used.

Mesocosm studies are important when studying amphibian evolutionary ecology, ecotoxicology, community ecology, and conservation, just to name a few (Semlitsch and Boone, 2009). However, mesocosm studies cannot be successful unless the tadpoles are properly cared for. While many studies claim that their mesocosms contained self-sustaining systems, we found that some human intervention was necessary to keep mortality low in the mesocosms. Minimal human intervention is important for eliminating confounding factors when looking at tadpole mortality in a study, some intervention is particularly important when mesococms are used for the purpose of conservation.

Many previous studies have setup mesocosms so that they have a substrate of leaf litter and rabbit chow (Semlitsch and Boone, 2009). However, this kind of set up is not environmentally relevant for the natural breeding environment of *Anaxyrus americanus* and *Anaxyrus fowleri* in Long Point, Ontario, who tend to lay their eggs in shallow, sandy bottom ponds or ephemeral pools that do not have high quantities of leaf litter. Martin et al (2015) found that the survivorship of American toad tadpoles was heavily influenced by the substrate in the mesocosms they were raised in. Toad tadpoles that were raised using the traditional methods used to raise frog tadpoles were not found to fair well, and suffered high mortality (Caut et al, 2012; Arribas et al, 2014; Arribas et al, 2015).

In order to study how toad tadpoles influence their immediate environment, toad tadpoles need to be able to survive in the environment. It was thus necessary to develop an environment in the mesocosms that could support toad tadpoles, an endeavor that had never been undertaken prior.

Thesis Outline

In this thesis, I explore how toad tadpoles influence the aquatic ecosystem that surrounds them at broad and specific levels, when tadpoles are active and after they have left an aquatic ecosystem, and across multiple years. This in-depth study allows insight into the full scope of toad tadpoles in their ecosystem, and the multitude of impacts that toad tadpoles can have in different yearly conditions. I utilize toad tadpoles in mesocosms that replicate their natural environment using methods that I developed, so as to raise the tadpoles with high survivorship and in naturalistic conditions. I examine the net effects of toad tadpole on their aquatic environment in Chapter 1, assessing how nutrient concentration, algal biomass, and zooplankton community composition change when tadpoles are present or absent. In Chapter 2, I take a closer look at how toad tadpoles influence algal communities specifically, including how tadpole presence impacts which species dominate, and if tadpoles act as keystone grazers. In Chapter 3, I determine how different species of toad tadpoles influence each other in terms of survival, weight at, and time to metamorphosis, and how these interactions may change aquatic communities. Finally, in Chapter 4, I address how the impact of toad tadpoles changes with interannual variation between years. Throughout the thesis, I showcase the importance of toad tadpoles in their ecosystems, and the complexities of species loss in small aquatic ecosystems.

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Chapter 1 | The net effects of toad tadpoles on their environment

Submitted to Freshwater Biology

Ecological effects? Consequences of Amphibian Tadpoles (Anura, Bufonidae) on Small Freshwater Ecosystems

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Abstract

1. Ecosystem interactions, especially in low diversity systems, can be dramatically altered by the loss or gain of even a single species. Toad tadpoles often live in low diversity ponds, but the effects these tadpoles might have on these ecosystems are largely unknown.
2. Here we investigate the net effects of the presence or absence of tadpoles of American toads *Anaxyrus americanus*, and Fowler's toads *A. fowleri*, on abiotic and biotic ecological factors in experimental aquatic mesocosms.
3. Total phosphorus concentration, periphyton biomass, and zooplankton diversity were significantly affected by the presence vs. absence of toad tadpoles, with effects varying depending upon whether one or the other or both species were present.

4. These results demonstrate that amphibian larvae may exert considerable influence upon pond and wetland community ecology.

Introduction

Ecosystem function can be altered by a loss or gain of biological diversity, especially in ecosystems that had little diversity to begin with (Naeem et al., 1995). In such cases, even a single species can profoundly influence ecosystem function, potentially resulting in dramatic impacts on food web structure, species interactions, species composition, nutrient cycling, and primary production (Petchy et al., 2004; Thebault et al., 2007; Gruner et al., 2008). The presence or absence of key species can be expected to alter ecosystem dynamics in different ways depending upon their position in the food web, especially should they change food web length (Oksanen et al., 1981, Mittelbach, 2012). In many small, freshwater ponds with comparatively low biodiversity, amphibian larvae may often comprise the main form of vertebrate biomass, especially in locations where fish are not present (Wood and Richardson, 2010). In particular, many North American bufonid toads preferentially breed in shallow, sandy, oligotrophic pools that support relatively simple food webs. Their tadpoles often represent the highest trophic level in these low diversity systems and, thus, their presence or absence can be expected to have cascading effects on lower trophic communities and ecosystem function (Petchy et al., 2004; Thebault et al., 2007).

Although the effects of many environmental factors on amphibian larvae have been extensively studied (as shown in Semlitsch and Boone, 2009, and the introduction to this thesis), much less is known about how amphibian larvae, in turn, affect their environment (Whiles et al., 2006; Arribas et al., 2015). Thus the growth and development of tadpoles, both in the natural ponds and in mesocosm, have been shown to be influenced by the presence of competitors and predators (Relyea and Auld, 2005; Ford and Green, 2023), food availability (Castano et al, 2010; Enriquez-Urzelai et al, 2013), water chemistry (Thurnheer and Reyer, 2001; Smith et al, 2007), water body permanency (Van Buskirk, 2009; Marquez-Garcia et al, 2010), water depth (Calich and Wassersug, 2012), pond substrate (Marques and Nomura, 2018), and sediment and nutrient additions (Wood and Richardson, 2009). Yet tadpoles should not be considered mere spectators to the biotic and abiotic factors that influence their lives. They, too, can be ecological drivers and thereby play crucial roles in regulating the aquatic communities that surround them (Flecker et al., 1999, Altig et al., 2007; Colón-Gaud et al., 2009; Wood and Richardson 2010).

Studies of the effects *of* tadpoles on freshwater ecosystems are relatively few compared to studies of effects *on* tadpoles and have been confined to field experiments in neotropical streams (Flecker et al., 1999; Connelly et al., 2008; Colón-Gaud et al., 2009), a set of laboratory microcosm experiments by Caut et al. (2012), and an experiment involving tadpoles and their predators in mesocosms by Arribas et al. (2014). These investigations have provided evidence to indicate that amphibian larvae may affect numerous ecosystem properties, including sedimentation rates (Flecker et al., 1999), macrophyte, periphyton, phytoplankton and macroinvertebrate abundances (Arribas et al., 2014; Whiles et al., 2006; Connelly et al., 2008, Wood and Richardson, 2010; Buck et al. 2012; Colón-Gaud et al., 2009), and/or zooplankton community composition (Hamilton et al., 2012; Buck et al. 2012; Arribas et al., 2014). However, a more general understanding of tadpole presence/absence upon aquatic ecosystems will benefit from investigations on more species of anuran tadpoles in more environments than just these, particularly species inhabiting temperate zone pond environments where amphibian tadpoles may be especially significant components of ecological communities (Whiles et al., 2006; Altig et al., 2007; Wood and Richardson, 2010) and which do not require supplemental nutrition.

Although Arribas et al., (2014) employed European spadefoot tadpoles, genus *Pelobates*, in their experiments, North American bufonid toad tadpoles, genus *Anaxyrus*, can also serve as suitable experimental agents for studies of small freshwater systems, particularly as they are less prone to carnivory than *Pelobates* (Pfennig, et al., 1993).

Tadpoles could affect nutrient concentrations, algal biomass, or zooplankton community composition in mesocosms in several ways. For example, tadpoles, as they metabolize and grow, secrete nitrogenous and phosphorus-containing compounds as components of their waste (Tattersall and Wright, 1996; Norlin et al., 2016), thus their presence should elevate the net amount of total nitrogen and phosphorus in the local environment. Because tadpoles primarily consume periphyton (Kupferberg 1997; Ranvestel et al. 2004; Altig et al., 2007; Connelly et al. 2008; Wood and Richardson, 2010, Hamilton et al., 2012), they are likely to reduce periphyton biomass (Whiles et al., 2006; Connelly et al., 2008, Wood and Richardson, 2010; Buck et al., 2012), resulting in more available nutrients for the phytoplankton community (Leibold and Wilbur, 1992; Hamilton et al., 2012; Costa and Vonesh, 2013; Arribas et al., 2014; Rowland et al., 2017). This, in turn, may cause a net increase in prey for zooplankton, which should lead to increased zooplankton abundance and/or diversity (Dodson, 1989; Anderson and Kneitel, 2015). Tadpoles also demonstrate trophic plasticity, shifting what and how they eat based on food availability and the presence of predators or competitors (Seale, 1980; Altig et al., 2007; Caut et al., 2012; Arribas et al., 2015). Furthermore, as tadpoles shift their diet as they grow and develop over time, or in the presence of competing species, cascading effects on other aquatic communities, such as zooplankton, should result.

Hence, we examine here the net effects of American toad (*Anaxyrus americanus*) and/or Fowler's toad (*A. fowleri*) tadpoles on associated abiotic factors and aquatic communities in experimental mesocosms. This is done to determine if, as may be expected, these tadpoles serve as drivers of change in certain environmental factors within freshwater pond environments and influence nutrient concentrations and biotic communities in response to their presence or absence.

Methods

Experimental setup

We conducted an outdoor mesocosm experiment to compare response variables between mesocosms that contained tadpoles, and those that did not. We designated mesocosms as single species *A. americanus* (n=6), single species *A. fowleri* (n=6), mixed species which contained 50 tadpoles of each species (n=5), and no tadpoles (control) (n=5). The experiment was conducted over 40 days, from May 30 to July 9, 2018. Mesocosms were sampled for response variables roughly every 10 days, weather permitting, and these response variables included total phosphorus (TP; ug/L), total nitrogen (TN; mg/L), phytoplankton and periphyton chlorophyll *a* concentration (chla, µg/L), and crustacean zooplankton communities (number of individual taxa/L). We used Rubbermaid structural foam cattle watering tanks as mesocosms in which to raise the tadpoles. These mesocosms contain algae and zooplankton, and mimic natural shallow, sandy, oligotrophic ponds (Ford and Green, 2021) in which the toads preferentially breed. We used mesocosms that were 100 US gallons, measuring 63.50 cm L × 78.74 cm W × 134.6 cm H. Each mesocosm had a shade screen lid secured in place with a bungee cord. We arranged 30 mesocosms in three rows of 10 in the maintenance yard of Long Point Provincial Park from May to August in 2018 (Ford and Green, 2021). We assigned experimental groups to mesocosms haphazardly.

We prepared mesocosms with a sand substrate collected from dug up, local terrestrial sand, and filled with mechanically filtered drinking water from the municipality of Port Rowan which we left to dechlorinate. We then inoculated the water with 1L of local pond water containing, on average, 789.47 zooplankton/m^3, or about 12 zooplankton a liter. The pond water used was selected as the pond was known to support American toad tadpoles. We left the mesocosms to sit for two weeks before the addition of tadpoles. We did not add any other food or nutrients to the mesocosms. The low nutrient, shallow, sandy habitat this created mimicked the local ponds that the Fowler's toads and American toads breed in.

We collected fertilized *A. americanus* eggs from a nearby wetland following an observed breeding chorus. We took care not to remove all eggs, or all of one clutch, from the area, so as not to deplete the wetland of tadpoles. We removed a pair of *A. fowleri* from an unsuitable breeding area in a local parking lot, and placed them in a breeding mesocosm which contained 10 cm of water, sand mounded to one side, and live plants. We left the *A. fowleri* pair overnight to deposit their eggs and we released them the following evening. We collected *A. fowleri* eggs from this breeding mesocosm. In total, we had two clutches of *A. americanus* eggs and one clutch of *A. fowleri* eggs.

We allowed the tadpoles to hatch and mature to Gosner stage 26 (Gosner, 1960) in designated nursery mesocosms before transferring them to experimental mesocosms at a density of 100 tadpoles per mesocosm, or one tadpole per 3.7 L.

Sample collection and analysis

We collected initial water, phytoplankton, periphyton and zooplankton samples immediately before the addition of tadpoles to the experimental mesocosms. Subsequently, we took three samples at approximately two-week intervals, with Sample 3 being collected as the toadlets began emerging from the water in the mesocosms. We recorded the initial sample collection as experimental day zero, with the exception of the *A. fowleri* mesocosms, which we stocked on experimental day five due to Fowler's toads breeding later in the season, and thus reaching Gosner stage 26 later than the American toads. We collected Sample 1 on experimental day 16, Sample 2 on experimental day 26, and Sample 3 on experimental day 40.

Nutrient samples

We collected water samples to quantify dissolved and particulate nutrient concentrations: total nitrogen (TN; mgL^{-1}) and total phosphorus (TP; µgL^{-1}) at each sampling time, roughly every 10 days. Total Nitrogen included nitrite, and nitrite and nitrate combined. Total Phosphorus included dissolved phosphorus, orthophosphate and dissolved orthophosphate. These samples were analyzed at the GRIL- Université de Montréal (UdeM) analytical laboratory. Total Nitrogen (TN) was analyzed using automated colorimetry (US EPA 353.2). We add 3.5 mL of a persulfate solution (60g potassium persulfate + 32 g sodium hydroxide diluted with DI water to 1L) to 25 mL of sample. We placed samples in a sterilizer for 45 min at 121°C and 15 psi. We ran samples through a Lachat Quickchem 8500 with a detection limig of 4 ug N/L.

We analyzed Total Phosphorus using persulfate digestion (US EPA 365.3). For Total Phosphorus (TP), we add 0.3 g of potassium persulfate to 25mL of sample placed samples in a sterilizer for 45 min at 121°C and 15 psi. We ran samples through an Astoria 2 by Astoria-Pacific with a detection limit of 0.7 ug P/L.

Phytoplankton and periphyton sampling

We collected phytoplankton samples between 8:30 am and 10:00 am. As the phytoplankton was at the surface during this time, we scooped up a a 500 mL sample using a labeled 1 L mason jar from about 10 cm below the surface of the water. We immediately stored samples in the dark. We took two samples from each tank for phytoplankton. Beginning with Sample 1, we took duplicates one day apart due to an insufficient amount of time to filter all samples in one day. We first filtered samples through 1.2 µm glass fiber paper filters (Wacom) using a Gask portable vacuum pump. We recorded the volume of water filtered. We folded filters, wrapped them in tin foil, and kept them frozen at -20^{o}C for later chlorophyll extraction and pigment analysis. We filtered all samples within 18 hours of collection.

We collected periphyton by scraping it off the side of the tank. We always collected periphyton from the South-facing wall of the mesocosm to ensure all samples had received an equal amount of light. We pressed a 90^{o} elbow pipe against the side of the tank to create a seal, and modified a toothbrush so that it matched the curve of the pipe was used to scrape periphyton off the side of the mesocosm so that it was free in the water held in the pipe. We gathered approximately 200 mL of periphyton and water using a baster. We filtered, stored, extracted and analyzed the samples in the same way as phytoplankton samples.

Chlorophyll *a* extraction and analysis

We measured comparative phytoplankton and periphyton biomass by determining chlorophyll *a* concentrations, in µg/L, in our samples. Chlorophyll *a* is a commonly used proxy measure of algal biomass (Huot et al., 2007). To do so, we extracted chlorophyll *a* in 8 mL of 95% ethanol that had been heated to 70°C. We then incubated the samples for one hour before running them through a Trilogy® fluorometer (Turner Designs, Inc., San Jose, CA) We transferred 1 mL of the sample to a 2 mL cuvette, acidified them with two drops of 10% HCl, and measured their fluorescence twice, once before acidification and once after acidification (Mayer et al, 1997). We converted raw fluorescence data to estimated chlorophyll *a* concentration through standard curves. Our standard curves were made by obtaining the raw fluorescence values of samples with known chlorophyll a concentration and creating a formula in excel using this curve where we could enter the raw fluorescence vales and obtain chlorophyll a concentration (Holm-Hansen et al, 1965).

Zooplankton diversity

We collected zooplankton using a 20 cm diameter Wisconsin plankton net with a mesh size of 80 µm. We lifted the plankton net vertically through the water column of the mesocosms to create a cylinder of 0.0152 m^3. Zooplankton were sprayed off the net and into a sample tube using water in a spray bottle. After being added to the sample tube, zooplankton were filtered once again through a 70 µm mesh, before being submerged in club soda while still in the mesh. Following anesthesia with club soda by to better maintain the shape of the zooplankton (Gannon and Gannon, 1975), samples were sprayed off the mesh using a spray bottle filled with 70$ ethanol, and preserved in 70% ethanol as a now concentrated sample. We enumerated zooplankton species from whole samples to the lowest taxonomic level possible using a dissecting microscope and identification keys by Balcer et al (1984), Witty (2004) and Haney et al. (2013).

We used the Shannon Diversity Index, which takes into account both species richness and species evenness (reference), as a quantitative measure of zooplankton diversity. We calculated Shannon Diversity for all mesocosms in each experimental group (tadpoles present, tadpoles absent, *A. americanus* only tadpoles present, *A. fowleri only* tadpoles, and both species of tadpoles present) at each sample time to test if tadpole presence increased or decreased the diversity of zooplankton. As some zooplankton could only be identified down to the taxonomic family level, Shannon Diversity was calculated at the family level.

Statistical analysis

Two-sample tests and ANOVAs

To begin our analysis, we first tested for significant changes in dissolved nutrient concentrations, chlorophyl a concentration, and zooplankton from one sampling period to the next using simple linear regression on scatterplots. We then tested for significance differences in the slopes of these regressions depending upon tadpole presence vs. absence using analysis of co-variance (ANCOVA). We used the same procedure to test for effects of *A. americanus* tadpoles only, *A. fowleri* tadpoles only and of the two species together. Faced with limited statistical power for parametric tests with these reduced datasets, we also used Spearman's rank correlation, and its coefficient ρ (rho), rather than p, as a non-parametric substitute. Spearman's Correlation does not assume a linear relationship and allows for mixed data types, which was important as our response variables were continuous but our explanatory variable – tadpole presence vs. absence – was categorical.

To test for significant differences in response variables according to tadpole presence vs. absence at any point in time after the start of the experiment, rather than over the whole experiment, we used two-tailed *t*-tests for each response variable and ANOVAs for all response variables.

The amount of variance amongst total phosphorus concentration through time was compared using Levine's test of homogeneity of variance to determine if tadpole presence reduced the variance in phosphorus between replicate mesocosms through time.

When data did reasonably fit a normal distribution at all time points, which was only the case for periphyton biomass, an additional 2-tailed t-test was conducted. This t-test compared the mean periphyton biomass at each time point in each experimental group (single species *A. americanus* mesocosms, single species *A. fowleri* mesocosms, and mixed species mesocosms) to the control mesocosms to test if tadpole presence decreased the biomass of periphyton in the mesocosms.

After we confirmed that there were relationships between our response variables and tadpole presence and absence, we followed up with modified ANCOVAs to look for differences between each response variable at each time point. However, even when using the R function "coeftest" to make our ANCOVAs robust to high and unequal variance, the data did not meet the assumptions of the ANCOVAs. We found significant interactions between some response variables and the sample times (nitrogen concentration: control and initial time point, $p = 0.009$ phytoplankton biomass, control and initial time point, $p = 0.01$; zooplankton diversity, Fowler's toad tadpoles and initial time point, $p < 0.001$), making the ANCOVAs non-informative.

Multivariate correlational analyses

Following the aforementioned series of tests, to assess the effect of the four experimental groups (American toad tadpoles, Fowler's toad tadpoles, both species of tadpoles, and control) on each of our response variables (nutrient concentration, algal biomass, and zooplankton community composition), we employed a Generalized Linear Mixed Model (GLMM). We chose to use GLMMs to account for repeated measures taken from the same mesocosms, and to incorporate random effects.

As our sample size was low, there was risk of over-fitting any GLMM that included more than 2 predictors. To avoid this, we ran a separate GLMM for each response variable at each sample time to eliminate "sample time" as a predictor. Additionally, as we took repeated measures from the same mesocosm, our sample times were not truly independent. To account for this, we calculated the proportional change from our baseline measurement before any tadpoles were added to the mesocosms - the "Initial" sample. We used proportional change from the Initial timepoint instead of proportional change from the previous timepoint to assess net changes overall, not just changes in 2-week increments. Finally, to account for any biases due to the positions of the mesocosms, we used row as a random effect in the models. We did not use the specific mesocosm as a random effect as, since we eliminated "sample time" as a predictor to avoid over fitting, there was only one sample from each mesocosm per model, which would make the mesocosm account for 100% of the data should it be used as a random effect, which would not be useful. We instead used the row the mesocosms were in to account for any bias potentially stemming from the mesocosms location in the experimental array, and there were more mesocosms per row than per column. We assumed a Gaussian distribution for continuous data with small sample size. Our GLMMs each included a continuous response variable (nitrogen concentration, phosphorus concentration, phytoplankton biomass, periphyton biomass, or zooplankton abundance) and one of four variations of the categorical, explanatory variable, *tank kind* (American toad tadpoles, Fowler's toad tadpoles, both species, or control). Additionally, each GLMM had a categorical random effect of mesocosm row (row 1, row 2, row 3). We also included a null model was the same except for the exclusion of *tank kind* as a variable. In instances where any data were missing in a time series, we omitted that series from analyses. This removed two, single-species Fowler's toad mesocosms for which an initial, total phosphorus concentration datum was missing, from GLMM analysis.

After running each model for each response variable at each sample time and noting any significant correlations, we used Akaike's Information criterion, AIC, (Symods and Moussalli, 2011) to determine the most informative model or models. As we only included one response variable in each model due to our small sample size and the risk of over-fitting the model, we only compared the AIC values for each model and its corresponding null model. If the data fit our model better, as indicated by a lower AIC value, this indicated that said response variable may correlate with one of our experimental groups. If the data fit the null model better, we determined that said response variable is unlikely to correlate with one of our experimental groups.

We performed all statistical procedures, including the generation of charts and graphs, using R vers. 3.3.3 (R-project, 2017).

Results

Our results indicated that the presence or absence of tadpoles had strong effects on total phosphorus concentration, periphyton biomass, and zooplankton diversity. The net effects exerted *A. americanus* tadpoles and *A. fowleri* tadpoles differed from one another, despite these species being closely related and thought to be ecologically comparable. In addition, the net effects exerted by tadpoles in single species mesocosms differed dramatically from those in mixed species mesocosms.

Nutrients

Over time, total nitrogen, including both dissolved and particulate nitrogen in the water, trended downwards regardless of tadpole presence or absence (**Figure 1.2A**). However, the presence of toad tadpoles did not significantly correlate with total nitrogen concentration in single species *A. americanus* (rho = 0.08, p = 0.059)*, A. fowleri* (rho = -0.07, p = 0.62)*,* or mixed species mesocosms (rho = -0.07, p = 0.60). There was no significant difference in the average slope of total nitrogen among any of the treatments when compared to control mesocosms (β = -212.9) (experimental group: single species *A. americanus* mesocosms: β = -212.9, df = 7, p = 0.52; *single species A. fowleri* mesocosms: β = -138.6, df = 4, p = 0.16; mixed species mesocosms: β = -161.4, df = 6, p = 0.14).

By contrast, total phosphorus concentrations, including both dissolved and particulate phosphorus in the water, was altered by tadpole presence. While there was no significant correlation between total phosphorus concentration and tadpole presence (t = -1.69, df = 78, p = 0.09), total phosphorus concentrations were more variable when tadpoles were absent (**Figure**

1.2B). There was more variation in control mesocosms than single species *A. americanus* mesocosms ($F = 7.32$, df = 43, $p < 0.01$; Levene's test of homogeneity). This increase in variation led to higher extremes of total phosphorus concentration when tadpoles were absent, with concentrations ranging from 10 to 45 ug/L when tadpoles were absent, but remaining between 10 and 30 ug/L when tadpoles were present (**Figure S1.4**).

When biases based on mesocosms position and starting conditions were taken into account using a GLMM, tadpole presence had no detectable effect on nitrogen concentration between the initial time point and sample 1 (treatment: residual deviance = 1.70, df = 14, AIC = 13.7; null: null deviance = 1.15, df = 19, AIC = 12.4), or the initial time point and same 2 (treatment: residual deviance = 0.17, df = 14, AIC = -24.3; null: null deviance = 0.19, df = 19, AIC = -28.4), as the null models had the lower AIC and there were no significant effects. However, nitrogen concentration was significantly higher in mesocosms with both species of tadpoles compared to control mesocosms (mean = 0.14, standard error = 0.14, t = 2.67, p = 0.018) between the initial time point and sample 3, and here the null model was rejected due to having a higher AIC (treatment: residual deviance = 0.09, df = 14, AIC = -36.0; null: null deviance = 0.17, df = 19, AIC = -31.2).

When running the GLMMs for phosphorus concentration, there was a small but significant effect of tadpole presence, with phosphorus concentration being marginally significantly lower in single species *A. americanus* mesocosms compared to control mesocosms (mean = -0.80, standard error = 0.37, $t = -2.14$, $p = 0.049$). While not being significant, mesocosms with both species of tadpoles (mean = -0.77, standard error = 0.39, $t = -1.93$, $p = 0.073$) and single species *A. fowleri* (mean = -0.90, standard error = 0.42, $t = -2.10$, $p = 0.054$)

were close to having significantly lower phosphorus concentration compared to control mesocosms. Similarly, the AIC values for the model and null model were very close, with the treatment model being only slightly lower, thus rejecting the null (treatment: residual deviance = 5.29, df = 14, AIC = 44.1; null: null deviance = 8.67, df = 19, AIC = 45.8).

Between the initial and sample 2 time points, there was no longer a detectable trend of tadpole presence or absence on phosphorus concentration (treatment: residual deviance = 6.29, df = 14, AIC = 47.6; null: null deviance = 7.49, df = 19, AIC = 47.6). Between the initial and sample 3 time points, the null was rejected (treatment: residual deviance = 5.18, df = 14, AIC = 43.7; null: null deviance = 9.25, df = 19, AIC = 46.9), but no significant trends were detected amongst the experimental groups.

Phytoplankton and Periphyton

Total phytoplankton biomass, as indicated by chl*a* concentrations, was negatively correlated with tadpole presence in single species *A. fowleri* mesocosms (rho = -0.37, *p* = 0.03), but this correlation was not significant in other mesocosm groups (*A. americanus*: rho = -0.18, *p* = 0.26; Both species: rho = -0.31, *p* = 0.051). However, the phytoplankton chla trends through time were notably different in control mesocosms. While chlorophyll *a* concentration fluctuated through time in single species *A. americanus, A. fowleri* and mixed species mesocosms, it steadily increased in the control mesocosms (**Figure 1.3A**).

Periphyton chlorophyll *a* concentration was negatively correlated with tadpole presence in *A. americanus* mesocosms (rho = -0.35, *p* = 0.02), *A. fowleri* mesocosms (rho = -0.36, *p* = 0.03), and mixed species mesocosms (rho = -0.37, *p* = 0.02). On average, *A. americanus* presence decreased periphyton chlorophyll *a* concentration by 49%, and *A. fowleri* presence decreased periphyton chlorophyll *a* concentration by 31% (**Figure 1.3B**). A two tailed t-test revealed that periphyton chlorophyll *a* concentration was lower in *A. americanus* mesocosms than control mesocosms at the initial time point (*t* = 2.5, df = 9, *p* = 0.034) and Sample 3 (*t* = 3.4, df = 9, *p* = 0.0078).

When biases based on mesocosms position and starting conditions were taken into account using a GLMM, tadpole presence had no detectable effect on periphyton biomass between the initial time point and sample 1 (treatment: residual deviance = 80.67, df = 15, AIC = 101.8; null: null deviance = 95.30, df = 20, AIC = 97.4), initial time point and sample 2 (treatment: residual deviance = 56.07, df = 15, AIC = 94.2; null: null deviance = 84.21, df = 20, AIC = 92.4), or initial time point and sample 3 (treatment: residual deviance = 38.73, df = 15, AIC = 86.4; null: null deviance = 48.75, df = 20, AIC = 84.8).

Similarly, GLMMs showed that tadpole presence had no detectable effect on phytoplankton biomass between the initial time point and sample 1 (treatment: residual deviance = 80.67, df = 15, AIC = 80.9; null: null deviance = 95.30, df = 20, AIC = 75.8) and initial time point and sample 2 (treatment: residual deviance = 126.71, df = 15, AIC = 111.3; null: null deviance = 182.45, df = 20, AIC = 110.31). Between the initial and sample 3 time points, the null was rejected (treatment: residual deviance = 38.7, df = 15, AIC = 105.3; null: null deviance = 48.75, df = 20, AIC = 107.17), but no significant trends were detected amongst the experimental groups.

Zooplankton

Zooplankton Shannon diversity positively correlated with tadpole presence in *A. americanus* mesocosms (rho = 0.32, *p* = 0.03) and *A. fowleri* mesocosms (rho = 0.32, *p* = 0.04) (**Figure 1.4**). In mesocosms with tadpoles, the zooplankton community was dominated by cladocerans, particularly *Daphnia* and *Bosmina* species, at Sample 2, but this spike in cladocerans did not occur in the control mesocosms (**Figure S1.1**).

In terms of zooplankton abundance, rather than diversity, GLMMs revealed no detectable trend between zooplankton abundance and tadpole presence or absence between the initial and sample 1 time point (treatment: residual deviance = 30.73, df = 9, AIC = 67.3; null: null deviance = 52.66, df = 14, AIC = 67.0), between the initial time point and sample 2 (treatment: residual deviance = 1324.4, df = 9, AIC = 123.7; null: null deviance = 1835.9, df = 14, AIC = 120.9), or between the initial time point and sample 3 (treatment: residual deviance = 14.98, df = 9, AIC = 56.54; null: null deviance = 22.55, df = 14, AIC = 52.6).

Due to the high number of zeroes in the dataset for zooplankton diversity, due to there at times being no zooplankton in the sample, or only one species in the sample, it was not possible to run a legitimate GLMM to test for changes in zooplankton diversity when tadpoles are present or absent.

Discussion

Toad tadpoles exert cascading changes in phosphorus concentration, algal biomass, and zooplankton community composition. In small freshwater ponds, these significant influences on their immediate surroundings indicate that tadpoles likely function as ecosystem engineers.

Tadpole guild structure was important in determining the net effects of tadpoles on small freshwater experimental ecosystems.

Net effects

As tadpoles excrete both nitrogen and phosphorus in their waste, we predicted that nutrient concentrations would increase when tadpoles were present. However, both total nitrogen and total phosphorus concentrations, which included both dissolved and particulate nutrients in the water column, showed a decreasing trend through time. This finding indicates that the tadpoles, even at a density of 1 tadpole/3.7L, did not add nutrients to the mesocosms faster than algal communities could uptake these nutrients. While there was often no significant difference in total nitrogen between experimental mesocosms (with tadpoles) and control mesocosms (without tadpoles), there was significantly more of an increase in nitrogen in mesocosms with both species of tadpoles at the sample 3 time point. When the data were assessed visually, little change could be noted between nitrogen concentrations in different mesocosms through time. More visual and statistical differences were present when considering phosphorus concentrations. Single species *A. americanus* mesocosms had significantly less fluctuation in total phosphorus levels compared with control mesocosms. While this trend was only significant with single species *A. americanus* mesocosms, the trend holds true in single species *A. fowleri* mesocosms and mixed species mesocosms, indicating that tadpoles may have a regulatory effect on total phosphorus levels, including phosphorus dissolved in the water and in particulate matter in the water column.

All mesocosms with tadpoles had reduced periphyton chlorophyll *a* concentration at Sample 2 and Sample 3 time points compared with control mesocosms, and periphyton biomass negatively correlated with tadpole presence in single species *A. americanus* and *A. fowleri* mesocosms. This is not surprising, as these tadpoles are thought to primarily consume periphyton, but this reduction in periphyton did result in some stark visual and structural differences between mesocosms containing tadpoles and those that did not, such as an increase in filamentous algae in control mesocosms (**Figure 1.4**).

A. americanus and *A. fowleri* tadpoles demonstrated some differences in the net effects they exerted on the environment. Only *A. fowleri* tadpole presence negatively correlated with phytoplankton biomass. Phytoplankton biomass demonstrated a different trend over time in all mesocosms with tadpoles compared with control mesocosms. Notably, in mesocosms containing tadpoles, the phytoplankton biomass fluctuated up and down over time, but in the control mesocosms phytoplankton biomass only increased through time. This result indicates that the tadpoles cause intermittent decreases in phytoplankton biomass, which do not seem to occur in the control mesocosms.

In single species *A. americanus* and *A. fowleri* mesocosms, phytoplankton biomass was positively correlated with periphyton biomass. We expected that phytoplankton biomass would negatively correlate with periphyton biomass, as these two communities compete for nutrients (Leibold and Wilbur, 1992; Hamilton et al., 2012; Costa and Vonesh, 2013; Arribas et al., 2014; Rowland et al., 2017). Thus, as the tadpoles decreased periphyton, more nutrients would be available for phytoplankton communities, leading to an increase in biomass. As tadpoles decreased periphyton dramatically in the mesocosms, it is possible that they relied more heavily on suspension feeding than we anticipated, leading to this positive correlation between periphyton and phytoplankton. Alternatively, as total nitrogen levels decreased over time in the mesocosms, there may simply have been an insufficient amount of nutrients remaining to see a dramatic rise in phytoplankton following a decrease in periphyton.

Zooplankton community composition was altered by tadpole presence. Zooplankton Shannon diversity was positively correlated with tadpole presence in single species *A. americanus* and *A. fowleri* mesocosms. The zooplankton community in single species *A. americanus, A. fowleri,* and mixed species mesocosms became dominated by cladocerans, particularly *Bosminidae* and *Daphnidae,* at Sample 2, but this spike in cladoceran abundance did not occur in the control mesocosms. The presence of tadpoles thus not only altered the diversity of the zooplankton community, but how those communities changed through time.

While a shift in primary productivity would influence zooplankton populations, there is also a possibility of direct predation on zooplankton by tadpoles (Hamilton et al., 2012). Tadpoles are widely considered to be herbivorous, but this idea has often been challenged (Altig and Johnston, 1989; Whiles et al., 2006; Altig et al., 2007; Schiesari et al., 2009; Caut et al., 2013; Arribas et al., 2015; Rowland et al., 2017; Montaña et al., 2019). It is possible that *A. fowleri* and *A. americanus* tadpoles selectively feed on certain zooplankton species, as was found with *Incillus valliceps* tadpoles (Jacobson et al., 2017), thus altering community composition through this selective predation.

While tadpole presence has sometimes been found to decrease zooplankton abundance (Seale, 1980), our results indicated no change in the abundance of zooplankton with tadpole presence or absence. Our results also counter studies that found no effect on the amphibian community (Buck et al., 2012) or that tadpole presence reduced zooplankton diversity and led to a copepod dominated community (Arribas et al., 2014). It is important to note, however, that these studies were conducted on different amphibian species in different ecosystems and communities, which likely plays an important role in determining the net effect of a species' presence.

Single species vs mixed species mesocosms

There were notable differences between the single species *A. americanus* and *A. fowleri* mesocosms and the mixed species mesocosms, despite the mixed species mesocosms containing 50 *A. americanus* and 50 *A. fowleri* mesocosms. In mixed species mesocosms, neither tadpole presence nor absence significantly correlated with phytoplankton biomass, periphyton biomass, or zooplankton diversity. As is especially apparent with zooplankton diversity, communities in mixed species mesocosms began to act markedly different from single species mesocosms between Sample 1 and Sample 2. This timing coincided with a suspected predation event in the mixed-species mesocosms, where the population of *A. fowleri* tadpoles was greatly reduced by the presence of *A. americanus* tadpoles. This speculated shift in the diet of the *A. americanus* tadpoles may explain the differences between the response of phytoplankton, periphyton, and zooplankton in the mixed-species mesocosms, as it would have altered food web dynamics and changed the cascading effects of tadpole presence.

Our results demonstrate not only the impact of tadpole presence or absence in shallow, sandy, oligotrophic pools, but also the importance of the amphibian guild composition in determining ecosystem interactions. Different amphibian species can have starkly different effects on ecosystems, varying in both the direction and magnitude (Kupferberg, 1997; Costa and Vonesh, 2013; Luhring, 2013; Arribas et al., 2014), and the addition of other amphibian species can change these interactions altogether (Arribas et al., 2014). The composition of this amphibian guild may dramatically alter the top-down effects exerted by the tadpoles, considering the amount of trophic plasticity tadpoles have been shown to possess (Seale, 1980; Altig et al., 2007; Caut et al., 2013; Arribas et al., 2015).

Caveats

Our findings highlight the need for more research to understand mechanisms underlying the ecological responses to toad tadpoles that we observed. We have shown clear trends that tadpoles presence and absence can affect ecosystems in mesocosms, but our results are indicative rather than conclusive due to our small sample size and high variance. Future studies should include more replicates of each experimental group or focus on only one species of tadpole to account for more replicates, within the same season. With these additional replicates, future studies could include statistical tests with more power and with more predictor variables, which could capture more of the variation within these complex ecosystems. Additionally, many factors were not captured in this single season study, such as how these net effects could change annually. The reduction in periphyton biomass caused by tadpoles resulted in structural and aesthetic changes in the mesocosms. These changes may have important implications for natural ponds inhabited by these tadpoles, which remain to be validated. While limited research has been done on the ecological effects of amphibian assemblages on abiotic and biotic responses in small freshwater ecosystems that contain many different families, additional research is warranted on more closely related communities, and the unique contributions of individual species, in order to determine if functional redundancy is occurring.

Conclusion

Our study demonstrates the importance of guild structure in determining the net effects of toad species assemblages on small freshwater experimental ecosystems. In sum, we found that toad tadpoles exert cascading changes in phosphorus concentration, algal biomass, and zooplankton community composition. This suggests that they have a strong impact on their immediate environment, likely acting as ecosystem engineers, in small freshwater pond ecosystems where they occur in nature.

Acknowledgements

We thank Bruce Pauli from Environment and Climate Change Canada (ECCC) for advice on tadpole rearing; N. Jreidini, A. Zerafa, V. Tawa, A. Botsko, M. Martini, K. Kwaku, A. Brimacombe, C. Berruezo I Llacuna, S. Ford, T. Ford, S. Thivierge, J. Purdy, A. Malet, L. Meehan J. Cuffaro, H. McCurdy-*Adams (Wildlife Preservation Canada), and A. Pereira for assistance with tadpole rearing and mesocosm maintenance; R. Wassersug for discussions; and Ontario Parks, the Ontario Ministry of Natural Resources and Forestry (OMNRF), ECCC, and the staff at Long Point Provincial Park, for logistical support. This research was funded by research grants to DMG from the Natural Science and Engineering Council (NSERC) of Canada and OMNRF, a doctoral scholarship from FRQNT to JF, and was carried out in accordance with McGill University Animal Use Protocol 4569 and permits issued by OMNRF and ECCC. We also acknowledge the traditional owners of the land on which we work; Long Point Provincial Park is situated on the traditional territories of the Mississauga and Haudenosaunee nations; and McGill University is located on the unceded territories of the Haudenosaunee and Anishinaabeg nations.*

Author Contributions

Jessica Ford: Conceptualization (lead); data curation (lead); formal analysis (lead); methodology (lead); writing –original draft (lead); writing –review and editing (lead).

Alexandrea Farquhar: data curation (supporting); formal analysis (supporting)

Alison M. Derry: methodology (supporting); writing –review and editing (supporting)

David M. Green: Supervision (lead); writing –review and editing (supporting).

Funding Information

Funding was provided by the following sources: Fonds de Recherche du Québec—Nature et Technologies, Ontario Ministry of Natural Resources and Forestry, Natural Sciences and Engineering Research Council of Canada.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

Data Availability Statement

All data is available upon request.

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Figures

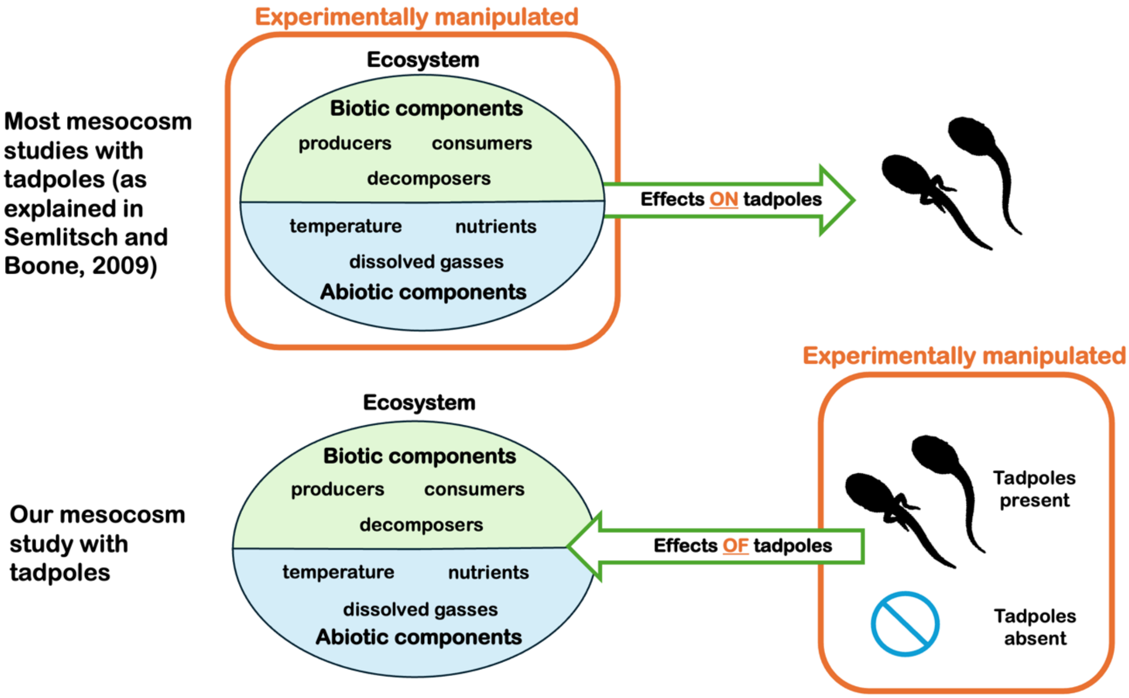

Figure 1.1: Demonstrating the difference between our mesocosm study to how most mesocosm studies with tadpoles are conducted.

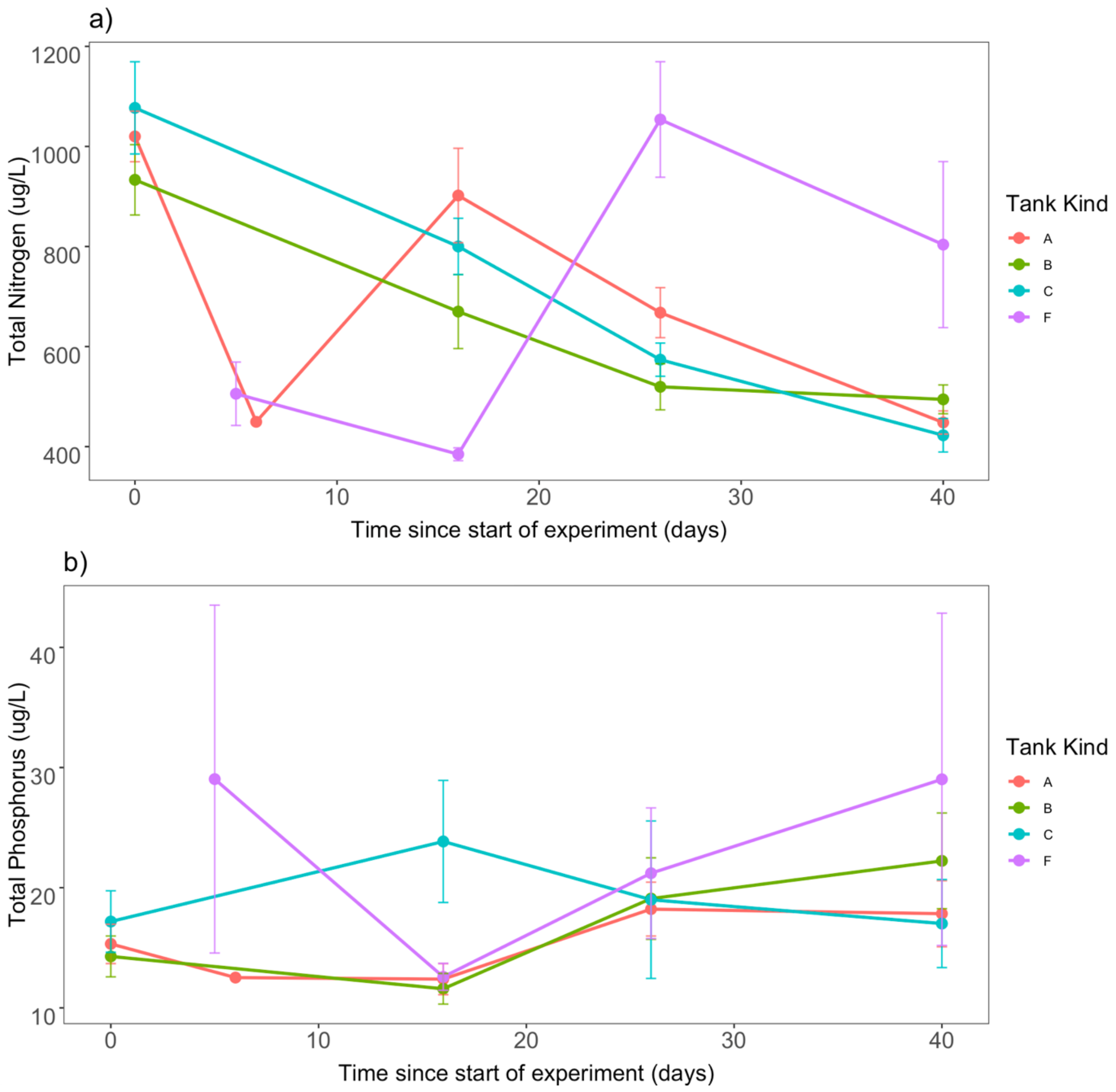

Figure 1.2: Total nitrogen and total phosphorus concentrations in single species *A. americanus* (A), single species *A. fowleri* (F), mixed species (B), and control mesocosms that contain no tadpoles (C). As Fowler's toads bred later in the season, they were only added to the mesocosms at experimental day 5. The increased variation in single species *A. fowleri* mesocosms is likely attributable to increased suspended solids in those samples

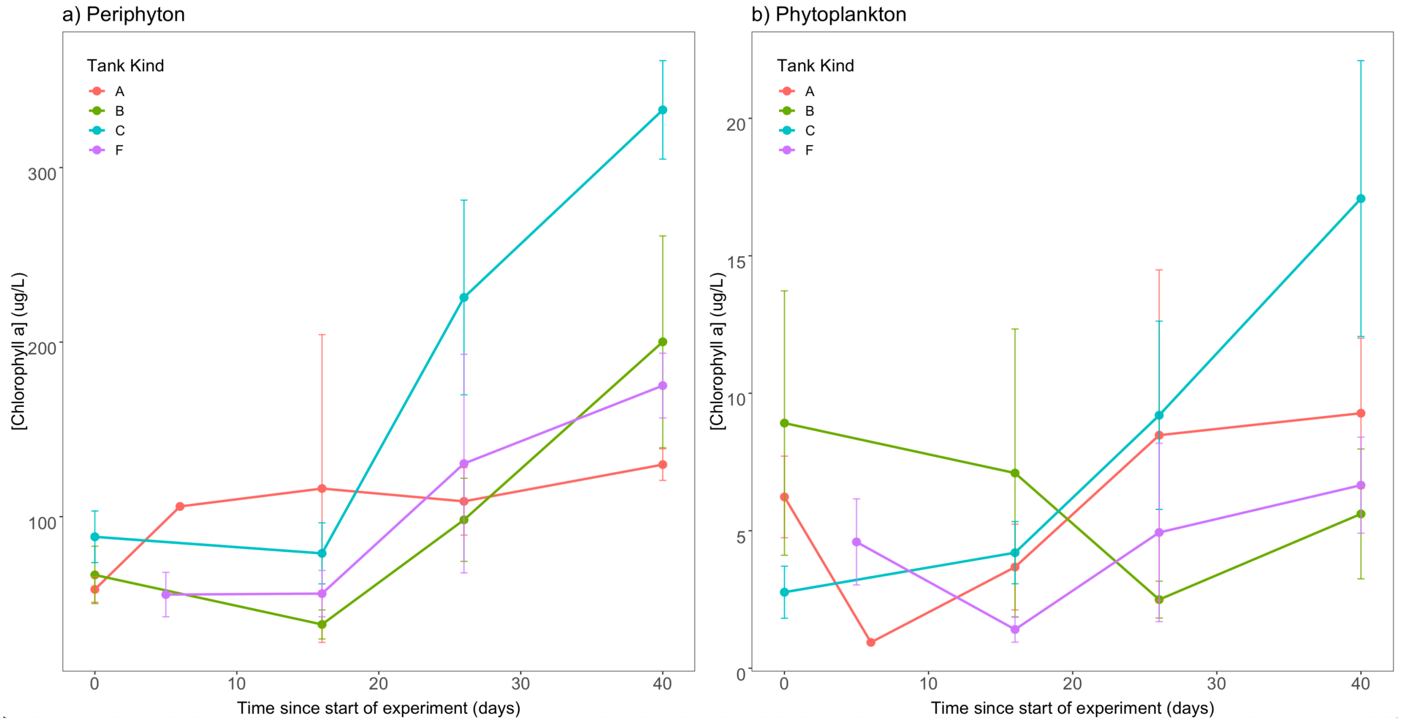

Figure 1.3: Periphyton and phytoplankton chlorophyll *a* concentration in single species *A. americanus* (A), single species *A. fowleri* (F), mixed species (B), and control mesocosms that contain no tadpoles (C). As Fowler's toads bred later in the season, they were only added to the mesocosms at experimental day 5.

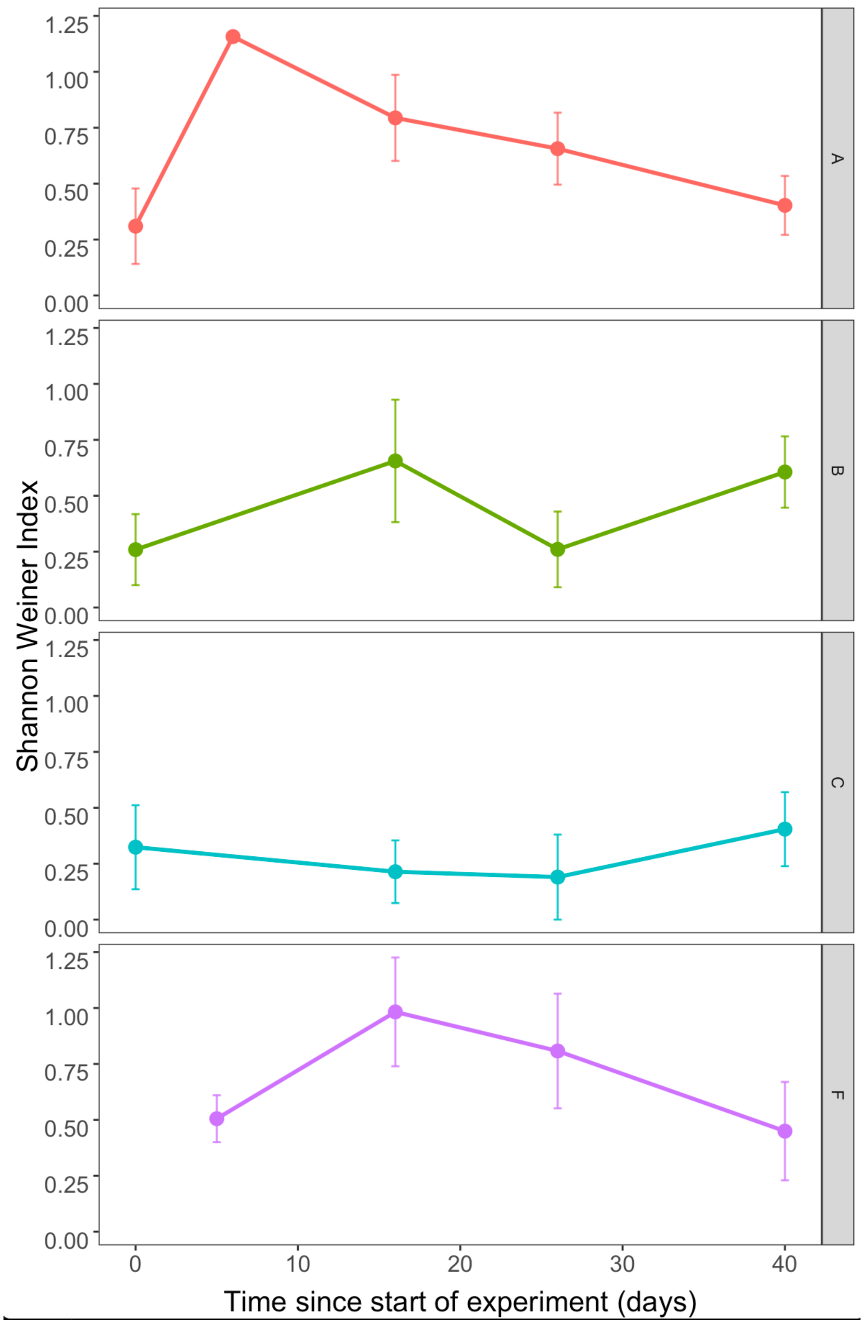

Figure 1.4: Zooplankton Shannon diversity in single species *A. americanus* (A), single species *A. fowleri* (F), mixed species (B), and control mesocosms that contain no tadpoles (C). As Fowler's toads bred later in the season, they were only added to the mesocosms at experimental day 5.

Figure 1.5: Two mesocosms that had been established for the same amount of time and established in the same way, but only the "with tadpoles" mesocosm was stocked with 100 *A. fowleri* tadpoles. This image shows an extreme case in the stuctural and aestheitc differences when tadpoles are present or absent. In the "with tadpoles" mesocosm there is only a thin film of periphyton presence, whereas in the "no tadpoles" mesocosm is full of filamentous periphyton and algal mats on the waters surface.

Supplemental

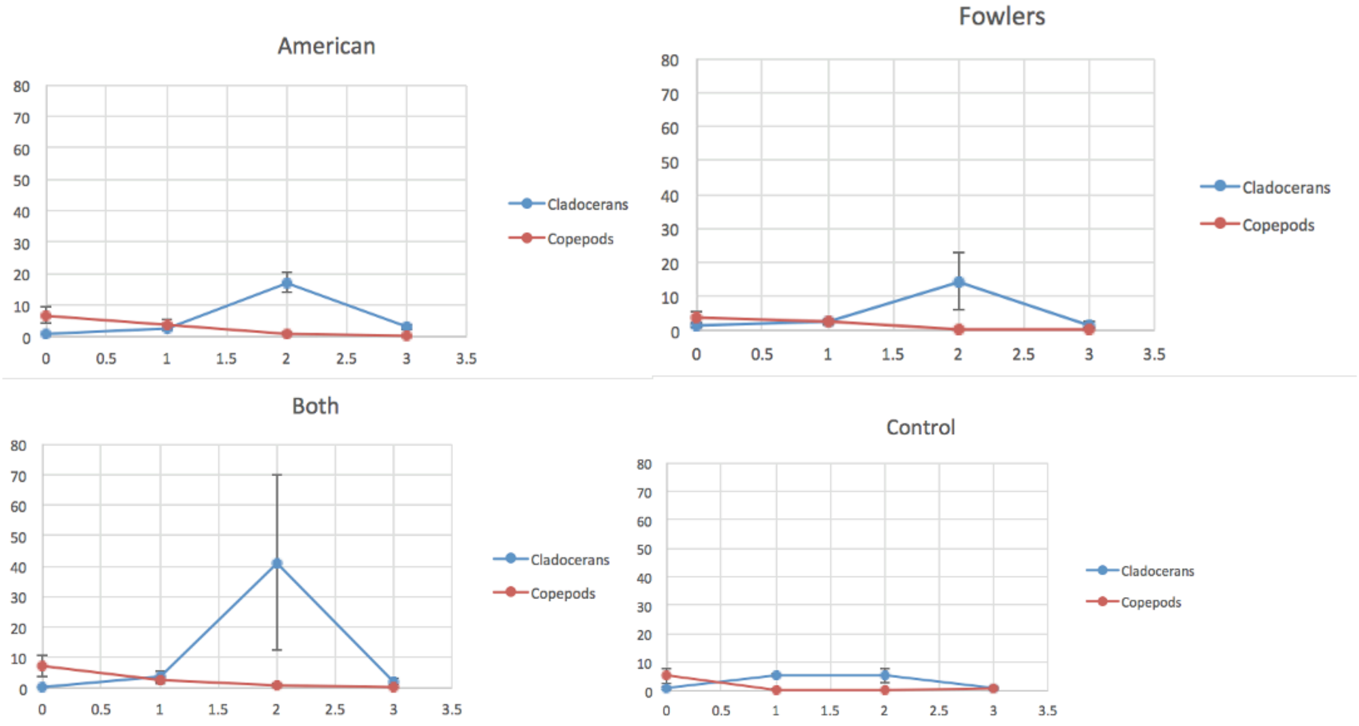

Figure S1.1: The abundance of cladoceran and copepod individuals in single species *A. americanus* (A), single species *A. fowleri* (F), mixed species (B), and control mesocosms that contain no tadpoles (C).

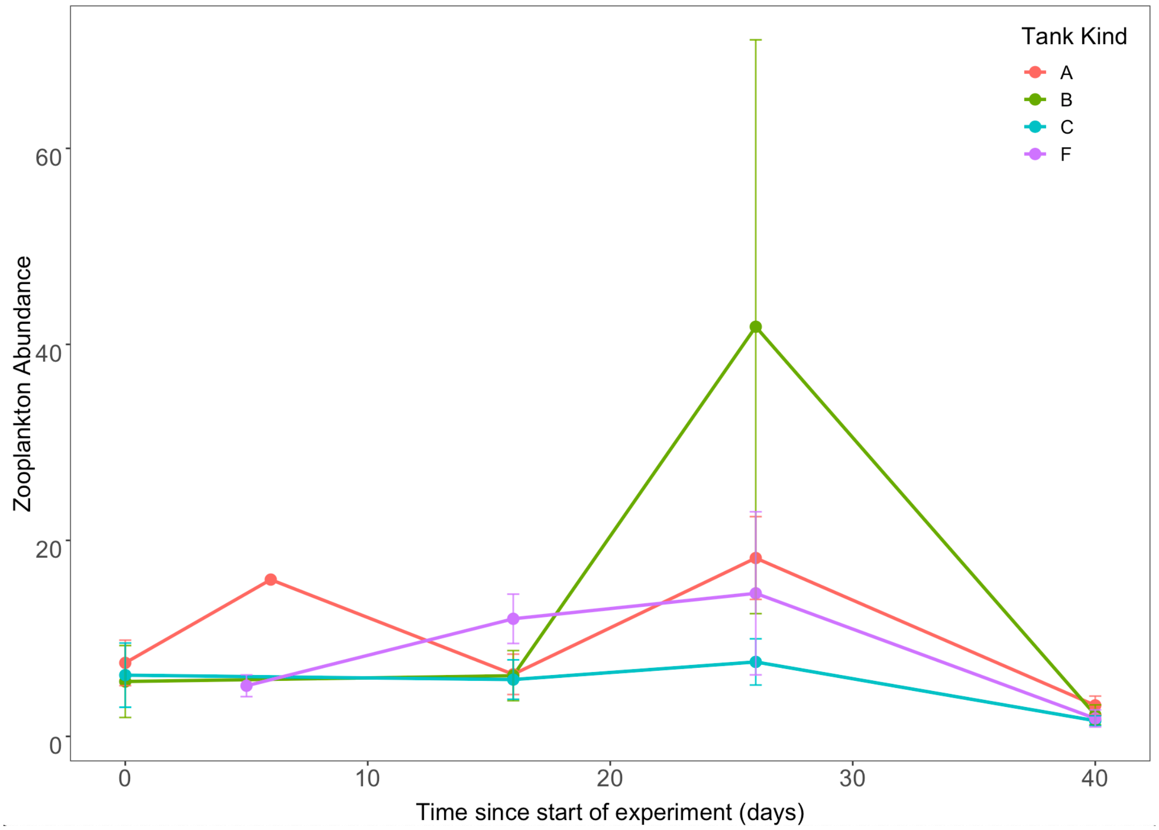

Figure S1.2: The abundance of zooplankton individuals in single species *A. americanus* (A), single species *A. fowleri* (F), mixed species (B), and control mesocosms that contain no tadpoles (C). Cladoceran communities in all mesocosms containing tadpoles were dominated by Daphmiidae and Bosmina species.

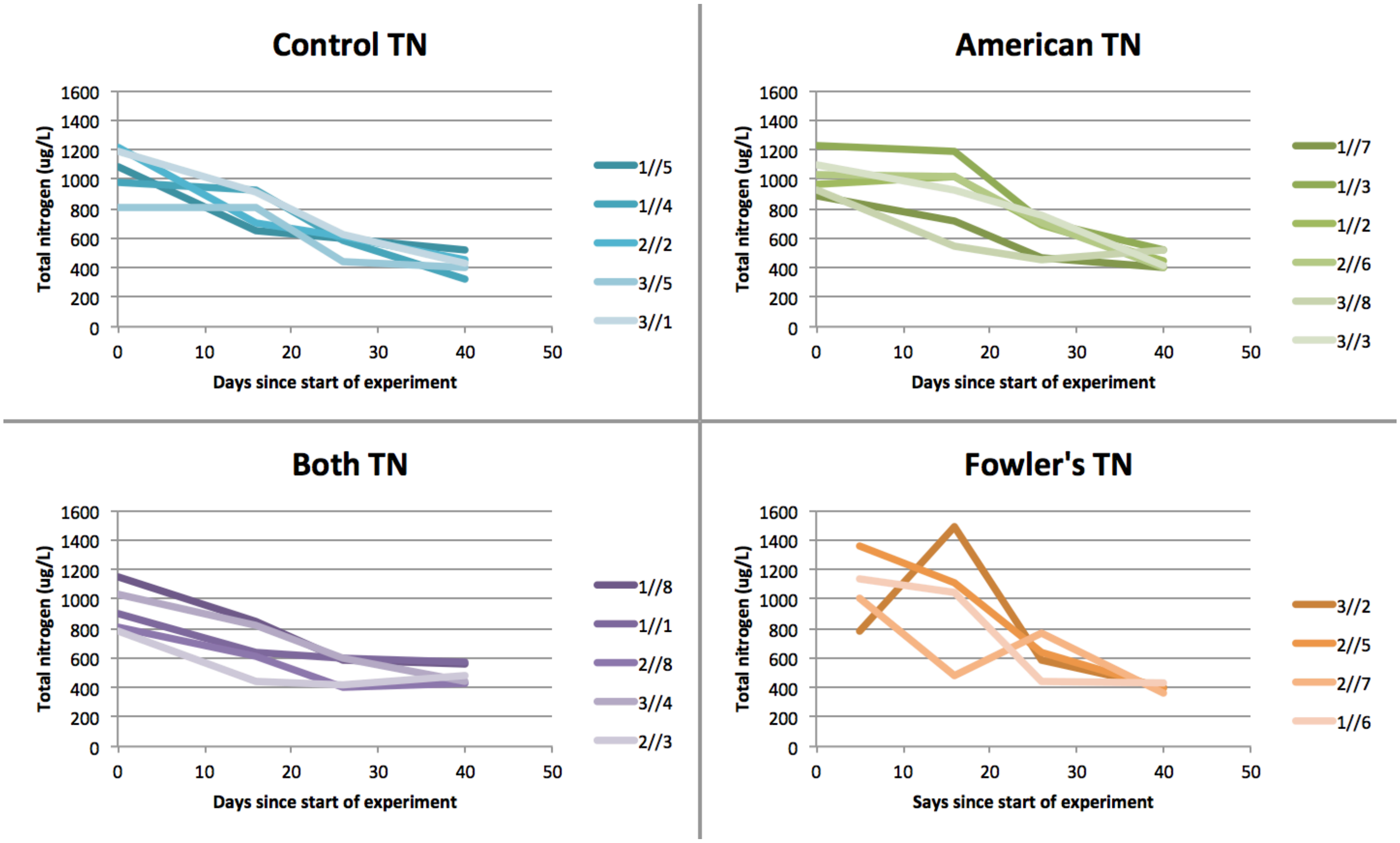

Figure S1.3: The concentration of total nitrogen in each mesocosm in each treatment group, showing the variation between mesocosms.

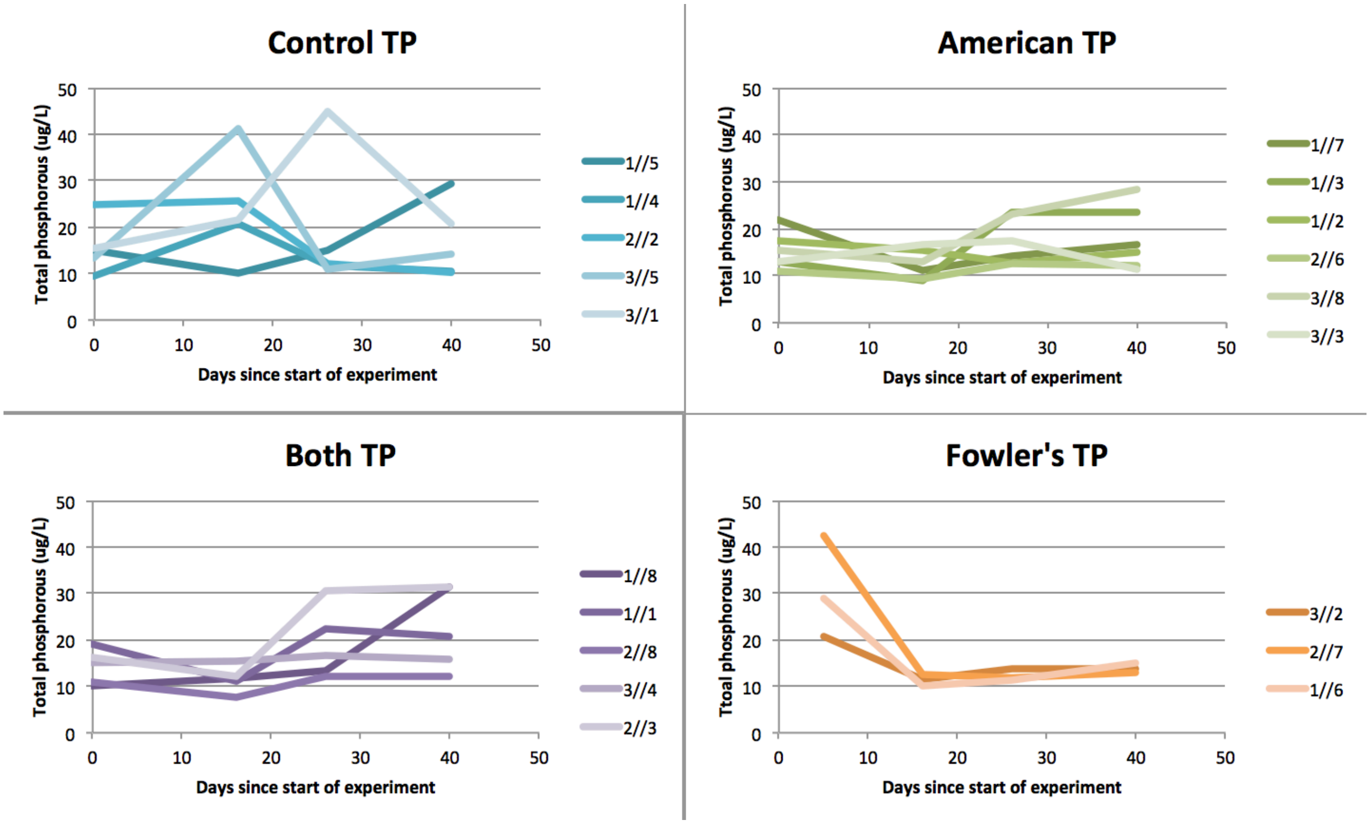

Figure S1.4: The concentration of total phosphorus in each mesocosm in each treatment group, showing the variation between mesocosms. Two single species Fowler's toad mesocosms were omitted from this graph as the initial time point sample went missing, and thus the time series data was incomplete.

Connecting Statement Between Chapters 1 and 2

Tadpoles have profound impacts on their aquatic environment. In Chapter 1, I demonstrate how toad tadpoles in outdoor mesocosms impact the concentration of nitrogen and phosphorous, the biomass of periphyton and phytoplankton, and the diversity of zooplankton. American and Fowler's toad tadpoles reduce the variation of total phosphorus through time, and the presence of toad tadpoles increased the biodiversity of zooplankton. Toad tadpoles had the most noticeable effect on periphyton and phytoplankton biomass. In Chapter 1 I showed that toad tadpoles prevent a constant rise in phytoplankton biomass, and Fowler's toads reduced periphyton biomass by 30%, while American toads reduce periphyton biomass by 50%. Phytoplankton and periphyton are not a monoculture, however, and contain plentiful populations of algal species with multiple ecological roles and functions, competing with one another for resources. In Chapter 2, I address this complexity by assessing how toad tadpoles impact algal communities at the broader Class level, and then zooming in to the species level. Chapter 2 allows us to see not only how much algae is reduced by tadpoles presence, but what tadpole grazing does to algal communities, which species are impacted and which are not, and how the algal community responds to tadpole grazers of different species and at different densities.

Additionally, Chapter 1 assesses the impacts of toad tadpoles until they reach metamorphosis, but tadpoles may leave a legacy effect after metamorphosis. To test for a legacy effect, in Chapter 2 I include an additional sample time post-metamorphosis, to determine if algal communities return to their original state after tadpoles leave the aquatic environment. This study has implications for understanding the importance of grazers in aquatic ecosystems, and determining how algal communities respond to different grazing pressures.

Chapter 2 | What tadpoles eat and what they don't; the impact of a larval anuran grazer on algal communities

Prepared for Oecologia

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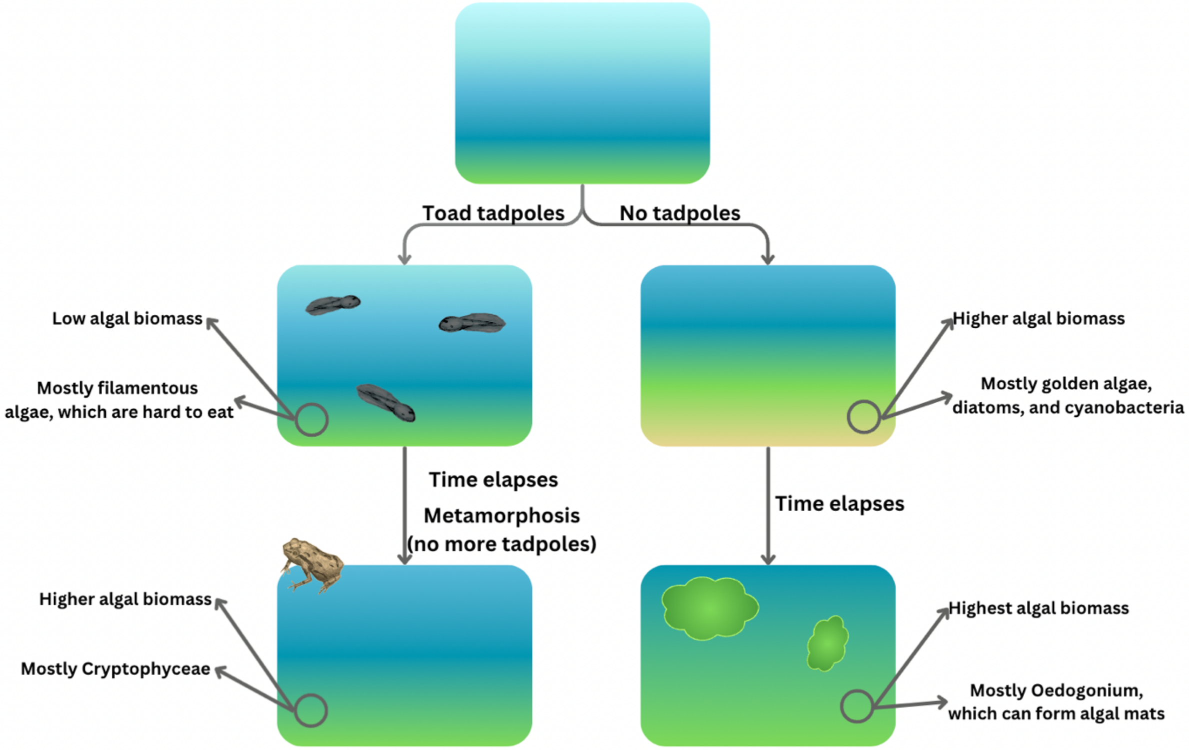
Abstract

Aquatic algal communities can be heavily influenced by the presence of grazers. Grazers can lead to decreases in overall algal biomass, as well as increases in particular algal species. Tadpoles tend to be the main form of grazer biomass in pond systems and may act as keystone herbivores, having a large effect on algal populations in ponds. While tadpoles are expected to influence the biomass of pond algae, little is known about their influence on algal community structure. We assess the influence of multi and single species tadpole grazing communities on algal communities in mesocosms, to determine how this basal plant community may respond to grazing. We used toad tadpoles as they are thought to be generalist grazers, consuming both periphyton and phytoplankton. Different species of toad tadpoles, the density and composition of the tadpole community, the presence or absence of toad tadpoles, and the prior presence of toad tadpoles before metamorphosis, all resulted in different periphyton and phytoplankton communities. Tadpole grazer community composition shaped the algal community, even after the tadpoles underwent metamorphosis and left the mesocosms, demonstrating the importance of tadpoles as keystone herbivores and ecosystem engineers.

Graphical Abstract



Algal communities and biomass over time in mesocosms with and without tadpole grazers

Introduction

Small freshwater ponds are common environmental features that, by definition, lack thermal stratification and large fluctuations in water level, and do not have the large mass of water characteristic of lake ecosystems (Paczuska et al, 2002). Despite being relatively simple water bodies compared to lakes, freshwater ponds act as reservoirs of algal biodiversity, and collectively represent large amounts of algal diversity and biomass on a landscape scale (Paczuska and Paczuski, 2015). Algal populations in these small ponds are impacted primarily by nutrient availability and the presence of grazers, which can, also, impact nutrient availability (Friedl, 1974; Grimm, 1988) and reduce total algal biomass (McCormick and Stevenson, 1991). However, by grazing down competing populations, grazer presence can also result in the increase of certain algal populations, even when total algal biomass is lowered (McCormick and Stevenson, 1991).

In freshwater pond systems, tadpole grazers often constitute the main form of vertebrate biomass, especially when fish are not present (Seale, 1980). Tadpoles play a crucial role in regulating the aquatic communities that surround them, even acting as ecosystem engineers (Flecker et al, 1999, Altig et al, 2007; Colón-Gaud et al, 2010; Wood and Richardson 2010). While the effects of many environmental factors on amphibian larvae have been extensively studied, comparatively little is known about how amphibian larvae affect their environment (Whiles et al., 2006; Arribas et al., 2015). From the studies that have been conducted, amphibian larvae may influence periphyton and phytoplankton abundance (Whiles et al., 2006; Connelly et al., 2008, Wood and Richardson, 2010; Buck et al. 2012), Tadpoles can have direct effects on algal communities, as they feed on both periphyton and phytoplankton (Seale, 1980; Arribas et al, 2014). Tadpoles preferentially eat periphyton while rasping or grazing, but will also consume phytoplankton while suspension feeding (Hamilton et al, 2012). The net effect of tadpole grazing may differ depending on species composition, and tadpole presence can either increase or decrease (Kupferberg, 1997; Flecker et al, 1999; Wood and Richardson 2010) primary productivity. For instance, the presence of tadpoles of the Foothill Yellow-legged frog (*Rana boylii*) was associated with increased primary productivity whereas the presence of tadpoles of the Pacific tree frog (*Hyla regilla*) was associated with decreased primary productivity in a California river (Kupferberg, 1997).

Direct consumption, a top-down effect, is not the only way in which tadpoles may influence primary producers. Indirect, bottom-up effects of tadpole presence could also alter the environment to make them more suitable for primary producers. For instance, the presence of European western spadefoot (*Pelobates cultripes*) tadpoles was found to decrease macrophyte biomass, likely due to an increase in water turbidity (Arribas et al, 2014). However, when *P. cultripes* tadpoles, which are strong and voracious competitors, were removed from the system, plant biomass increased in the presence of the remaining tadpole community, potentially due to increased nutrient concentration in the system (Arribas et al, 2014). Tadpoles boost available nitrogen, an important and often limiting nutrient for plants in aquatic systems, by secreting ammonia as a waste product (Seale, 1980), which may contribute to an increase in phytoplankton and periphyton biomass (Seale, 1980, Rowland et al, 2017). Nutrients secreted and excreted by tadpoles as waste may influence detritivore communities in addition to primary producers (Iwai and Kagaya, 2007), and the feeding activities and movement of tadpoles may also help disperse the nutrients within small pond systems (Iwai and Kagaya, 2007). Furthermore, aside from direct predation and indirect nutrient stocking and shifts in turbidity, tadpoles may also shape primary producer communities through influencing competition between phytoplankton and periphyton (Leibold and Wilbur, 1992; Hamilton et al, 2012; Costa and Vonesh, 2013; Arribas et al, 2014; Rowland et al, 2017

Additionally, organisms that undergo metamorphosis, such as amphibians, may leave an impact on the ponds they once inhabited even after they have left. These legacy effects in ponds that once held amphibians include continued changes in algal biomass (Rowland et al, 2017). Algal biomass can remain lower in ponds that once held amphibians, compared to ponds that never held amphibians, even weeks after salamander larvae and tadpoles have metamorphosed (Blaustein et al, 1996; Rowland et al, 2017). These legacy effects of amphibians would result in different habitats for late summer occupants in temperate environments, which can alter the succession and colonization of other species (Connel and Slayter, 1997; Rowland et al, 2017).

The complex relationship between tadpole grazers and primary producers makes this association an excellent study system to assess the impact of consumers on primary producer communities. This is especially true in relatively simple aquatic systems with few species and low nutrients, where the outcome of tadpole presence can be discerned from other environmental factors. In Long Point, Ontario, Fowler's toads (*Anaxyrus fowleri*) breed in shallow, sandy, low nutrient ponds, where they tend to be the only vertebrate species present (Wood and Richardson, 2010). The closely related American toad (*Anaxyrus americanus*) is a generalist species that will occasionally also breed in these ponds, sometimes at the same time as *Anaxyrus fowleri* (Green, 1982). Depending on how large the clutch of tadpoles is, how many clutches there are, and how many tadpoles survive, the density of toad tadpoles within these individual ponds can also vary.

We mimicked such natural ponds in experimental mesocosms to assess how the presence, species, and density of toad tadpoles impact the biomass and community composition of phytoplankton and periphyton communities. If toad tadpoles are indiscriminate grazers, then algal communities should respond similarly to tadpole grazing regardless of species, given these species have similar grazing styles. As well, if tadpoles leave legacy effects in their ecosystems, then algal communities should remain different in mesocosms which had tadpoles, versus mesocosms which did not, even after metamorphosis.

Methods

To assess the result of tadpole grazing on algal communities and biomass, we raised American Toad and Fowler's Toad tadpoles in 100 US gallon Rubbermaid© structural foam cattle watering tanks measuring 63.50 cm L × 78.74 cm W × 134.6 cm H as mesocosms in Long Point Provincial Park in Long Point, Ontario. All mesocosms were covered with a 70% shade cloth to prevent insects and other animals from entering. We followed rearing methods outlined in Ford and Green (2021) to rear the toad tadpoles. We used 20 mesocosms to house tadpoles: four with 50 Fowler's toad tadpoles, four with 100 Fowler's toad tadpoles, five with 50 American toad tadpoles, four with 100 American toad tadpoles, and three with 50 tadpoles of each species. Four mesocosms were used as controls; these were established for the same amount of time but never received any tadpoles. Four remaining mesocosms were used to house tadpoles before they were added to experimental mesocosms.

We collected two clutches of American toad eggs from natural ponds on May 4th and May 16th, 2021 and one clutch of Fowler's toad eggs was collected from an amplectic pair on May 19th,

2021. One clutch of Fowler's toad eggs was located as Fowler's toads are endangered in Canada, and did not have large breeding choruses in 2021.

Mesocosms were initially inoculated with one litre of local pond water on May 8th, 2021, five days after being filled with chlorinated, mechanically filtered, and ultraviolet irradiated tap water, in order to give the chlorine time to evaporate and the sand substrate time to settle. This inoculate, as well as any spores in the local sand substrate, were the sources from which the algal communities could populate the mesocosms. Care was taken to evenly mix and distribute the sand substrate and inoculate so that all mesocosms would get the same composition. The inoculate was also poured through the shade cloth covering the mesocosms to remove any large debris. The inoculate consisted primarily of diatoms (Bacillariophyceae), golden algae (Chrysophyceae), green algae (Chlorophyceae), and cyanobacteria (Cyanophyceae). Algal communities were left to establish for 3 weeks. On May 29th, 2021, tadpoles at Gosner stage 26 (Gosner, 1960) were added to the experimental mesocosms.

Algal samples were collected at 3 time points: right before the tadpoles were added to the mesocosms (Initial), after the tadpoles had been feeding on the algae for about a month (Sample 2), and about 2 weeks after metamorphosis (Post-Tads). While control mesocosms never had any tadpoles, samples were collected from them at these same time points.

Phytoplankton and periphyton communities were collected separately. Phytoplankton samples were collected by scooping 70 mL of water from just below the surface in the mesocosms between 9:00 am and 10:00 am. Periphyton samples were collected by scraping it off the side of the mesocosm. Periphyton was always collected from the Northern wall of the mesocosm (the one facing South). An elbow pipe was pressed against the side of the mesocosm, and a modified toothbrush was inserted in the pipe and used to scrape periphyton off the side of the mesocosm. A 50 mL sample of this scraped periphyton in water was collected. All algal samples were preserved with dilute Lugol's solution and kept in a cardboard box at room temperature to later be analyzed for community composition using inverted light microscopy. Species identification was completed at the Interuniversity Research Group in Limnology (GRIL) at the Université du Québec à Montréal. Due to the cost of this analysis, we only analyzed 3 replicates from each experimental group at each sample time point.

We calculated Shannon diversity, species evenness, species richness, abundance (cell/L), and biomass (ug/L) at the Class level for each group and sample time point for the phytoplankton and periphyton communities. To assess if tadpole presence in our experimental groups significantly changed the periphyton and phytoplankton communities, both when tadpoles were present and after they left, we conducted a Generalized Linear Mixed Model (GLMM) statistical analysis. To account for biases due to different Initial conditions in the mesocosms, we used the change in either Shannon diversity, evenness, or species richness from the Initial time point instead of the absolute value of our response variables. We also accounted for potential biases due to where the mesocosms were located in our experimental grid by using the row of the mesocosms within the experimental grid as a random effect. We ran separate GLMMs for periphyton and phytoplankton data, as these were different datasets, and for the Sample 2 and Post Tads time points as these encompassed rather different conditions - Sample 2 had actively feeding tadpoles, and Post Tads samples were collected after tadpoles had left the mesocosms. Analyzing the Post Tads time point separately also allowed us to identify evidence of legacy effects of tadpoles after they had metamorphosed and left the mesocosms. Due to our small sample sizes, doing separate GLMMs reduces the risk of overfitting our model. As such, each GLMM included a response variable of either the change in Shannon diversity, evenness, or species richness, and an explanatory variable of treatment group (100 American toad tadpoles, 100 Fowler's toad tadpoles, both species of tadpoles, 50 American toad tadpoles, 50 Fowler's toad tadpoles) compared to control mesocosms, which had no tadpoles, as the reference group.

To narrow down which species in our dataset of over 80 periphyton and phytoplankton species were most associated with each experimental group, we conducted an Indicator Species Analysis (ISA) at the species level using abundance (cell/L) and biomass (μg/L) to determine which phytoplankton or periphyton species were characteristic of, and thus most associated with, each group at each sample time point. The ISA and the Indicator Value (indVal) was used to determine which species had strong associations with treatment groups using the relative abundance of a species and a randomization test to evaluate the probability of association (Severna and Sykes, 2020). The IndVal metric combines the specificity (how often that species occurs in a specific experimental group) and fidelity (how likely it is that a species will be found in all replicates of an experimental group) (Severna and Sykes, 2020) of different algal species in each experimental condition and the control. This ISA thus allowed us to determine which species changed the most, and which were most associated/characteristic of specific experimental groups, eliminating noise from abundant but common species in all groups. Species identified by the ISA were placed into two categories: grazer resistant or non-grazer resistant. Species were considered to have grazer resistance if they were filamentous or gelatinous (McCormick and Stevenson, 1991) to determine if the presence of tadpoles (the grazers) altered the grazer resistance of the algal communities.

All statistics were performed in R v. 4.1.1 (R Core Team, 2021).

Results

The presence tadpole communities was demonstrably associated with changes in the algal communities in comparison to the control communities, but tadpole presence did not have one uniform effect on all algal communities.

Tadpole presence or absence

A difference in algal abundance was clearly visible between mesocosms with tadpoles compared to those without (Figure 2.1). This was especially notable at Sample 2, when the toad tadpoles had been in the mesocosms for about four weeks. Control mesocosms containing no tadpoles had the highest phytoplankton cell abundance (mean cell count = 1896.3 cell/L, standard deviation = 1589.4) followed by mesocosms containing 50 toad tadpoles (50 American toad tadpoles: mean cell count = 779.3 cell/L, standard deviation = 99.6; 50 Fowler's toad tadpoles: mean cell count = 865.0 cell/L, standard deviation = 244.6), while mesocosms containing 100 toad tadpoles had the lowest algal cell abundance (100 American toad tadpoles: mean cell count = 288.3 cell/L, standard deviation = 175.3; 100 Fowler's toad tadpoles: mean cell count = 365.3 cell/L, standard deviation = 76.9). The most abundant algal Class was Chrysophyceae, which demonstrated the same trends in cell abundance (**Figure 2.2**), with control mesocosms having the highest algal cell abundance (mean cell count = 1349.0 cell/L, standard deviation = 1873.7), mesocosms containing 50 tadpoles having the second highest cell abundance (50 American toad tadpoles: mean cell count = 642.7 cell/L, standard deviation = 60.0; 50 Fowler's toad tadpoles: mean cell count = 360.7 cell/L, standard deviation = 570.2), and mesocosms containing 100 toad tadpoles having the lowest algal cell abundance (100 American toad tadpoles: mean cell count = 67.0 cell/L, standard deviation = 61.0; 100 Fowler's toad tadpoles: mean cell count = 100.3 cell/L, standard deviation = 63.4).

Algal diversity indices (Shannon Diversity, evenness, and richness) changed differently over time in our different experimental groups, as confirmed by a GLMM. Phytoplankton diversity increased significantly more from Initial conditions to Sample 2 conditions in mesocosms containing 50 Fowler's toad tadpoles (GLMM: mean = 1.43 ± 0.42, *t* = 3.35, *p* = 0.0065) compared to control mesocosms. Phytoplankton richness, however, decreased significantly more from Initial conditions to Post Tads conditions in mesocosms where both species of tadpoles (GLMM: mean = -11.33 ± 2.20, *t* = -2.29, *p* = 0.0021), 100 Fowler's toad tadpoles (GLMM: mean = -10.95 ± 4.65, *t* = -2.35, *p* = 0.038), or 50 American tadpoles (GLMM: mean = -20.26 ± 4.97, *t* = -4.07, *p* = 0.0018) were present compared to control mesocosms (**Figure 2.3**).

Even more significant differences were apparent regarding periphyton species. Periphyton diversity increased significantly more from Initial conditions to Post Tads conditions in mesocosms containing both species of tadpoles (GLMM: mean = 0.75 ± 0.29, *t* = 2.54, *p* = 0.027) and 100 Fowler's toad tadpoles (GLMM: mean = 1.18 ± 0.036, *t* = 3.62, *p* = 0.004) compared to control mesocosms. Furthermore, periphyton evenness increased significantly more from Initial conditions to Post Tads conditions in mesocosms containing both species of tadpoles (GLMM: mean = 0.22 ± 0.093, *t* = 2.37, *p* = 0.037) and 100 Fowler's toad tadpoles (GLMM: mean = 0.30 ± 0.10, *t* = 2.86, *p* = 0.015) compared to control mesocosms. Periphyton richness also increased significantly more from Initial conditions to Post Tads conditions in mesocosms containing 50 American toad tadpoles (GLMM: mean = 10.50 ± 6.52, *t* = -3.14, *p* = 0.0097) compared to control mesocosms. Conversely, periphyton richness decreased significantly more from Initial conditions to Sample 2 conditions in mesocosms containing 50 American toad tadpoles (GLMM: mean = -20.47 ± 6.52, *t* = -3.14, *p* = 0.0097) compared to control mesocosms (**Figure 2.4**).

At the taxonomic Class level, community composition varied between mesocosms that contained tadpoles, and mesocosms that did not. At Sample 2, all mesocosms with toad tadpoles were dominated by periphyton Chlorophyceae, where in mesocosms with 50 American toad tadpoles 72% of the algal community was Chlorophyceae, in mesocosms with 100 American toad tadpoles it was 63%, in mesocosms with both species of tadpoles it was 67%, in mesocosms with 50 Fowler's toad tadpoles it was 66%, and in mesocosms with 100 Fowler's toad tadpoles 68% of the algal community was Chlorophyceae. In the Control mesocosms, however, only 37% of the algal community was Chlorophyceae, and the community was dominated by Trebouxiophyceae (47% of the algal community). After tadpoles had metamorphosed and left the mesocosms, all mesocosms, even those that never contained tadpoles (control), were dominated by Chlorophyceae. Chlorophyceae was also one of the only periphyton Classes to increase in abundance between Initial and Sample 2 in the mesocosms along with Zygnematophyceae, while other Classes (Trebouxiophyceae, Cyanophyceae, Bacillariophyceae, Cryptophyceae, and Ulvophyceae) abundance decreased between Initial and Sample 2 in the mesocosms containing tadpoles.

At the species level, as supported by the Indicator Species Analysis, high *Oedogonium inconspicuum* (Chlorophyceae) abundance was characteristic of Control mesocosms, which never contained any tadpoles, in the late summer (post-tads) time point (ISA: *p*=0.0054, indVal=0.926). Similarly, high *Oedogonium inconspicuum* biomass was also characteristic of control mesocosms during the post-tads time point (ISA: *p*=0.0198, indVal=0.753) (**Table 2.1 and Table 2.2**). In all mesocosms that previously had tadpoles, there was no such association with *O. inconspicuum,* meaning that high *O. inconspicuum* abundance and biomass was not characteristic of mesocosms that once had tadpoles, even after metamorphosis. *Oedogonium inconspicuum* biomass was extremely low during the Initial and Sample 2 time points, only blooming in the post-tads mesocosms, and to a much greater extent in control mesocosms (**Figure 2.5**).

Moreover, at Sample 2, *Fragilaria crotonensis* (Bacillariophycae) and *Chromulina microplankton* (Crytophyta) were significantly associated with Control mesocosms (p=0.0074, indVal=0.888; and p=0.0029, indVal=0.773, respectively) (**Table 2.3 and Table 2.4**). Also at Sample 2, *Stigeoclonium polymorphum* (Chlorophyceae) abundance was significantly associated with single species mesocosms containing either 100 Fowler's or 100 American toad tadpoles (p=0.027, indVal=0.755). *Protoderma viride* (Ulvophyceae) abundance was significantly associated with single species mesocosms containing either 100 Fowler's or 50 American toad tadpoles (p=0.0228, indVal=0.735) (**Table 2.3 and Table 2.4**).

When species identified by the ISA were separated by those that had suspected grazer resistance and those that did not and were plotted in a pie chart, it was apparent that mesocosms that never held tadpole species contained primarily non-grazer resistant periphyton, whereas mesocosms containing any density and composition of tadpole species contained primarily grazer resistant periphyton (**Figure 2.6**).

Between species differences

Our evidence showed that American toad tadpoles and Fowler's toad tadpoles did not have the same impact on algal communities. In mesocosms with only Fowler's toad tadpoles, regardless of tadpole abundance, the abundance of planktonic diatoms (Bacillariophyceae) decreased between Sample 2 and Post Tads time points. Bacillariophyceae abundance between Sample 2 and Post Tads decreased by 9.3 ± 11.6 algal cells on average in mesocosms with 50 Fowler's toad tadpoles, and 0.3 ± 4.1 algal cells on average in mesocosms with 100 Fower's toad tadpoles. However, in those mesocosms with American toad tadpoles, also regardless of their abundance and whether or not Fowler's toad tadpoles were also present (i.e. both species mesocosms), Bacillariophyceae abundance increased between the same two time points. Bacillariophyceae abundance between Sample 2 and Post Tads increased by 10 ± 8 algal cells on average in mesocosm with both species of tadpoles, 5 ± 6.5 algal cells on average in mesocosms with 50 American toad tadpoles, and 5.3 ± 8.5 algal cells on average in mesocosms with 100 American toad tadpoles.

Meanwhile, in mesocosms with 50 American toad tadpoles, 100 American toad tadpoles, or 50 Fowler's toad tadpoles, Bacillariophyceae abundance in periphyton decreased between Sample 2 and Post Tads, but increased in mesocosms with both species of tadpoles and those with 100 Fowler's toad tadpoles between the same two time points. Bacillariophyceae abundance between Sample 2 and Post Tads decreased by 14.7 ± 15 algal cells/L on average in mesocosm with 50 American toad tadpoles, 7.7 ± 12.5 algal cells/L on average in mesocosms with 100 American toad tadpoles, and 8.3 ± 9.6 algal cells/L on average in mesocosms with 50 Fowler's toad tadpoles. Conversely, Bacillariophyceae abundance between Sample 2 and Post Tads increased by 14.7 ± 15 algal cells/L on average in mesocosm with 100 Fowler's toad tadpoles, and 0.67 ± 4.6 algal cells/L on average in mesocosms with both species of tadpoles.

Additionally, the abundance of conjugating green algae, such as Desmids and *Spirogyra* (Zygnematophyceae) in periphyton increased in mesocosms with either no tadpoles (mean = 52.0 cell/L ± 71.1) or with only Fowler's toad tadpoles (50 Fowler's toad tadpoles: mean = 25.3 cell/L ± 43; 100 Fowler's toad tadpoles: mean = 30.3 cell/L ± 51.6) between Sample 2 and Post Tads time points. However, in all mesocosms containing American toad tadpoles, whether or not Fowler's toad tadpoles were also present, these algae remained extremely rare, with Zygnematophyceae abundance in mesocosms with American toad tadpole and mixed species mesocosms representing only 0.87% of the Zygnematophyceae in all mesocosms.

Our results also indicated significant differences in the algal communities between the tadpole species and tadpole densities in the mesocosms. Trends in periphyton and phytoplankton diversity, evenness, and richness differed through time depending on tadpole species, tadpole density, and tadpole community composition (**Figures 2.3 and 2.4**). For instance, phytoplankton diversity increased significantly more in mesocosms with 50 Fowler's toad tadpoles than in control mesocosms between the Initial time point and Sample 2 (GLMM: mean = 1.43 ± 0.42, $t = 3.35$, $p = 0.0065$), but this trend wasn't significant in mesocosms with a higher density of the same species of tadpoles, or mesocosms with the same density of American toad tadpoles. Further, periphyton diversity increased significantly more in mesocosms with both species of tadpoles (GLMM: mean = 0.75 ± 0.29, $t = 2.54$, $p = 0.027$), and mesocosms with 100 Fowler's toad tadpoles (GLMM: mean = 1.18 ± 0.036, $t = 3.62$, $p = 0.004$) between the Initial and post-tads time points, but this trend was not significant in mesocosms with only American toad tadpoles at any density, or mesocosms with 50 Fowler's toad tadpoles.

The community composition of algal species characteristic with our experimental groups differed between each species, each community composition, and each density of tadpoles in the mesocosms (**Figure 2.7**). For instance, the periphyton community in all experimental groups and the control mesocosms were dominated by *Fragilaria capucina* at the Initial time point, but at Sample 2, each experimental group was dominated by different indicator species. Periphyton communities in mesocosms with 100 American toad tadpoles were heavily dominated by *Stigeoclonium polymorphum,* mesocosms with 50 American toad tadpoles were dominated by *Protoderma viride*, mesocosms with both species of tadpoles were dominated by *Fragilaria capucina*, mesocosms with 50 Fowler's toad tadpoles were dominated by *Dictyosphaerium elegans*, mesocosms with 100 Fowler's toad tadpoles were dominated by *Protoderma viride* and *Stigeoclonium polymorphum*, and mesocosms with no tadpoles were dominated by *Fragilaria crotonensis, Fragilaria capucina,* and *Ochromonas sphagnalis.*

Discussion

Tadpole presence or absence

Toad tadpole presence in the mesocosms can reduce algal cell count for both periphyton and phytoplankton and alter algal communities regardless of which species of tadpoles are present. The presence toad tadpoles appears to favour the growth of filamentous algae such as *Stigeoclonium polymorphum* (Chlorophyceae) and *Protoderma viride* (Ulvophyceae), which grow in long tendrils often anchored to the substrate and can be difficult for certain grazers to consume (McCormick and Stevenson, 1991). This grazer resistance may explain why *S. polymorphum* was so abundant in mesocosms with grazing toad tadpoles, as tadpoles may not have been able to reduce *S. polymorphum* biomass due to the algae's grazer resistance. This may have left *S. polymorphum* with more space, nutrients, and light, as the tadpoles reduced the biomass of competitors (McCormick and Stevenson, 1991). Additionally, algae species in the genus *Stigeoclonium* often grow in the understory of the periphyton mat. If tadpole grazers consume the more loosely attached overstory of algae, the growth of *S. polymorphum* could be promoted. (McCormick and Stevenson, 1991). *Protoderma viride* has a filamentous structure similar to *S. polymorphum* and may benefit from tadpole presence in the same way.

Between species differences

American toad and Fowler's toad tadpoles, despite their similar mouthparts, had different effects on algal communities. This is not unheard of, as different species of tadpoles can also have differing effects on the relationship between phytoplankton and periphyton (Costa and Vonesh, 2013). For example, Red-eyed tree frog (*Agalychnis callidryas*) tadpoles reduce periphyton (and as a result increase phytoplankton) more intensely than Hourglass tree frog (*Dendropsophus ebraccatus*) tadpoles in experimental mesocosms (Costa and Vonesh, 2013). Although Hoverman et al. (2015) consider the mouthparts of Fowler's toad and American toad tadpoles to be indistinguishable, Hinckley (1882) had previously observed that Fowler's toad tadpole oral papillae were softer and flatter that those of American toad tadpoles, which she described as "rough and raised". Tadpole oral papillae, which surround the mouthparts, can help to hold food against the mouth while tadpoles rasp at algae but are also sensory and covered in taste buds (Bambeke, 1863; Nomura et al, 1979), which develop when tadpoles begin feeding

(Żuwała and Jakubowski, 1997). The number of taste buds on papillae, when they develop, and the positioning of papillae around the mouthparts all suggest that they would be used for food selection and the assessment of food quality (Żuwała and Jakubowski, 1997) as has been demonstrated in tadpoles of the Pacific tree frog (*Hyla regilla*), Foothill yellow-legged frog (*Rana boylii*), and American bullfrog (*Lithobates catesbeiana*) (Kupferberg, 1997). It follows that Fowler's toad and American toad tadpoles likely are not indiscriminate grazers at all, and select for specific algae to consume, resulting in their observed differing influences on algal communities. Thus, despite being closely related and similar in appearance, Fowler's toad and American toad tadpoles are not functionally redundant in pond ecosystems.

Legacy Effects

The presence of tadpoles in an ecosystem can have demonstrable, lingering effects even after metamorphosis has occurred and the tadpoles have left. The common, filamentous alga, *Oedogonium inconspicuum*, for example, was hardly detectable in mesocosms until it exhibited a conspicuous bloom in abundance in late summer **(Figure 2.5),** but only dominated the community in control tanks, which had not previously contained tadpoles **(Figure 2.8)**. This constitutes a form of legacy effect (Cuddington, 2011) in which the prior presence of tadpoles, even after they have undergone metamorphosis and no longer exist in the mesocosms, still exerts an inhibitory effect on the growth and abundance of another species. Positive legacy effects may also occur. Shannon diversity, evenness, and richness of periphyton communities in mesocosms that once held toad tadpoles could significantly differ both positive and negatively from control mesocosms after tadpole metamorphosis (**Figure 2.3 and Figure 2.4**). These observations of changes in algal biomass and alterations in the composition of algal communities compared to control mesocosms where tadpoles were never present echo those of Rowland et al. (2017), who found that tadpoles of the southern leopard frog, *Lithobates sphenocephalus*, also confer legacy effects on periphyton biomass in the ponds they once inhabited.

The prior presence of tadpoles in small aquatic ecosystems may also significantly continue to influence habitat heterogeneity and community composition after metamorphosis in the same ecosystem (Rowland et al, 2017). For instance, we show that after metamorphosis, the periphyton community in mesocosms that once contained tadpoles held more grazer resistant genera, such as *Stigeoclonium* and *Protoderma* (**Figure 2.7**). Also, we demonstrate that *Oedogonium inconspicuum* was only able to bloom to a high extent in the late summer, after metamorphosis, in mesocosms that never contained tadpoles (**Figure 2.5**, **Figure 2.8**). Thus the effect of the presence and then absence of tadpoles may conform to a "facilitation" model of ecological succession (Connel and Slayter, 1997), whereby the success of later species depends upon the prior presence of earlier species in the same ecosystem. Such legacy impacts specifically of tadpoles on environments post-metamorphosis, though, have rarely been studied previously.

Implications and future studies

We have demonstrated that Fowler's toad and American toad tadpoles, while their presence is ephemeral, are extremely important keystone herbivores in mesocosms that mimic natural ponds. These toad tadpoles reduce algal cell abundance and alter periphyton and phytoplankton communities with their presence. Toad tadpole presence alters which algal classes dominate communities, as well as what algal species are most closely associated to different conditions. Additionally, differences in the algal community remain even after toad tadpoles have metamorphosed and left the mesocosms, leaving behind a legacy effect. While these general changes are true when tadpoles are present or absent, Fowler's toad tadpoles and American toad tadpoles, despite being closely related, are not functionally redundant. In addition to differences in the algal community when tadpoles are present or absent, the algal community is different depending on the species of tadpole present, the density of tadpoles present, and whether there are one or two species of tadpole present. This deviation between the algal communities in all these different tadpole compositions in mesocosms indicates that there would be a large number of differences between the algal communities in natural ponds, which have far more natural variation than the mesocosms. This difference in ponds containing different species or different densities of tadpoles would create a massive amount of habitat heterogeneity, promoting greater biodiversity across the wetland landscape (Tews et al, 2004; Shi et al, 2010; Lorenzón et al, 2016).

These effects make toad tadpoles in mesocosms an excellent study system with which to evaluate the response of algal communities to consumer loss. Taking advantage of this system in future studies may enable us to determine how the rest of the aquatic community responds to tadpole grazer loss, and the associations between these other aquatic community members, such as zooplankton and algal communities. The selectivity of grazers is also open to further examination to determine if the algae being selected for or against has different nutritional components or physical adaptions to promote or reduce grazing. As well, the mechanisms with which grazers exert this pressure on algal communities, and whether this effect is top-down or bottom-up, could also be examined in future studies. Future studies could also examine the effects of different, closely related algal grazers on communities at different densities, as we have demonstrated that even species expected to be functionally redundant may not be.

Overall, we provide evidence that aquatic grazers, such as tadpoles, can be extremely important in influencing algal biomass, phytoplankton communities, and periphyton communities at both the Class and Species levels. We also provide evidence indicating that the different species, community composition, and densities of grazers can all alter the algal community, not only grazer presence or absence. Future studies on this topic could expand on our preliminary study, as we only had two species and two densities of grazers, and a relatively small sample size, but still show interesting differences in algal composition between grazer species and densities. Further research on species that are assumed to be non-selective aquatic grazers is warranted, as well as research that examines changes in algae at the community level, not only the biomass level, as we demonstrate interesting changes in algal community composition. Such studies would aid in our understanding of the role of grazers in ecosystems, even when the impact of these species is not immediately apparent.

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Tables

Table 2.1: Results of an indicator species analysis (ISA) using the biomass of phytoplankton species in mesocosms with different tadpole communities, and when tadpoles were present or absent. The sample time “Initial” indicates that this was right before tadpoles were added, “Sample 2” was during a time when tadpoles were actively feeding, and “Post Tads” was after tadpoles had metamorphosed and left the mesocosms.

Sample time	Species present (tadpole density)	Significantly associated species	ISA results	
			IndVal	*p*-value
Initial	American toad (100)	none	-	-
	American toad (50)	*Cryptomonas borealis*	0.586	0.0214
	Fowler’s toad (100)	none	-	-
	Fowler’s toad (50)	none	-	-
	Mixed species (100)	none	-	-
	Control (0)	none	-	-
Sample 2	American toad (100)	none	-	-
	American toad (50)	none	-	-
	Fowler’s toad (100)	none	-	-
	Fowler’s toad (50)	*Fragilaria capucina* (degrading)	0.716	0.0319
	Mixed species (100)	none	-	-
	Control (0)	none	-	-
Post Tads	American toad (100)	none	-	-
	American toad (50)	none	-	-
	Fowler’s toad (100)	*Pseudanabaena limnetica*	0.824	0.0074
	Fowler’s toad (50)	none	-	-
	Mixed species (100)	none	-	-
	Control (0)	*Oedogonium inconspicuum*	0.753	0.0198

Table 2.2: Results of an indicator species analysis (ISA) using the cell counts of phytoplankton species in mesocosms with different tadpole communities, and when tadpoles were present or absent. The sample time "Initial" indicates that this was right before tadpoles were added, "Sample 2" was during a time when tadpoles were actively feeding, and "Post Tads" was after tadpoles had metamorphosed and left the mesocosms.

Sample time	Species present (tadpole density)	Significantly associated species	ISA results	
			IndVal	*p*-value
Initial	American toad (100)	none	-	-
	American toad (50)	none	-	-
	Fowler's toad (100)	*Calonensis bacillum*	0.713	0.0165
		Sururella ovata var. *apiculata*	0.698	0.0318
	Fowler's toad (50)	none	-	-
	Mixed species (100)	none	-	-
	Control (0)	*Nitzschia acicularis* (degrading)	0.87	0.0083
	Mixed and American (100)	*Nitzschia acicularis* (degrading)	0.716	0.0272
Sample 2	American toad (100)	none	-	-
	American toad (50)	none	-	-
	Fowler's toad (100)	none	-	-
	Fowler's toad (50)	none	-	-
	Mixed species (100)	none	-	-
	Control (0)	None	-	-
Post Tads	American toad (100)	none	-	-
	American toad (50)	none	-	-
	Fowler's toad (100)	none	-	-
	Fowler's toad (50)	none	-	-
	Mixed species (100)	none	-	-
	Control (0)	*Oedogonium inconspicuum*	0.926	0.0061

Table 2.3: Results of an indicator species analysis (ISA) using the biomass of periphyton species in mesocosms with different tadpole communities, and when tadpoles were present or absent. The sample time "Initial" indicates that this was right before tadpoles were added, "Sample 2" was during a time when tadpoles were actively feeding, and "Post Tads" was after tadpoles had metamorphosed and left the mesocosms.

Sample time	Species present (tadpole density)	Significantly associated species	ISA results	
			IndVal	*p*-value
Initial	American toad (100)	none	-	-
	American toad (50)	none	-	-
	Fowler's toad (100)	none	-	-
	Fowler's toad (50)	none	-	-
	Mixed species (100)	*Fragilaria capucina*	0.0891	0.0067
	Control (0)	none	-	-
	Mixed and Control	*Eunotia tenella*	0.0734	0.0302
Sample 2	American toad (100)	***Stigeoclonium polymorphum***	**0.561**	**0.0099**
	American toad (50)	*Protoderma viride*	0.799	0.0032
	Fowler's toad (100)	none	-	-
	Fowler's toad (50)	*Dictyospharium elegans*	0.666	0.0499
	Mixed species (100)	none	-	-
	Control (0)	*Fragilaria crotonensis*	0.812	0.0027
		Chromulina mikroplankton	0.736	0.0080
		Ochromonas sphagnalis	0.651	0.0255
		Aphanothece clathata var. *brevis*	0.642	0.0420
Post Tads	American toad (100)	*Rhodomonas minuta*	0.898	0.0062
	American toad (50)	none	-	-
	Fowler's toad (100)	none	-	-
	Fowler's toad (50)	none	-	-
	Mixed species (100)	none	-	-
	Control (0)	none	-	-

Table 2.4: Results of an indicator species analysis (ISA) using the cell count of periphyton species in mesocosms with different tadpole communities, and when tadpoles were present or absent. The sample time "Initial" indicates that this was right before tadpoles were added, "Sample 2" was during a time when tadpoles were actively feeding, and "Post Tads" was after tadpoles had metamorphosed and left the mesocosms.

Sample time	Species present (tadpole density)	Significantly associated species	ISA results	
			IndVal	*p*-value
Initial	American toad (100)	none	-	-
	American toad (50)	*Pseudendoclonium basiliense*	0.719	0.0161
	Fowler's toad (100)	none	-	-
	Fowler's toad (50)	none	-	-
	Mixed species (100)	none	-	-
	Control (0)	none	-	-
	Fowler's (100) and American (50)	*Caloneis bacillum*	0.752	0.0395
		Synedra ulna	0.732	0.0489
Sample 2	American toad (100)	none	-	-
	American toad (50)	none	-	-
	Fowler's toad (100)	none	-	-
	Fowler's toad (50)	none	-	-
	Mixed species (100)	none	-	-
	Control (0)	*Fragilaria crotonensis*	0.888	0.0087
		Chromulina mikroplankton	0.773	0.0042
	American (100) and Fowler's (100)	***Stigeoclonium polymorphum***	0.755	0.0282
	Fowler's (100) and American (50)	*Protoderma viride*	0.735	0.0207
	Control (0) and American (50)	*Gloeocystis ampla*	0.665	0.0491
Post Tads	American toad (100)	*Rhodomonas minuta*	0.898	0.0071
	American toad (50)	none	-	-
	Fowler's toad (100)	none	-	-
	Fowler's toad (50)	none	-	-
	Mixed species (100)	none	-	-
	Control (0)	none	-	-

Figures

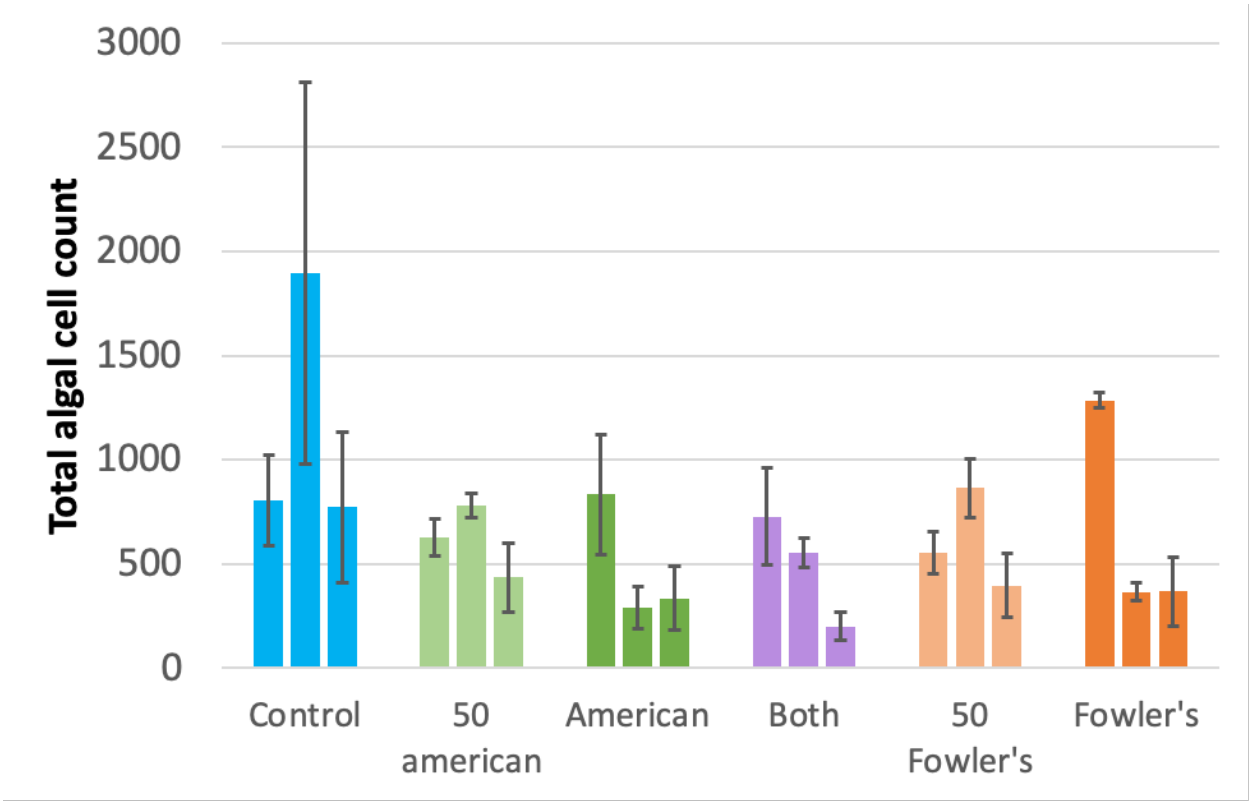

Figure 2.1: The total cell count of all phytoplankton species found in each composition of mesocosm through time. The first column of each colour represents the "Initial" sample time, followed by "Sample 2" and "Post Tads".

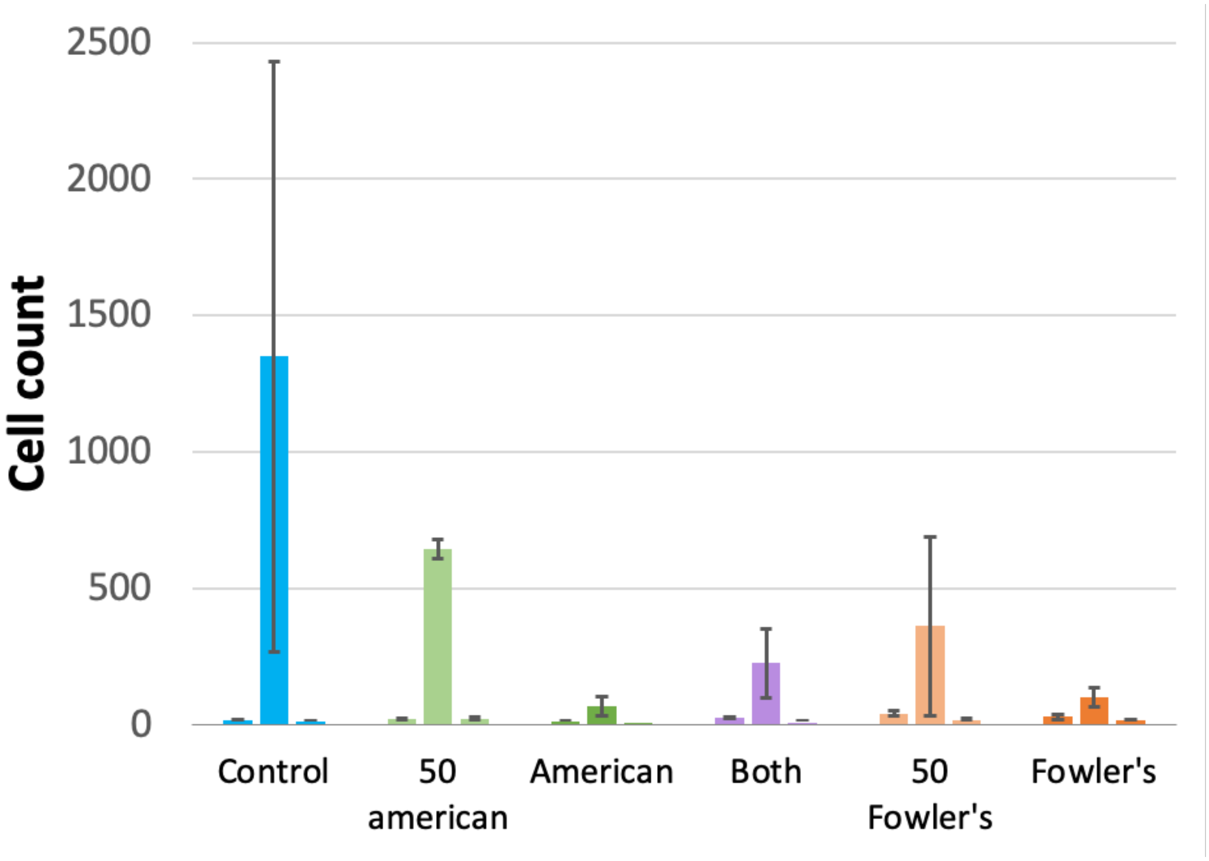

Figure 2.2: The cell count of phytoplankton in the Class Chlorophycae found in each composition of mesocosm through time. The first column of each colour represents the "Initial" sample time, followed by "Sample 2" and "Post Tads".

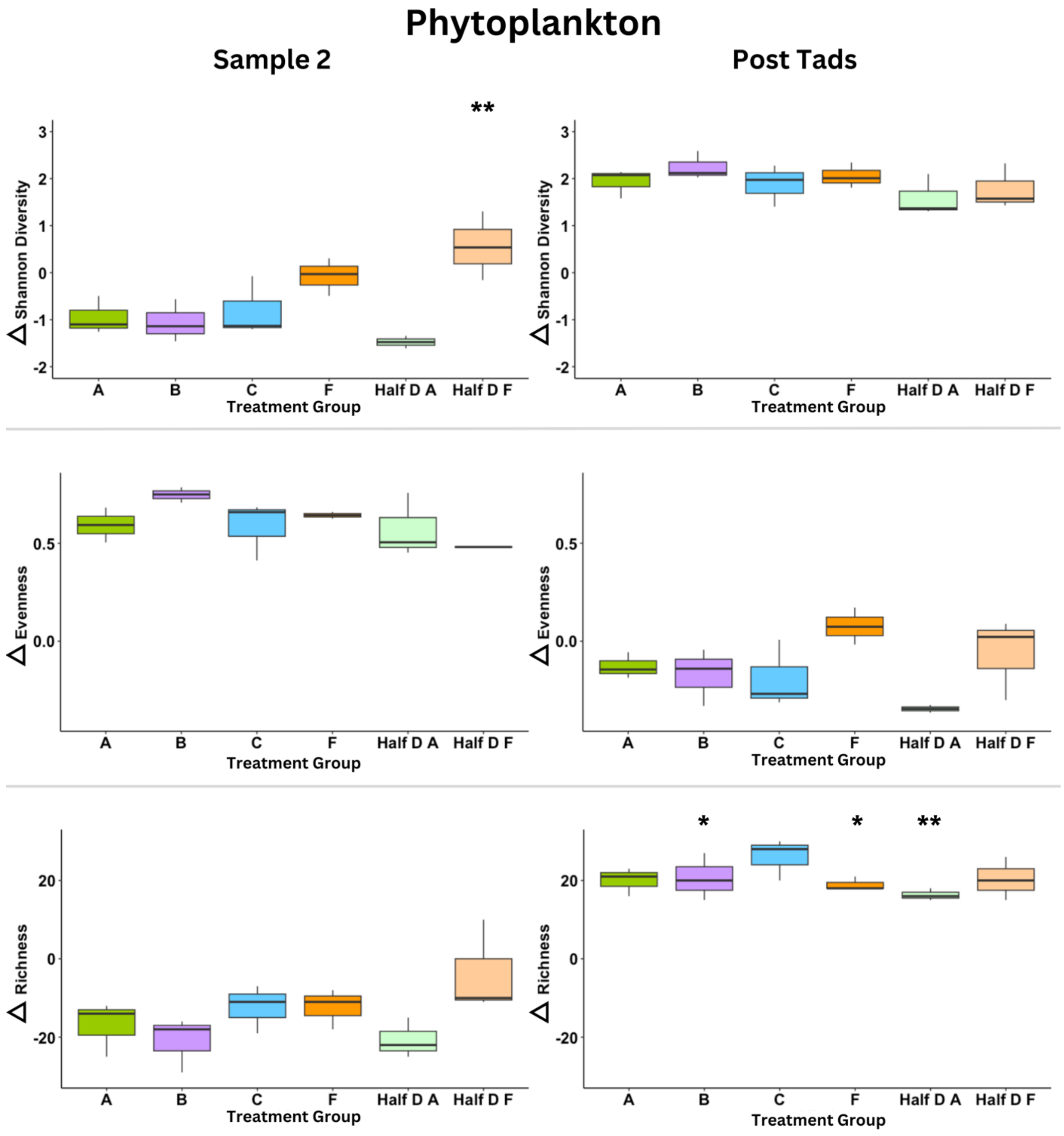

Figure 2.3: Boxplots showing the change in phytoplankton Shannon diversity, evenness, and richness from the Initial time point to the Sample 2 (actively feeding tadpoles) and Post Tads (after metamorphosis) time points. Asterix indicate significant differences from control mesocosms as per a GLMM. Treatment groups are indicated as follows: A = 100 American toad tadpoles, B = Both species of tadpoles, C = Control (no tadpoles), F = 100 Fowler's toad tadpoles, Half D A = 50 American toad tadpoles, Half D F = 50 Fowler's toad tadpoles.

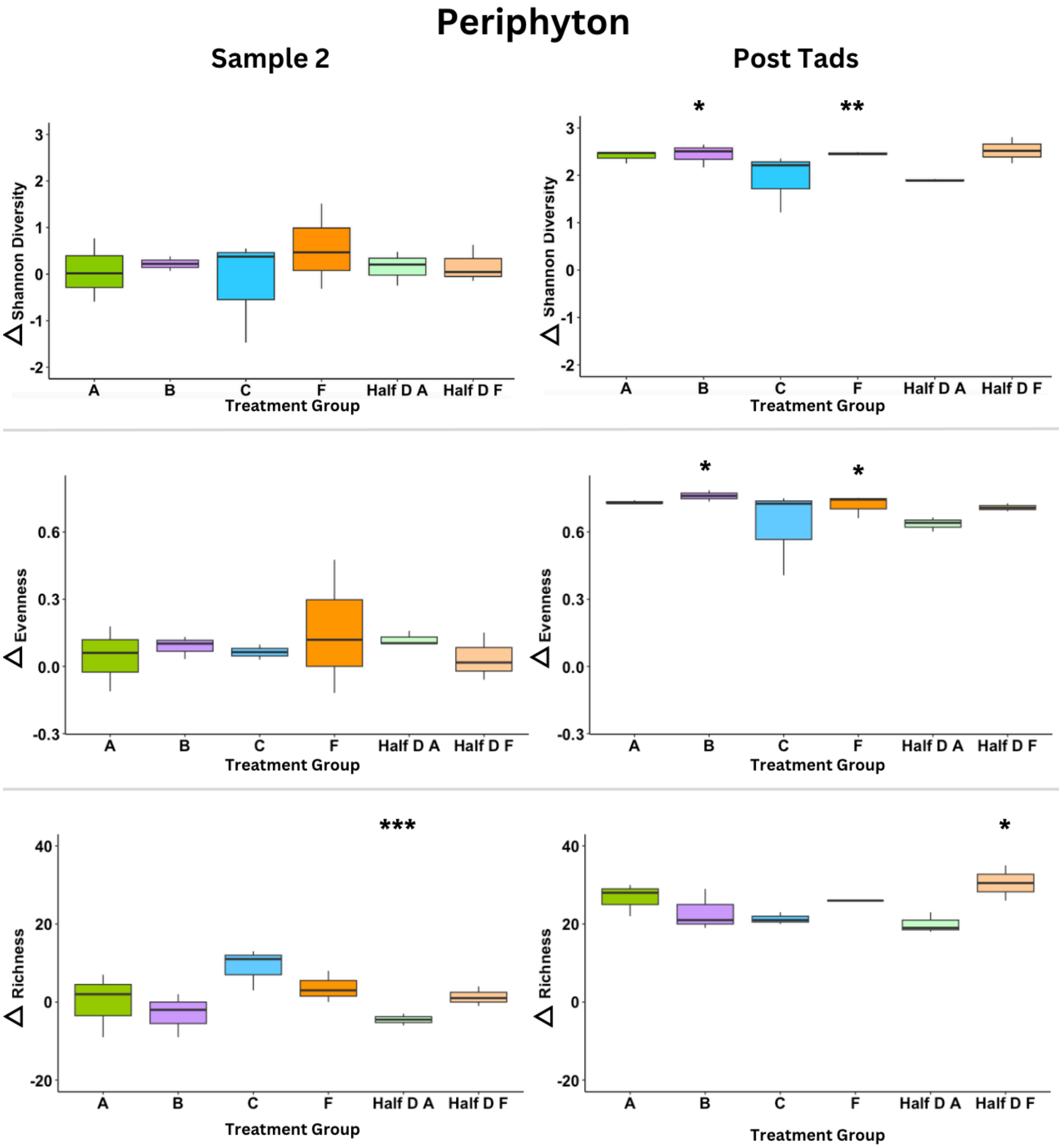

Figure 2.4: Boxplots showing the change in periphyton Shannon diversity, evenness, and richness from the Initial time point to the Sample 2 (actively feeding tadpoles) and Post Tads (after metamorphosis) time points. Asterix indicate significant differences from control mesocosms as per a GLMM. Treatment groups are indicated as follows: A = 100 American toad tadpoles, B = Both species of tadpoles, C = Control (no tadpoles), F = 100 Fowler's toad tadpoles, Half D A = 50 American toad tadpoles, Half D F = 50 Fowler's toad tadpoles.

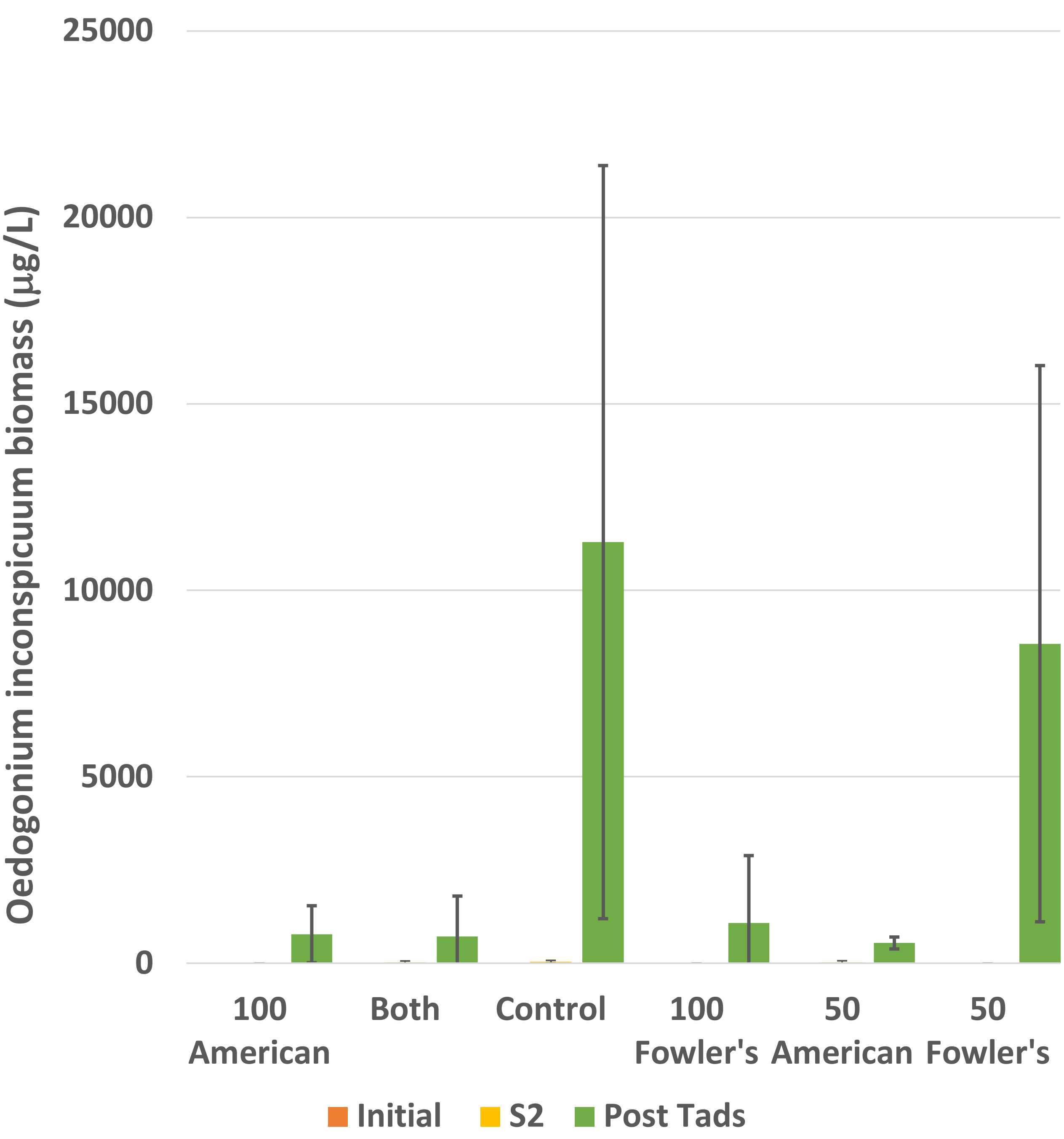

Figure 2.5: The biomass of *Oedogonium inconspicuum* in mesocosms with different tadpole communities through time. Initial was just before tadpoles were added to the mesocosms, S2 indicates Sample 2, where tadpoles were actively feeding in the mesocosms, and Post Tads was after tadpoles had metamorphosed and left the mesocosms. All 3 sample times are included in the bar graph.

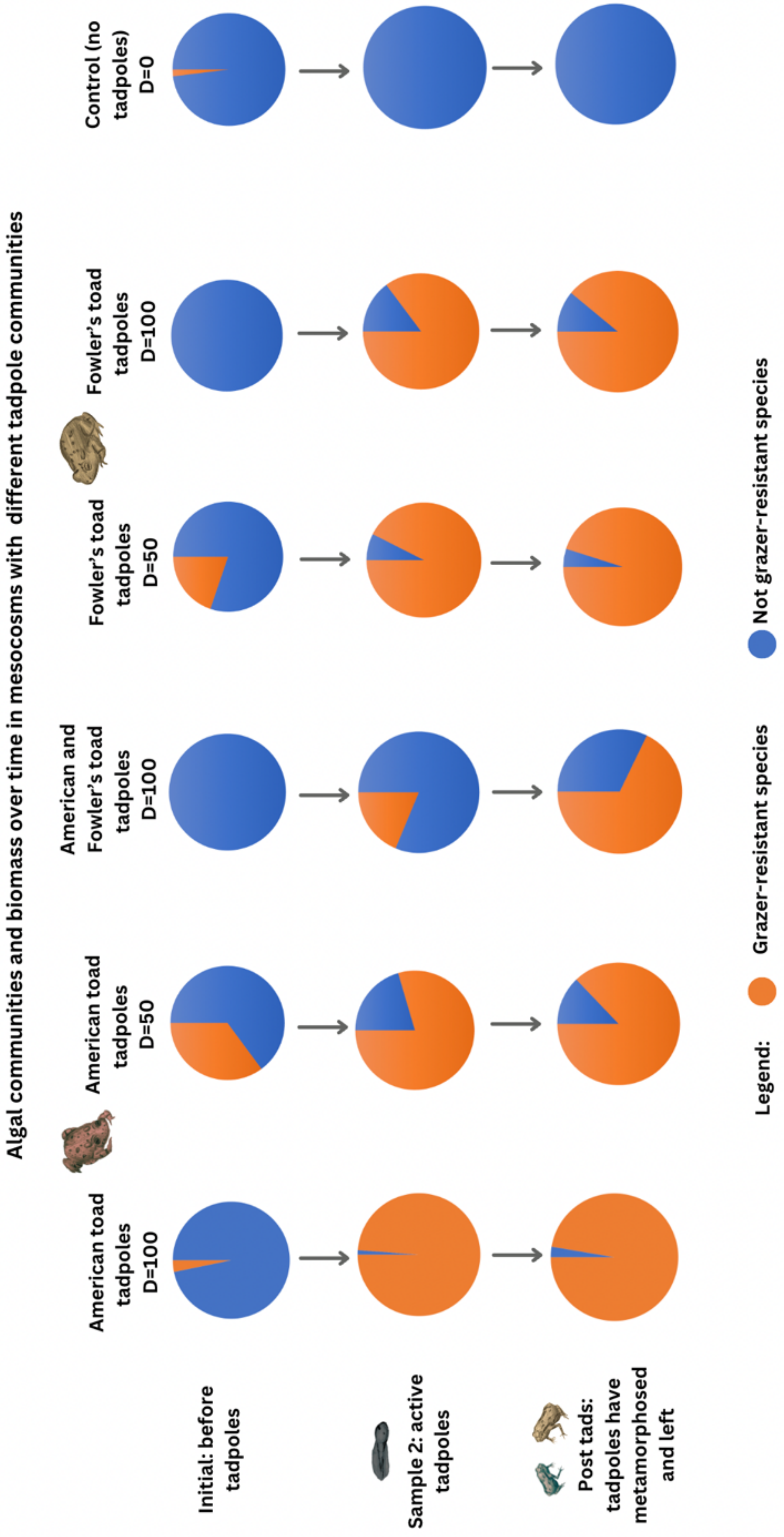

Figure 2.6: The biomass of different periphyton species identified by the indicator species analysis in mesocosms with different tadpole communities through time. Filamentous or gelatinous periphyton species were categorized as grazer resistant.

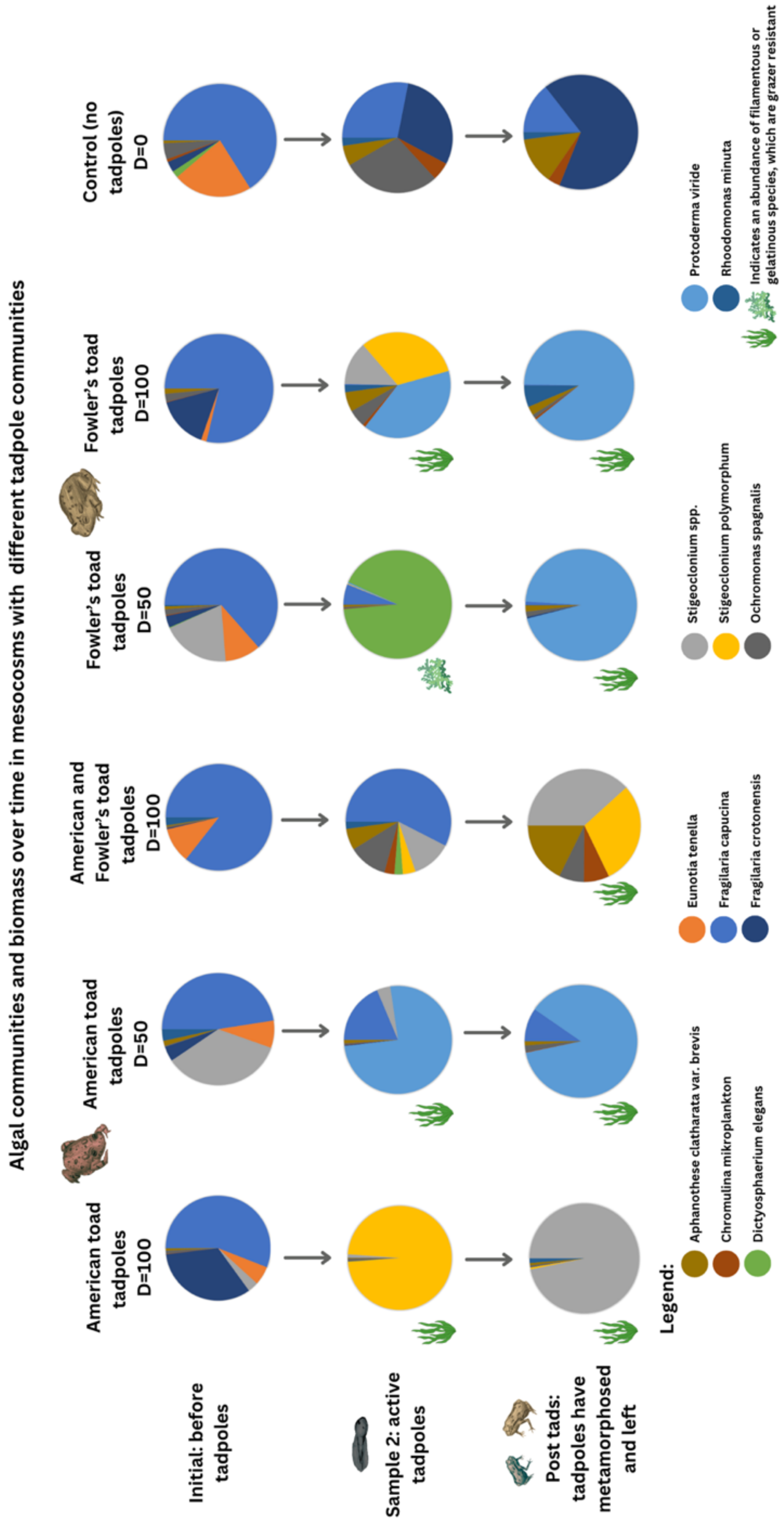

Figure 2.7: The relative biomass of different periphyton species identified by the indicator species analysis in mesocosms with different tadpole communities through time.

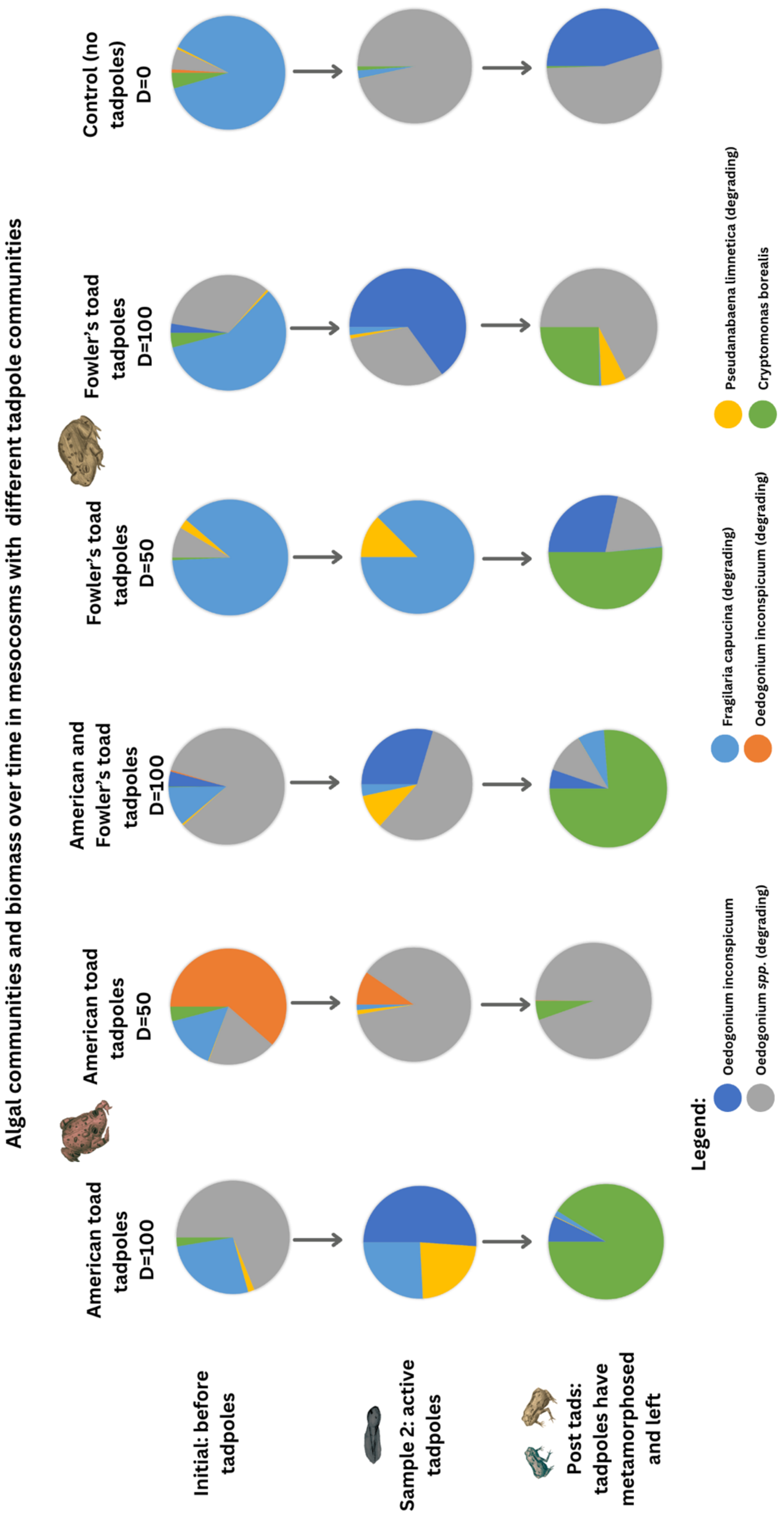

Figure 2.8: The relative biomass of different phytoplankton species identified by the indicator species analysis in mesocosms with different tadpole communities through time

Connecting Statement Between Chapters 2 and 3

In Chapter 1 I noted that the changes in concentration of nitrogen and phosphorus, agal biomass, and zooplankton diversity followed different trends in mesocosms containing both American and Fowler's toad tadpoles, compared to the trends when either species were on their own. In Chapter 2, this pattern continued, with mesocosms containing both American and Fowler's toad tadpoles having algal communities that differed from those in single species mesocosms at any density. The unique effect of a mixed tadpole community in mesocosms, even when tadpoles are at the same density as single species mesocosms, suggests that toad tadpoles are behaving differently when in the presence of heterospecifics. These different community interactions could be due to competition for food resources between the tadpoles, or predation threats from one tadpole species on another.

To delve deeper into the interactions occurring between American and Fowler's toad tadpoles in the mixed species mesocosms, in Chapter 3 I assess the impact of being raised in a mixed species environment on the tadpoles themselves. When tadpoles emerge as toadlets, their time to metamorphosis, weight at metamorphosis, and survival to metamorphosis are all indicators of later fitness. Determining how toad tadpoles interact with each other provides insight into how a change in intraguild interactions could result in cascading impacts down the food web. This study has implications for understanding tadpole community interactions, and understanding if competitive exclusion is occurring between the common American toad and locally endangered Fowler's toad.

Chapter 3 | Inter-annual variation in amphibian larval interspecies interactions

Published in Ecology and Evolution, 2023

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Abstract

The outcomes of species interactions can vary by life stage, year, and surrounding environmental conditions. Amphibian species are expected to compete most strongly during their tadpole stage when they exist in the highest densities. Changes in arrival timing, surrounding aquatic communities, and yearly conditions could all affect the outcome of larval competition. In Long Point, Ontario, the Fowler's toad (*Anaxyrus fowleri*) is at the northern edge of its range and overlaps with the more common American toad (*Anaxyrus americanus*). Both species breed in ponds that encounter high interannual variation. To determine if these species compete strongly, and if this effect was replicated across multiple years, we raised both species as tadpoles together, and apart, in mesocosms in 2018 and 2021. We measured survivorship to, weight at, and time to metamorphosis for both species in both years. We determined that the presence of American toad tadpoles consistently had a detrimental effect on Fowler's toad tadpoles, even though this effect presented itself differently across years. Our study suggests that competitive exclusion by American toads could be occurring at the edge of the Fowler's toad's range. This study further demonstrates the importance of studying communities across multiple years to understand the full scope of species interactions.

Introduction

According to the competitive exclusion principle, two species with identical niches cannot coexist, as one species will eventually drive the other to extinction (Hardin, 1960; Levin, 1970; Hening and Nguyen, 2020). Species are more likely to compete strongly if their niche overlap is large, or if they are competing for the same food resource (Hardin, 1960; Levin, 1970; Hening and Nguyen, 2020). Ecologically similar species will thus compete strongly when habitat or food availability is reduced, or where the edges of their ranges overlap, as has been shown in sea birds in marine environments (Bonnet-Lebrun et al, 2021) and foxes in terrestrial environments (Elmhagen et al, 2017; Gosselink et al, 2003).

However, which species dominates may not be predictable, as many factors can alter the outcome of interspecies interactions, including temporal variation (Hutchinson, 1961; Rudolf, 2019). Non-equilibrium conditions caused by temporal variation can allow ecologically similar species to have competitive advantages at different times, enabling coexistence where it would not occur otherwise (Grainger et al, 2019; Hening and Nguyen, 2020). Accounting for temporal changes in species interactions over time is thus essential for understanding species coexistence and community structure (Chesson, 2000; Angert et al, 2009; Rudolf, 2019). This temporal variation, or inter-annual variation, is often caused by year effects, such as changes in weather and environmental conditions (Dakos et al, 2009; Werner et al, 2020). Year effects can exert profound impacts on community assembly, community composition, and ecological dynamics (Rudolf, 2018; Werner et al, 2020). Despite the importance of inter-annual variation, changes in the outcome of species interactions between years are rarely studied or considered (Rudolf, 2018; Rudolf, 2019; Werner et al, 2020).

Small aquatic ecosystems, such as ponds, can be heavily affected by inter-annual variation in precipitation, as this can change hydroperiod and pond size, with ensuing effects on the interactions between inhabitants (Reinhardt et al, 2015). Ponds are dynamic and often ephemeral habitats that support high biodiversity and serve as refuge sites for many species (Reinhardt et al, 2015; Hill et al, 2021). Inter-annual variation in species composition has been observed in ponds even in successive years, affecting the phenology of phytoplankton community blooms and their interactions with keystone herbivores in the zooplankton community (Winder and Schindler, 2004). Temporal variation in climatic conditions can also change the establishment times of invertebrate communities in ponds and alter their interactions with larval amphibian communities, as well as how the larval amphibians interact with one another (Reinhardt et al, 2015). This change in larval amphibian communities can, in turn, have profound feedback effects on the surrounding algal and zooplankton communities (Hamilton et al, 2012; Buck et al 2012; Arribas et al, 2014). Understanding the impacts of inter-annual variation in relation to larval amphibian interactions, such as competition, is thus essential to comprehending the ecology of small aquatic ecosystems.

The tadpoles of pond-breeding anurans are important components of many small aquatic ecosystems (Hamilton et al, 2012; Buck et al, 2012; Arribas et al, 2014). Competition between closely related tadpole species can influence breeding site selection (Buxton and Sperry, 2017), the structure of tadpole communities (Faragher and Jaeger, 1998; Stein et al, 2017), and the success of larval development (Wilbur, 1987). Tadpoles within ponds may be at very high densities, and often share the same primary food source of periphyton (Connelly et al. 2008; Wood and Richardson, 2010; Hamilton et al., 2012), facilitating strong competition between species (Wilbur, 1980; Pechmann, 1995; Altwegg, 2003; Gazzola and Buskirk, 2014). Tadpoles are ephemeral, and the timing of adult breeding in ponds can change each year, altering competition dynamics (Alfred and Wilbur, 1985; Lawler and Morin, 1993; Rudolf, 2018). Furthermore, the ponds in which tadpoles live will change in response to weather conditions (Florencio et al, 2010; Reinhardt et al, 2015; Rudolf, 2018; Florencio, 2020), which can affect aquatic community structure and impact the outcomes of interspecies competition. Tadpoles, being the larval form of amphibians, do not have other forms of competition such as sexual competition for mates, which may complicate studies on resource competition. The effects of larval competition, and the resulting fitness of metamorphs, can be determined by time to metamorphosis, survivorship through metamorphosis, and the weight of metamorphs as they emerge (Dash and Hota, 1980; Bardsley and Beebee, 2001; Stein et al, 2017). These metrics can be measured in a relatively straightforward manner, making the model system of tadpole communities in ponds valuable for studying competition and inter-annual variation (Bardsley and Beebee, 2001).

Throughout much of eastern North America, Fowler's Toads, *Anaxyrus fowleri*, overlap in range with American Toads, *Anaxyrus americanus*, including in our study site of Long Point, Ontario. As adults, Fowler's toads tend to be associated with sand dune habitats whereas American toads are a generalist species (Petranka et al, 1994). However, both species will breed in shallow, sandy, nutrient-poor ponds and have been known to breed in the same ponds (Green, 1982) at locations where their ranges overlap, and even hybridize (Zweifel, 1968; Green 1984; Green and Parent, 2003). Under these circumstances, competition between tadpoles of these two species is highly probable.

American toad tadpoles may have an advantage over Fowler's toad tadpoles when they co-occur. As American toads breed roughly two weeks earlier in the spring than Fowler's toads (Volpe, 1955), their tadpoles are likely to be larger and at a later developmental stage than any

Fowler’s toad tadpoles present in the same pond. Because of this priority effect, we expected that American toad tadpoles would outcompete Fowler’s toad tadpoles for food resources, consisting mainly of periphyton (Connely et al, 2008; Wood and Richardson; 2010; Hamilton et al, 2012), possibly causing American toad tadpoles to metamorphose more quickly, with higher survivorship. (Woodward, 1987; Alford and Wilbur, 1985).

Under stable environmental conditions, niche partitioning due to the habitat preferences of breeding adult toads may occur to reduce competition among the different species of tadpoles. However, these toads do not live in stable conditions. The landscape of Long Point is a highly dynamic sand dune and marshland environment that is heavily influenced by fluctuating water levels and storm-driven waves from Lake Erie (Hebb, 2003). Thus, the two species may instead co-exist in an unstable environment subject to year effects. To test between these alternative scenarios, it is first necessary to establish whether the American toad tadpoles have a detrimental effect on Fowler’s toad tadpoles, consistent with competitive exclusion. If competitive exclusion is likely to occur with these toad species, then the tadpoles of one species should have a detrimental effect on the tadpoles of the other species. The disadvantaged species, which we expect to be the Fowler’s toad tadpoles, should exhibit lower survivorship, reduced size at metamorphosis, and prolonged time to metamorphosis when raised in mesocosms with American toad tadpoles compared to when raised alone. However, if inter-annual variation alters the outcome of such competitive interactions, then repetition of the experiment in different years could generate significantly different results.

Methods

To assess the result of competitive interactions and year effects, we raised American Toad and Fowler's Toad tadpoles in Rubbermaid© structural foam cattle watering tanks, which we used as outdoor mesocosms (Ford and Green, 2021), during 2018 and 2021 at Long Point Provincial Park, Ontario. The mesocosms measured 63.50 cm L × 78.74 cm W × 134.6 cm H and were covered with a shade cloth to prevent insects and other animals from entering. The experiment was initially conducted in 2018 but not repeated until 2021 due the late emergence of Fowler's Toads at the site in 2019, and closure of access to the site in 2020 due to the COVID-19 pandemic. The tadpoles we used were hatched from toad eggs collected from clutches laid in nearby local ponds or from egg clutches laid by amplectic pairs that we placed in a specialized breeding tank for 24 hours. In 2018, we collected three clutches of American toad eggs from a natural pond on May 3rd, and one clutch of Fowler's toad eggs was collected from an amplectic pair on May 10th. In 2021, two clutches of American toad eggs were collected from natural ponds on May 4th and May 16th and one clutch of Fowler's toad eggs was collected from an amplectic pair on May 19th. One clutch of Fowler's toad eggs were located each year as Fowler's toads are endangered in Canada and did not have any large breeding choruses in either 2018 or 2021.

We obtained data on air temperature (maximum, minimum, and mean daily temperatures, in ºC) from the Government of Canada (https://climate.weather.gc.ca) for the Long Point Weather Station (Latitude 42.53° N | Longitude 80.05° W, approximately 28 km from our study site). Rainfall (total monthly precipitation, in mm, and percent average precipitation) data was sourced from Agricorp for the hamlet of Charlotteville, Ontario (now known as Walsh, Ontario) approximately 20 km from our study site. We measured water temperature (ºC) between noon and

2:00 PM each day using an EcoSense oxygen probe (YSI, DO200) in 2018 and a HANNA multiparameter probe (HANNA Instruments, HI98194) in 2021. We assessed differences in air and water temperature across years using unaired *t*-tests.

Each year, we established mesocosms in which to raise tadpoles according to a standardized protocol (Ford and Green, 2021). We monitored the mesocosms continuously for changes in ammonia, nitrate, nitrite, oxygen, and signs of mortality (Ford and Green 2021). To ensure uniformity and prevent a spike in ammonia or nitrate from affecting tadpole success, we conducted partial, 20% water changes when ammonia or nitrate were above 0 ppm. Only two such water changes were needed in 2018, and none were required in 2021. We also set up additional mesocosms to house eggs and hatchling tadpoles, or for use during water changes. Any tadpoles remaining in additional mesocosms after the addition of tadpoles to experimental mesocosms were released at the point of origin (approximately 2.15 km from the mesocosm).

In 2018, we used 17 mesocosms to house tadpoles: six with 100 American toad tadpoles, six with 100 Fowler's toad tadpoles, and five with a mixture of 50 tadpoles of each species. In 2021, we used 20 mesocosms to house tadpoles: four with 50 Fowler's toad tadpoles, four with 100 Fowler's toad tadpoles, five with 50 American toad tadpoles, four with 100 American toad tadpoles, and three with 50 tadpoles of each species. The number of replicates varied between years as we had different amounts of experimental groups within the 30 mesocosms worked within each year.

When toadlets reached developmental stage 42 (Gosner, 1960) we removed them from the mesocosms, weighed them individually, recorded the presence or absence of a tail, and released them at point of origin. We determined time to metamorphosis by recording the first day a toadlet was found in each mesocosm. We tested for significant differences in weight at metamorphosis and time to metamorphosis between single and mixed species mesocosms using unpaired *t*-tests.

We calculated survivorship as the number of metamorphic toadlets of a species ultimately collected from a mesocosm compared to the number of tadpoles of that species originally placed in that mesocosm. To test whether tadpoles of one species had a detrimental effect on tadpoles of the other species, we used Generalized Linear Mixed Models (GLMMs). Our GLMMs used the response variable *tadpole survivorship*, explanatory variable *tank kind* (categorical, n= 2 in 2018 and n= 3 in 2021; levels in 2018: single species, mixed species; levels in 2021: single species, single species half density, mixed species) and random effect variables *tank number* (*n* = 17 In 2018 and n= 20 in 2021). We ran separate models for the Fowler's toad dataset and American toad dataset in both years. We tested the data for overdispersion and found Poisson distribution to be optimal (R package: lme4). If the Poisson distribution was overdispersed (indicated by the quotient of residual deviance by degrees of freedom being much greater than one), we tested again using quassipoisson and negative binomial distribution models. Akaike's information criterion (AIC) was used to determine the model that best fit the data (R package: DHARMa).

To determine if the year of the study, as well as the composition of mesocosms, had a significant effect on tadpole survivorship, we ran another GLMM. This GLMM had the response variable *tadpoles survivorship*, two explanatory variables, *tank kind* (this time combining 2018 and 2021, categorical, n= 3, single species, single species half density, mixed species) and *year* (categorical, n= 2, levels: 2018 and 2021), as well as the random effect *tank number* (n= 37).

All statistics were performed in R v. 4.1.1 (R Core Team, 2021).

Results

Yearly conditions varied between 2018 and 2021; 2018 was comparatively warm and dry, whereas 2021 was noticeably cooler and wetter (**Table 3.1**). The average daily maximum water temperature in the mesocosms was generally higher in 2018 than in 2021. In 2018, May, July, and August were 6.8 ºC, 4.6 ºC, and 4.4 ºC warmer, respectively, than in 2021. In 2021, June was marginally warmer than 2018, but by only 0.8 ºC. The average daily minimum air temperature was also slightly warmer in 2018 than in 2021. A *t*-test revealed that mean air temperature was significantly warmer in 2018 than in 2021 (*t*-test: $t = 5.1194$, df = 57 $p < 0.0001$). Mesocosm water temperatures were significantly warmer in 2018 in May (*t*-test: $t = 6.3133$, df = 298, $p < 0.0001$), July (*t*-test: $t = 7.0836$, df = 988, $p < 0.0001$), and August, (*t*-test: $t = 24.0248$, df = 67, $p < 0.0001$), and significantly warmer in 2021 in June (*t*-test: $t = 4.41477$, df = 794, $p < 0.0001$). However, it should be noted that while these differences were significant, they represent a difference of less than 4 degrees, and the large sample sizes may have resulted in an overpowered *t*-test.

2021 had notably more rainfall than in 2018, especially in July, which accumulated 160% of the average rainfall in 2021 but only 60% of the average rainfall in 2018, an approximately 2.7 times increase. This was accompanied by many intense rainstorms, sometimes lasting for days at a time. In 2021, June and July had 65.0 mm and 61.0 mm more rainfall, respectively, than in 2018. In May and August, however, there was 29.6 mm and 9.0 mm, respectively, more rain in 2018 than in 2021.

Fowler's toad tadpoles were negatively affected by the presence of American toad tadpoles in both 2018 and 2021, with the fitness parameters of time to, weight at, and survivorship to metamorphosis differing in the presence of competitors, but not in the same ways each year (**Table 3.2, Figure 3.1**). In 2018, while there was no significant difference between time to metamorphosis between American toadlets in single species or mixed species mesocosms, there was a significant difference between the time to metamorphosis of Fowler's toadlets in single species and mixed species mesocosms (**Table 3.2**). Fowler's toadlets took significantly longer to metamorphose in mixed species mesocosms than in single species mesocosms (*t*-test: t=3.934, df = 9, p = 0.0034). In 2021, although not significant, Fowler's toadlets from mixed species mesocosms still emerged later than in single species mesocosms with an initial density of either 50 or 100 tadpoles, with an average of 42.23 (± 4.90, n = 3) days compared to 36.3 (± 0.60, n = 3) and 38.0 (± 0.80, n = 4) days, respectively (**Table 3.2**). As these data were analyzed using an unpaired *t*-test, the high variance in time to metamorphosis of Fowler's toadlets in mixed species mesocosms may account for the lack of a significant difference despite the trend. Additionally, Fowler's toadlets emerging from mixed species mesocosms in 2021 only began metamorphosis after most of the American toadlets had left the mesocosms and did not decrease in weight at metamorphosis over time, which contrasts with all other mesocosms, where toadlet weight at metamorphosis, regardless of species, decreased over time (**Figure 3.2**). Similar data for 2018 is unavailable as too few Fowler's toad tadpoles survived to metamorphosis in the mixed species mesocosms to observe any trends. American toadlets in mixed species mesocosms and single species mesocosms showed no significant difference in time to metamorphosis in either 2018 or 2021.

The weight of emerging American and Fowler's toadlets also varied between treatment and year (**Table 3.2**). In 2018, Fowler's toadlets weighed significantly more in mixed species mesocosms compared with single species mesocosms (*t*-test: $t = 3.996$, df = 56.818, $p < 0.001$).

Similarly, American toadlets emerging from mixed species mesocosms weighed significantly more than their single species counterparts in 2018 (*t*-test: $t = -7.835$, df = 331.52, $p < 0.001$). In 2021, however, Fowler's toadlets emerging from mixed species mesocosms weighed significantly less than Fowler's toadlets from single species mesocosms (**Table 3.2)** with an initial density of 50 tadpoles (*t*-test: t = 15.804, df = 282.99, p < 0.0001) and 100 tadpoles (*t*-test: t = 10.622, df = 250.05, p < 0.0001). In addition, in 2021 American toadlets emerging from mixed species mesocosms also weighed significantly less than American toadlets emerging from single species mesocosms with an initial density of 50 tadpoles (*t*-test: t = 14.716, df = 350.1, p < 0.0001) and American toadlets from mesocosms with an initial density of 100 tadpoles (*t*-test: t = 2.177, df = 224.35, p = 0.0305).

Additionally, tadpole survivorship varied depending upon treatment and year (**Table 3.2**). In 2018, the average survivorship of Fowler's toad tadpoles to metamorphosis was significantly reduced in the presence of American toad tadpoles compared to mesocosms that contained only Fowler's toads (GLMM, Poisson distribution: $z = 2.084$, $p = 0.0372$). In 2021, however, survivorship of Fowler's toad tadpoles was not affected by the presence of American toad tadpoles, as all mesocosms produced high toadlet survivorship with values ranging from 95-100%. There was also no difference in Fowler's toad survivorship between mixed species mesocosms and single species mesocosms at either a density of 100 tadpoles per mesocosm (GLMM, Poisson distribution: $z = 0.122$, $p = 0.903$) or a density of 50 tadpoles per mesocosm (GLMM, Poisson distribution: $z = -0.087$, $p = 0.930$) in 2021. In this model, American toad tadpole survivorship did not significantly differ between mixed species and single species mesocosms either in 2018 (GLMM, negative binomial distribution: $z = 1.488$, $p = 0.147$) or in 2021 at densities of 100 tadpoles per mesocosm GLMM, negative binomial distribution:

(GLMM, negative binomial distribution: *z* = -0.943, *p* = 0.345) or 50 tadpoles per mesocosm (GLMM, negative binomial distribution: *z* value = -0.133, *p* = 0.894). All single species Fowler's toad and single species American toad mesocosms produced higher survivorship rates in 2021 than in 2018.

When running an additional GLMM involving the year of study, year also had a significant effect on the survivorship of Fowler's tadpoles (GLMM, quassipoisson equivalent distribution, z= 5.643, p<0.001) but not on the survivorship of American toad tadpoles (GLMM, Poisson distribution, z= 1.600, p= 0.109). However, in the GLMM involving both tank kind and year, American toad tadpole survivorship was significantly higher in mixed species mesocosms than in single species mesocosms (GLMM, Poisson distribution, z= -2.121, p= 0.034).

Discussion

While some species may exhibit competitive advantages over another, these roles are not always consistent (Rudolf, 2019). The outcome of species competition can be altered by phenological shifts and yearly conditions (Rudolf, 2019, Reinhardt et al, 2015). Our results indicate that year effects can create significant changes in the outcomes of species interactions. Specifically, Fowler's toadlet time to, weight at, and survivorship to metamorphosis when in the presence of American toadlets can vary significantly from one year to another (**Table 3.3)**. In terms of competitive exclusion, Fowler's toadlets reared in the presence of American toad tadpoles demonstrated either lower relative fitness with decreased survivorship or lower weight at metamorphosis. Thus, it may be beneficial for Fowler's toads to avoid breeding in ponds with American toads. However, while American toad tadpoles always appear to have a detrimental impact on co-occurring Fowler’s toad tadpoles in experimental conditions, the appearance of this effect can be notably different between years.

The mechanism of the American toad tadpole’s competitive advantage over Fowler’s toad tadpoles is unclear. There are many ways tadpoles can interact with competitors, ranging from increased toxin production (Bókony et al, 2017) to trophic plasticity (Altig et al, 2007; Caut et al, 2013; Arribas et al, 2015) and predation or cannibalism (Polis et al, 1989). It is possible that, in 2018, American toad tadpoles scavenged on Fowler’s toad tadpole remains or predated upon them directly, as Fowler’s toads had very low survivorship in the mixed species mesocosms, but no deceased tadpoles were ever found in the mesocosms despite daily checks. Notably, this phenomenon only occurred in the mixed species mesocosms and was not density dependent or a crowding effect, as mixed species and single species mesocosms had the same density of tadpoles.

While many species of tadpoles, including Bufonid tadpoles, are often considered to be herbivorous, it has been suggested that many species may consume animal matter such as zooplankton (Hamilton et al, 2012; Khan, 2014) and have trophic plasticity (Altig et al, 2007; Caut et al, 2013; Arribas et al, 2015). In 2018, American toad metamorphs were larger from mixed species mesocosms than single species mesocosms, possibly from predating or scavenging the remains of Fowler’s toad tadpoles. It has previously been noted that American toad tadpoles that scavenge on the remains of deceased conspecifics tend to develop faster than those that fed only on algae (Heinen and Abdella, 2005). Fowler’s toad tadpoles, on the other hand, are known to exhibit reduced survival to metamorphosis when in the presence of tadpoles of other species, including Gray Treefrogs (*Dryophytes chrysoscelis*) and Coastal Plain Toads *(Incilius nebulifer)*

(Parris and Cornelius, 2004; Vogel and Pechmann, 2010). Tadpoles of the closely related Woodhouse's Toad (*Anaxyrus woodhousii*) are known to take a longer time to reach metamorphosis and suffer lower survivorship when in the presence of larger tadpoles of the same species (Woodward, 1987). In addition, American toad tadpoles are known to have a higher survivorship, greater weight, and shorter time to metamorphosis when they are introduced to a mesocosm before other species (Alford and Wilbur, 1985), which may confer a fitness advantage over any co-occurring Fowler's toad tadpoles.

It was expected that disadvantaged tadpole species, in this case the Fowler's toad, would have lower survivorship and a lower weight at metamorphosis (Griffiths, 1991; Bardsley and Beebee, 1998; Cabrera-Guzmán et al, 2013). However, this is not what occurred, as the surviving Fowler's toadlets from mixed species mesocosms in 2018 were larger than their single species counterparts. It is possible that the reduced density of the surviving Fowler's toad tadpoles led to decreased intraspecific competition, resulting in their larger size. A similar result occurs when exposure to toxic Cane Toad (*Rhinella marina*) eggs decrease the survivorship, but increase the weight, of Ornate Burrowing Frog (*Platyplectrum ornatum*) metamorphs (Crossland et al, 2009). Fowler's toad tadpoles also had a lower weight at metamorphosis when raised with Gray Treefrog (*Dryophytes versicolor*) tadpoles than when raised alone (Parris and Cornelius, 2004). As well, Fowler's toad tadpoles grown at lower densities have been found to be larger as metamorphs, even when competitors are not present (Yagi and Green, 2016), indicating that intraspecific competition may be important in determining the growth of this species. Contrarily, when replicated in 2021, both Fowler's and American toadlets had a significantly lower weight at metamorphosis in mixed species mesocosms compared to single species mesocosms. As there was high survivorship in all mesocosms and thus no difference in tadpole density between single species and mixed species mesocosms, we can conclude that the lower weight of toadlets emerging from the mixed species mesocosms is likely due to intraspecific competition in 2021, and not diet or tadpole density.

Time to metamorphosis is also an indicator of fitness in toadlets, with toadlets emerging later being considered to have a lower fitness (Griffiths, 1991; Bardsley and Beebee, 1998). We noted that in single species mesocosms in 2021, toadlets emerging later in the season also tended to weigh less than their conspecifics who metamorphosed earlier from the same mesocosm. The mixed species mesocosms revealed a different trend, however. Not only did tadpoles metamorphose later in mixed species mesocosms, and only after most of the American toad tadpoles had completed metamorphosis, but they did not decrease in weight over time, a stark contrast to all other mesocosms (**Figure 3.2**). This could indicate that a competitive release occurred in 2021, allowing the Fowler's tadpoles to grow and reach metamorphosis only after the American toad tadpoles had left the mesocosms.

Notably, one higher fitness metric in toadlets may not compensate for another, lower one. Despite higher survivorship of Fowler's toad tadpoles in 2021 than 2018 when in mixed species mesocosms, their lower weight is still likely to be a detriment to their fitness, potentially leading to lower juvenile survival, lower reproductive success, and later age at reproduction (Woodward 1983, Smith 1987, Semlitsch et al. 1988). While some studies have found that newly metamorphed froglets can compensate for their small size with increased terrestrial growth (Bouchard et al., 2016), studies on Fowler's toads indicate that this species cannot (John-Alder and Morin, 1990; Yagi and Green, 2018), and thus this disadvantage as toadlets is likely to carry-over into adulthood.

The differences in species interactions between these two years have several possible causes. Phenological shifts in breeding time may impact competition outcomes, as tadpole developmental stage has been found to impact competition (Banks and Beebee, 1987) and the timing of tadpole hatching (Alfred and Wilbur, 1985; Lawler and Morin, 1993; Rudolf, 2018). Inter-annual variation also has the potential to alter pond ecosystems and species interactions. Year effects such as precipitation can alter the hydroperiod of ponds, shifting the interactions of species and of ecosystems (Reinhardt et al, 2015). Yearly changes in nutrient concentration or algal biomass in ponds could also result in different outcomes of competition and tadpole performance (Connelly et al. 2008).

Stable ecosystems are often the exception, and not the rule (Ricklefs and Schluter, 1993; Gómez-Rodríguez et al, 2010), and thus species interactions can change with shifts in their ecosystem. In the small pond ecosystems that Fowler's and American toad tadpoles inhabit, zooplankton and algal communities fluctuate in abundance and community structure within and between years, either with obvious climatic and environmental changes or without them (Dakos et al, 2009; Florencio et al, 2020). Tadpoles interact with zooplankton and algal communities, and changes in these communities or abundance would alter food availability to the tadpoles (Winder and Schindler, 2004; Connelly et al. 2008; Wood and Richardson, 2010, Hamilton et al., 2012), as well as the structure of their environment. Shifts in resources such as food sources can in turn alter the outcome of competition (Kupferberg, 1997; Rudolf and McCrory, 2018). With such changes in the surrounding aquatic communities and resources in lower guilds, it is logical that species interactions within the same guilds would also change, even within our semi-controlled conditions.

This study is an example of a shift in species interactions based on yearly conditions. While very few field studies are replicated, a striking 76% of those that are show significant inter-annual variation (Vaughn and Young, 2010). This inter-annual variation shouldn't be ignored, as stochasticity in pond environments can lead to higher biodiversity across the landscape (Chase, 2010) and alternate stable states (Chase, 2003; Chase, 2010). Inter-annual variation can also result in changes in community assembly, leading to shifts in community structure and function (MacDougall et al, 2008; Manning et al, 2018; Sarremejane et al, 2018; Werner et al, 2020). While we are beginning to see the importance of yearly conditions in studies, especially in plant communities, studies considering year effects on animals, particularly vertebrates, is lacking (Werner et al, 2020).

We ran our study twice, in semi-controlled conditions, and were still able to see drastically different results of species interactions. The effects of inter-annual variation are often treated as noise in more long-term studies, and overlooked (Rudolf, 2019). We show that a closer look at inter-annual variation is warranted when examining species interactions, as changes in yearly conditions, precipitation, surrounding aquatic communities, and phenology can all alter the outcome of competing species. Year effects are likely to become even more pronounced in coming years, as climate change results in more extreme weather events, accelerating the need to consider and examine inter-annual variation (Reinhardt, 2015; Rudolf, 2019; Stewart et al, 2019). It is necessary to replicate competition studies across years in order to obtain an improved understanding of how species may behave, develop, and interact differently in response to climatic changes and shifting conditions. Climate and environmental shifts such as habitat loss and food source variation are likely to alter competition dynamics in coming years, and studying inter-annual variation allows for a glimpse into resulting ecological impacts. This study system thus provides an excellent opportunity to observe the interactions between inter-annual effects and species competition, a dynamic relationship that holds potential relevance to a broad set of ecological systems.

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Tables

Table 3.1: Average air temperature, mesocosm water temperature, and rainfall data for Long Point, Ontario, in 2018 and 2021. Standard deviation is not shown for rainfall data as only total rainfall values were collected by Agricorp.

Year	Month	Average daily max air temp (°C) ± SD	Average daily mean air temp (°C) ± SD	Average daily min air temp (°C) ± SD	Average daily mean water temp (°C) ± SD	Total rainfall (mm)	Percent of normal rainfall (%)
2018	May	19.4 ± 4.7	14.7 ± 4.0	9.8 ± 4.0	23.9 ± 0.5	69.8	80
	June	21.5 ± 3.1	18.9 ± 2.6	16.4 ± 2.5	21.6 ± 2.9	93.4	120
	July	25.6 ± 2.2	23.2 ± 1.9	20.8 ± 2.0	27.9 ± 11.5	95.4	60
	August	24.8 ± 1.8	23.0 ± 1.5	21.2 ± 1.6	28.8 ± 0.7	83.0	120
2021	May	18.3 ± 5.6	12.5 ± 5.1	6.6 ± 5.0	17.1 ± 4.3	40.2	60
	June	25.1 ± 3.3	20.0 ± 3.2	15.0 ± 3.7	22.4 ± 2.5	158.4	120
	July	24.8 ± 2.2	20.6 ± 2.0	16.4 ± 2.1	23.3 ± 1.7	156.4	160
	August	28.3 ± 2.7	24.1 ± 2.7	19.9 ± 3.5	24.4 ± 0.7	74	-

Table 3.2: Average survivorship of American toad tadpoles and Fowler's toad tadpoles to metamorphosis in single species and mixed species mesocosms in 2018 and 2021.

Year	Species present	No. of mesocosms	Tadpoles per mesocosm	% Survivorship per mesocosm ± SD	Toadlet weight		Time to metamorphosis	
					Mean (g) ± SD	*n* (individuals)	Mean (days) ± SD	*n* (mesocosms)
2018	American toad	6	100	70.0 ± 23.20	0.09 ± 0.06	413	47.1 ± 2.93	6
	Fowler's toad	6	100	45.0 ± 17.09	0.12 ± 0.03	232	40.3 ± 2.50	6
	Mixed species: American toad	5	50	85.0 ± 11.78	0.12 ± 0.05	213	46.4 ± 2.07	5
	Mixed species: Fowler's toad	5	50	22.0 ± 11.72	0.14 ± 0.03	55	48.3 ± 4.19	5
2021	American toad	5	50	90.8 ± 9.01	0.18 ± 0.03	227	32.0 ± 0.00	5
	American toad	4	100	83.5 ± 13.77	0.14 ± 0.01	334	32.3 ± 0.50	4
	Fowler's toad	3	50	100 ± 0.00	0.25 ± 0.05	150	36.3 ± 0.60	3
	Fowler's toad	4	100	95.5 ± 9.00	0.21 ± 0.02	382	38.0 ± 0.80	4
	Mixed species: American toad	3	50	92.6 ± 9.45	0.13 ± 0.01	139	32.0 ± 0.00	3
	Mixed species: Fowler's toad	3	50	98.0 ± 2.00	0.17 ± 0.02	147	42.23 ± 4.90	3

Table 3.3: Summary of yearly condition trends and competition outcome for Fowler's toad tadpoles and American toad tadpoles in 2018 and 2021.

		2018	2021
Environmental variables	Overall	warmer and dryer	cooler and wetter
	Precipitation during May	low	low
	Precipitation during June	high	high
	Precipitation during July	low	high
Fowler's toadlet fitness metrics compared to single species mesocosms	Time to metamorphosis	longer	longer
	Weight at metamorphosis	higher	lower
	Survivorship	lower	same
American toadlet fitness metrics compared to single species mesocosms	Time to metamorphosis	same	same
	Weight at metamorphosis	higher	lower
	Survivorship	higher	higher

Figures

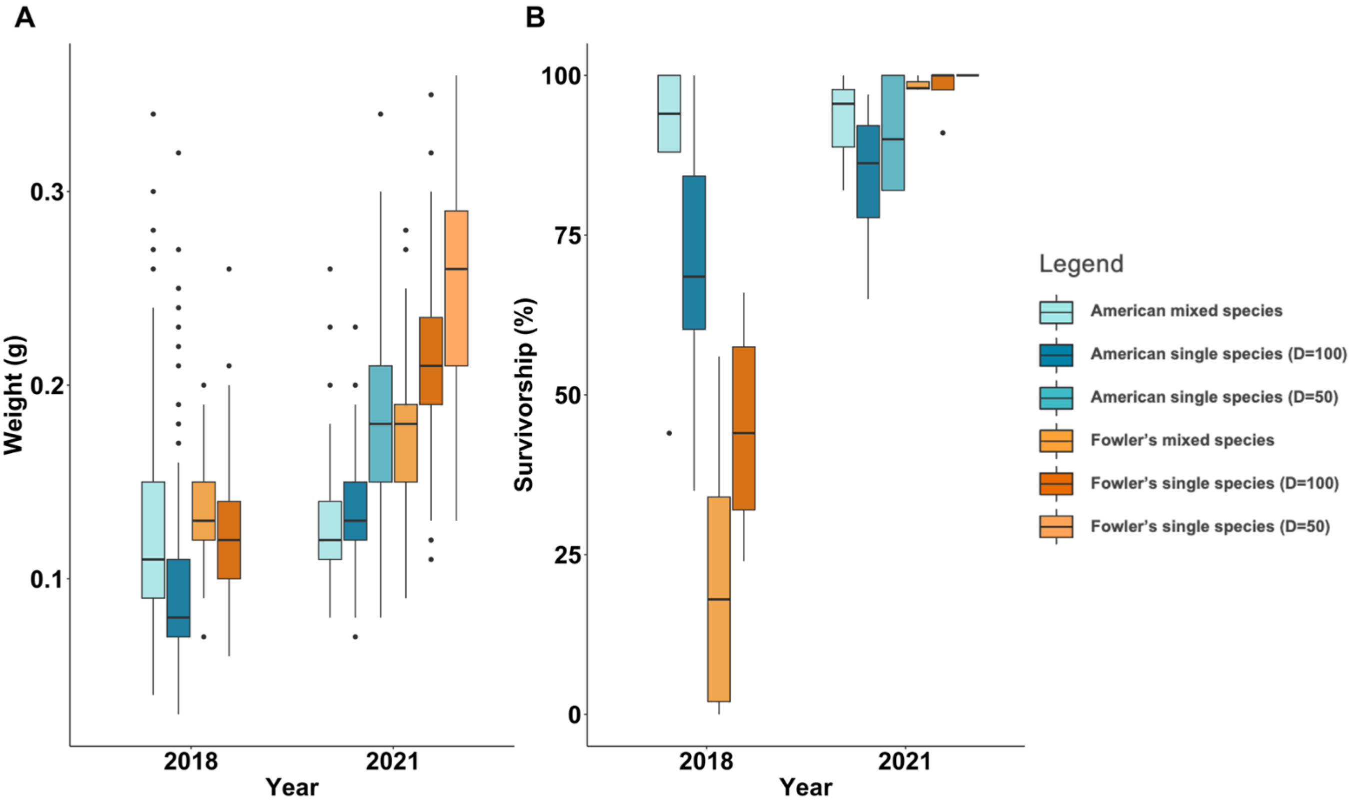

Figure 3.1: A) Average survivorship at metamorphosis of American toad tadpoles and Fowler's toad tadpoles in single species and mixed species mesocosms in 2018 and 2021. B) Average weight to metamorphosis of American toad tadpoles and Fowler's toad tadpoles in single species and mixed species mesocosms in 2018 and 2021.

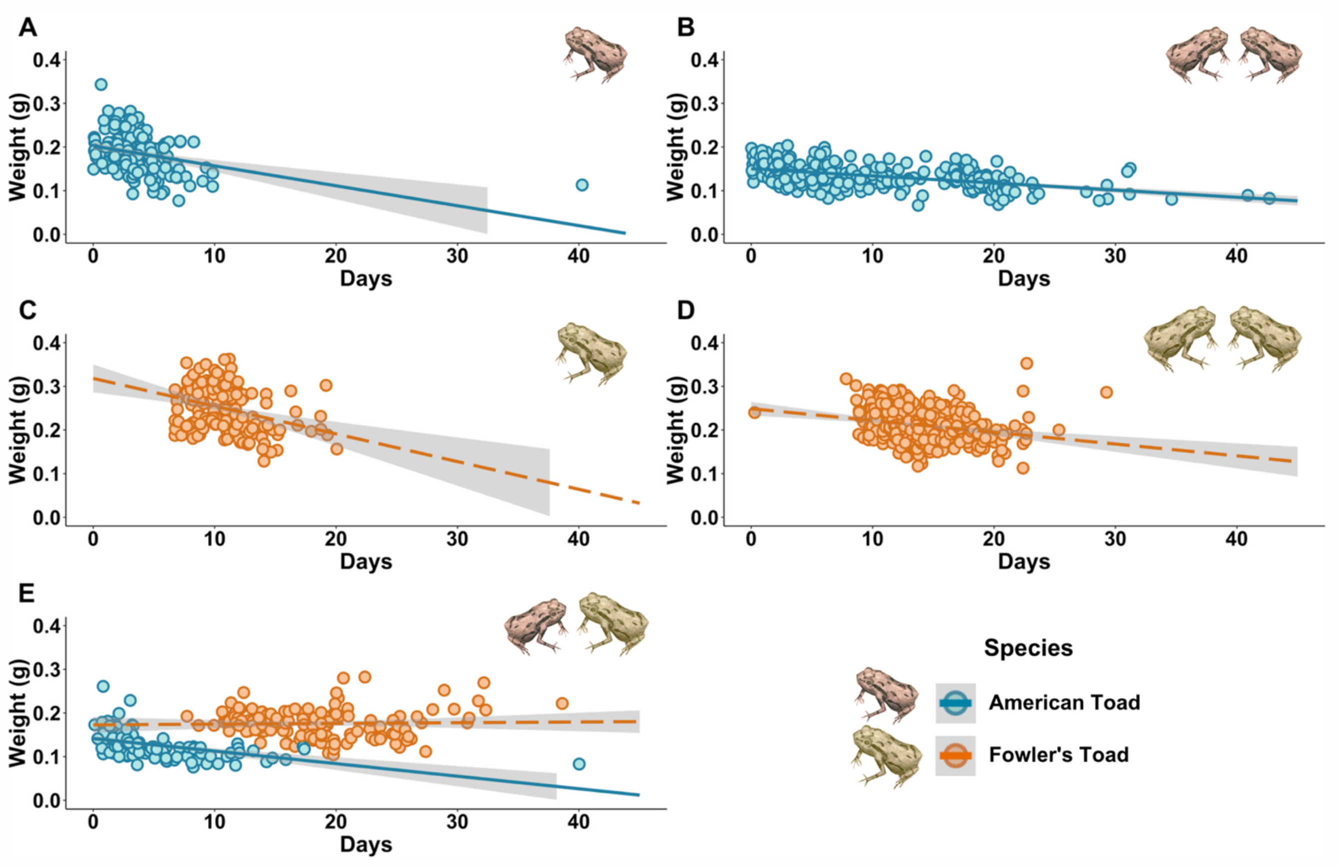

Figure 3.2: The weight of toadlets emerging from each experimental group of mesocosms in 2021, starting from the first day of toadlet emergence (June 16th). Each point represents an individual toadlet. A) Single species American toad tadpole mesocosms with a density of 50 tadpoles. B) Single species American toad tadpole mesocosms with a density of 100 tadpoles. C) Single species Fowler's toad tadpole mesocosms with a density of 50 tadpoles. D) Single species Fowler's toad tadpole mesocosms with a density of 100 tadpoles. E) Mixed species American toad and Fowler's toad tadpole mesocosms with a density of 100 tadpoles (50 of each species). *Illustrations by Jessica Ford.*

Connecting Statement Between Chapters 3 and 4

In Chapter 3, I show that American toad tadpoles have a detrimental effect on Fowler's toad tadpoles when raised in the same mesocosm, but the mechanism of this effect changed between the years of the study. In Chapter 1 and 2, I show that changes in tadpole community composition also changes the surrounding aquatic community through different interactions with the grazing tadpoles. These findings suggests that variation between years may change the way and amount that toad tadpoles effect their surrounding aquatic community. The interannual variation explored in Chapter 3 could also impact the concentration of nitrogen and phosphorus, algal biomass, and zooplankton diversity. While we know that temperature, precipitation level, and frost dates vary between years, the impact of this is generally considered noise, and not included in environmental studies.

To assess the impact of interannual variation on the impact of toad tadpoles on their environment, in Chapter 4 I determine if the impact of toad tadpoles was consistent across replicate years of 2018, 2019, and 2021. In all 3 years, the concentration of total nitrogen and total phosphorus, periphyton and phytoplankton biomass, and zooplankton diversity were analyzed when toad tadpoles were present and absent from experimental mesocosms. This study has implications for understanding the overlooked impact that interannual variation has on ecosystems, and indicates the importance of repeating environmental experiments across multiple years to attempt to understand the full scope of species interactions.

Chapter 4 | Listen to the noise; the importance of inter-annual variation when assessing ecological interactions

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Abstract

The structure of an ecosystem is rarely static. Particular species can be present or absent at different times, with sometimes strong ecological effects. Unpredictable weather events, including strong fluctuations in temperature and precipitation will also perturb ecosystems, potentially leading to significant inter-annual variation, especially in already dynamic systems such as small, freshwater ponds. In concert with this inter-annual, weather-related variation, the ecological consequences of the presence or absence of certain organisms that are known to have an impact on surrounding communities may vary across years. We assess if the ecological impact of toad tadpole presence or absence varies across years using experimental mesocosms and natural ponds. We recorded changes in temperature, precipitation, and nutrient concentration, which could all act as year effects. The impact of toad tadpoles varied within and across years in mesocosms, but no observed year effect was a consistent driver of these changes. Additionally, there was variation both within and across years in natural ponds, regardless of tadpole presence or absence, indicating that individual pond ecosystems were not consistent,

even in ponds that appeared very similar and close together in space. Our study highlights the importance of understanding interannual variation in order to comprehend the full scope of inter-species interactions, and contributes to a growing body of literature that emphasizes the importance of replicated ecological experiments.

Introduction

Ecosystems are constantly in flux (Yin and Rudolf, 2024). Changes in environmental conditions from one year to the next can result in extensive shifts in interspecific interactions (Riginos and Young, 2007; Veblen, 2008; Rudolf, 2018; Rudolf, 2019; Ford and Green, 2023), community structure (Pitt and Heady, 1978; Garcia et al, 1997; Bakker et al, 2003; Salmaso, 2005; Salmaso, 2010), and ecosystem function (Sala et al, 1988; Knapp and Smith, 2001). Interannual variation of this kind is often driven by year effects, which are variations in yearly conditions such as frost dates, precipitation, droughts, and water levels that can alter how communities are shaped and how they interact (Vaughn and Young, 2010; Werner et al, 2020).

Aquatic ecosystems are likely heavily influenced by year effects. Yearly changes in precipitation can result in changes in hydroperiod and pond fill time, which in turn can alter the richness, diversity, and biomass of phytoplankton and zooplankton in systems as diverse as saline lakes (García et al, 1997), freshwater ponds (Serrano and Toja, 2017), seasonal pools (Brooks, 2000; Florencio et al, 2020), and floodplain lakes (Simões et al, 2012). Yearly fluctuations in early season temperature in Canadian lakes, for example, are known to influence macrophyte biomass (Roony and Kalff, 2000). Yearly changes in conditions that alter nutrient deposition into lakes, such as precipitation impacting agricultural runoff or climate impacting timing of vertical mixing, also alter phytoplankton communities (Salmaso, 2005, 2010). Higher trophic levels are inevitably impacted by changes in these lower trophic levels, as noted in ostracod populations that fluctuate in accordance with yearly changes in algal biomass in lakes (Johnson and Weiderhom, 1992). Yet, despite their importance for understanding ecosystem functioning, and despite the studies listed above, year effects are rarely studied (Rudolf, 2018, 2019; Werner et al, 2020). A meta-analysis of over 500 scientific articles from ecological studies using field experiments or field studies between 1996 and 2008 found that only 5% of studies were replicated across years, with only one of them concerning a freshwater aquatic ecosystem (Vaughn and Young, 2010). This lack of replication may be due to resource constraints, time constraints (such as the length of a graduate degree) or an inability to commit resources for multi-year experiments (Vaughn and Young, 2010).

Freshwater ponds should provide an exceptionally good system in which to study inter-annual variation in ecosystem structure and ecological function. Freshwater ponds are dynamic and often ephemeral systems that can host a high proportion of regional biodiversity (Biggs et al, 2005; Williams et al, 2004; Reinhardt et al, 2015; Hill et al, 2021), and this biodiversity can shift between years (Hassall et al, 2012). Which species are present in ponds, and when those species arrive, can change the subsequent ecological interactions within the pond (Hassall et al, 2012; Zou and Rudolf, 2023). Among invertebrate populations in ponds, for example, establishment dates, interactions with higher trophic communities, and inter-specific interactions can all be affected by temporal variations in climatic conditions (Reinhardt et al, 2015). Shifts in the timing of phytoplankton community blooms in ponds have been observed to evoke changes in species composition of the zooplankton community even in successive years (Winder and Schindler, 2004). Although these between-year changes could be attributed to noise (Werner, 2020), they may actually be essential to how ecosystems work (Yin and Rudolf, 2024).

The ecologies of many freshwater pond habitats are influenced by the tadpoles of pond-breeding anurans (Hamilton et al, 2012; Buck et al, 2012; Arribas et al, 2014, Ford et al, *Chapter 1, this thesis*). Changes in tadpole communities can have profound feedback effects on local algal and zooplankton communities (Hamilton et al, 2012; Buck et al, 2012; Arribas et al, 2014). As amphibian larvae are so important in pond ecosystems, assessing their impact can be an excellent avenue by which to assess interannual variation and how that variation impacts species interactions across trophic levels, which is necessary knowledge for fully comprehending the ecology of small aquatic pond ecosystems.

Tadpoles of the North American toads in the genus *Anaxyrus* often live in relatively low diversity ponds where they comprise the main form of vertebrate biomass, especially in locations without fish (Wood and Richardson, 2010). Many North American toads preferentially breed in shallow, sandy, oligotrophic pools that have relatively simple food webs. In Long Point, Ontario, Fowler's toads (*Anaxyrus fowleri*) and American toads (*A. americanus*) breed in shallow, sandy, relatively low nutrient ponds. Long Point is an exceptionally dynamic ecosystem, with the structure and location of suitable breeding ponds changing each year, making this a valuable system in which to study interannual variation in aquatic ecosystems.

Tadpoles of both Fowler's toads and American toads have been found to exert significant effects on total phosphorus concentration through time, periphyton and phytoplankton biomass, and zooplankton community composition (Ford et al, *Chapter 1, this thesis*). Considering the importance of tadpoles in pond environments, it is plausible that the impact of their presence in relation to year effects may synergistically amplify interannual variation in species interactions across trophic levels, offering insights into the creation of ecological complexity among even fairly simple aquatic ecosystems. We used a combination of experimental aquatic mesocosms and natural ponds to assess if the impacts of Fowler's toad tadpoles and/or American toad tadpoles on their aquatic ecosystem was, or was not, consistent across years. If year effects influence small pond communities, then the responses of ecosystems to tadpole presence, and the structure of the ecosystems themselves, will vary between years. In addition, if year effects induce significant variation, then yearly replicates would not be statistically comparable, an important note for future experimental designs.

Methods:

Experimental setup – mesocosms

To assess the impact of toad tadpoles on aquatic ecosystems across years, we raised American toad and Fowler's toad tadpoles in outdoor mesocosms (Ford and Green, 2021) in 2018, 2019, and 2021 at Long Point Provincial Park, Ontario. We used Rubbermaid© structural foam cattle watering tanks as mesocosms which measured 63.50 cm L × 78.74 cm W × 134.6 cm H and were covered with a shade cloth to prevent insects and other animals from entering. Both American toads and Fowler's toads were present in the mesocosm study during 2018 and 2021, but due the late emergence of Fowler's toads at the site in 2019, only American toads were present in the mesocosm study that year. Due to the COVID-19 pandemic and the closure of access to the site, no experiments could be conducted in 2020. All tadpoles used were hatched from toad eggs collected from clutches laid in nearby local ponds or from egg clutches laid by amplectic pairs that we placed in a specialized breeding tank for 24 hours. In 2018, we collected three clutches of American toad eggs from a natural pond on May 3rd, and one clutch of Fowler's toad eggs from an amplectic pair on May 10th. In 2019, we collected one clutch of American toad eggs on May 2nd, and one clutch on May 4th, both from amplectic pairs that were placed in a bucket. In 2021, two clutches of American toad eggs were collected from natural ponds on May 4th and May 16th and one clutch of Fowler's toad eggs was collected from an amplectic pair on May 19th. Only one clutch of Fowler's toad eggs were located in 2018 and 2021 as Fowler's toads are endangered in Canada and did not have any large breeding choruses in either 2018 or 2021.

We obtained data on air temperature (maximum, minimum, and mean daily temperatures, in ºC) from the Government of Canada (https://climate.weather.gc.ca) for the Long Point Weather Station (Latitude 42.53° N | Longitude 80.05° W, approximately 28 km from our study site). Rainfall (total monthly precipitation, in mm, and percent average precipitation) data was sourced from Agricorp for the hamlet of Charlotteville, Ontario (now known as Walsh, Ontario) approximately 20 km from our study site. We measured water temperature (ºC) between noon and 2:00 PM each day using an EcoSense oxygen probe (YSI, DO200) in 2018 and a HANNA multiparameter probe (HANNA Instruments, HI98194) in 2019 and 2021. We assessed differences in air and water temperature across years using unpaired *t*-tests.

Each year, we established mesocosms in which to raise tadpoles according to a standardized protocol (Ford and Green, 2021). All tadpoles were raised in "nursery" mesocosms until Gosner stage 26 (Gosner, 1960). Any tadpoles remaining in "nursery" mesocosms after the appropriate number of tadpoles were transferred to experimental mesocosms were released at the point of origin, determined by where eggs or amplectic pairs were found (approximately 2.15 km from the mesocosm).

In 2018, we used 22 experimental mesocosms: six with 100 American toad tadpoles, six with 100 Fowler's toad tadpoles, five with a mixture of 50 tadpoles of each species, and 5 with no tadpoles (control). In 2019, we used 23 mesocosms to house tadpoles and of those, 7 housed 100 American toad tadpoles, and 5 mesocosms contained no tadpoles. In 2021, we used 24 experimental mesocosms: four with 50 Fowler's toad tadpoles, four with 100 Fowler's toad tadpoles, five with 50 American toad tadpoles, four with 100 American toad tadpoles, three with

50 tadpoles of each species, and 4 with no tadpoles. The number of replicates varied between years as we had different amounts of experimental groups and “nursery” or additional mesocosms within the 30 mesocosms worked with each year (Supplemental S.1).

Field sites

As Long Point is highly dynamic, with the location and existence of suitable breeding sites changing between years, we worked with different natural ponds in 2019 and 2021. In 2018, only mesocosms were included in the study. In 2019, we selected sites where we heard choruses of American toads, and nearby similar sites where we heard no choruses. Similarly, in 2021, we selected sites where we heard choruses of either American toads or Fowler’s toads. Both years, we used sites in the Port Rowan wetlands, in Long Point, and in the Long Point National Wildlife Area. In both years, we attempted so select sites with expected tadpole presence and absence that were within visual distance of each other (Supplemental S.2. for geographic locations).

Site 1 and Site 3 were not accessible in 2019 due to flooding, and Site 1.5 and Site 3.5 did not exist in 2019 as they were formed in a dune blowout in 2020. Additionally, Pond 4 and Pond 5 did not exist in 2021 due to insufficient rainfall.

Sample collection and analysis

We collected initial nutrient, phytoplankton, periphyton and zooplankton samples immediately before the addition of tadpoles to the experimental mesocosms. The three following samples were taken at approximately two-week intervals, with sample 1 collected when tadpoles were actively feeding, sample 2 when tadpoles were at roughly Gosner stage 36, and sample 3 as the toadlets began emerging from the water in the mesocosms. In 2021, the Post Tads sample time was collected 2 weeks after all tadpoles had left the mesocosms. As sample times depended on the development of the tadpoles, samples were not collected on the same dates each year.

Nutrient sampling

We collected surface water samples in 125 ml Nalgene tubes to be analyzed for total nitrogen and total phosphorus concentrations. We collected two samples per mesocosm at each sample time which were kept on ice until analysis.

Two subsamples were taken from each Nalgene tube, one of which was analyzed for total nitrogen and the other for total phosphorous. Samples were analyzed using sulfuric acid digestion by the Université de Montréal GRIL lab (Groupe de recherche interuniversitaire en limnologie).

Phytoplankton sampling

We collected phytoplankton samples between 8:30 AM and 10:00 PM in order to capture phytoplankton while it was near the surface. We collected a 500 mL sample using a labeled 1 L mason jar or opaque Nalgene 1 L bottle. All samples were immediately stored in the dark. We collected two samples from each mesocosm, and these duplicates were taken one day apart due to an insufficient amount of time to filter all samples in one day.

We filtered samples through 1.2 µm glass fiber paper filters (Whatman) using a Gask portable vacuum pump. We recorded the volume of water to be filtered. We folded the filters, wrapped them in tin foil, and kept them frozen at -20 ^{o}C for later chlorophyll extraction and pigment analysis. We filtered all samples within 18 hours of collection.

At a later date, we extracted algal samples in 8 mL of 95% ethanol that had been heated to 70^{o}C. We incubated samples for one hour before running them through a Trilogy Fluorometer. We transferred 1 mL of the sample to a 2 mL cuvette. Using a Trilogy Fluorometer, we measured samples for raw fluorescence, acidified samples with two drops of 10% HCl, and measured them once again to obtain before acidification and after acidification fluorescence.

Periphyton sampling

We collected periphyton by scraping it off the side of the tank. Periphyton was always collected from the South-facing wall of the mesocosm to ensure all samples had received an equal amount of light. We used a 90^{o} elbow pipe that was pressed against the side of the mesocosm to create a seal, and we used a toothbrush that had been modified to match the curve of the pipe to scrape periphyton off the side of the mesocosm, so that the periphyton was suspended in the water contained in the pipe. Approximately 200 mL of periphyton and water from within the elbow pipe was gathered using a baster.

In the field, due to a lack of any kind of wall to scrape periphyton off from, we collected sticks or other hard vegetation from the pond and scraped attached periphyton into 200 mL of pond water in a 1L mason jar. We measured the dimensions of the stick to record the surface area scraped.

We filtered, stored, extracted, and analyzed all periphyton samples in the same way as the phytoplankton samples.

Zooplankton sampling

We collected zooplankton using a 20 cm diameter student plankton net with a mesh size of 80 µm. We lifted the plankton net vertically through the water column of the mesocosms to create a cylinder of 0.0152 m^3. We then attached a 125 mL Nalgene tube to the base of the net, and the net was rinsed with water from a spray bottle so that all the zooplankton was rinsed into the Nalgene tube, which was then decanted into two 90 ml Nalgene bottles. We anesthetized zooplankton with club soda. We filtered zooplankton a second time through a 74 µm mesh screen and rinsed off using 70% ethanol to concentrate and preserve the sample.

We identified zooplankton species to the lowest taxonomic level possible using a dissecting microscope. Whole zooplankton samples were counted at one time – no subsamples were taken. We took photos of each zooplankton found in the sample with a microscope camera. All samples were returned to their 90 mL bottle and topped off with ethanol for long term storage.

Statistical and visual analysis

Nutrient samples

We plotted total Nitrogen and total Phosphorus for each experimental group per sample time. Due to the high variance between mesocosms and our low sample size, we focused on the general, observable trends of the nutrient concentrations to compare if trends held true for different experimental groups between years. Within years, we used a Levine's test of homogeneity to test if the variation of nutrient concentration amongst mesocosms was higher when tadpoles were absent, as was noted in 2018 (Chapter 1, *this thesis*)

Phytoplankton and periphyton samples

We used a standardized curve of raw fluorescence and known chlorophyll *a* values to determine the concentration of chlorophyll *a* in each sample. Again, due to the high variance between mesocosms and our low sample size, we focused on the general observable trends of algal biomass to compare if trends held true for different experimental groups between years.

For each experimental group each year, we determined if nutrient concentration was correlated with chlorophyll *a* concentration. We also visually compared these observed trends in nutrients and algal biomass between years to determine if there was interannual variation in top-down or bottom-up effects of tadpoles.

Zooplankton samples

We determined species richness, species evenness, and Shannon-Wiener diversity for each experimental group each year. We also determined which species were most common in the mesocosms each year. We visually compared these trends in richness, evenness, and diversity through time between years to observe if there was interannual variation in zooplankton community structure.

All statistical analyses were done in R v. 4.1.1 (R Core Team, 2021). Means are reported as mean ± standard error.

Results

Yearly climatic conditions

Although we had originally thought the 3 years of our study could be used as replicates, we realized that this was not the case. The slightly different experimental setups and sample sizes in the different years resulted in an uneven dataset. Any attempt to remedy the uneven dataset by removing samples (Zuur et al, 2009) left us with so little statistical power that our results would have been unreliable. Our uneven dataset, combined with the drastically different yearly conditions we uncovered, and our small sample size, as the years could not be considered replicates, made most statistical tests inappropriate with this dataset. However, our data were still valuable, if not in the manner originally intended. Observable trends were still discernable and could be described even if they were not wholly amenable to rigorous statistical analysis.

Comparatively, between the months of May and August, 2018 was warm and dry (mean air temperature: 20.0 °C ± 4.0, average monthly rainfall = 85.4 mm ± 11.7), 2019 was cool and wet (mean air temperature: 18.3 °C ± 5.1, average monthly rainfall = 126.0 mm ± 15.7), and 2021 was warm and damp (mean air temperature: 19.3 °C ± 4.9, average monthly rainfall = 107.3 mm ± 60.0) (**Table 4.1**). In fact, 2019 was a record-breaking year in terms of rainfall in Long Point and surrounding areas (Agricorp, 2019), with 503.8 mm falling between the months of May and August, causing roads and marshes in Long Point to flood and become inaccessible. Compared to 2018, 2019 had 61% more rain in May, 45% more rain in June, 50% more rain in July, and 36% more rain in August. Additionally, compared to 2021, 2019 saw 180% more rain in May, and 52% more rain in August. However, there was more rain still in 2021 than 2019 in June (17% increase) and July (10% increase).

Compared to other years, 2019 was also cold with the average air temperature being significantly lower in 2019 than 2018 in May (mean air temperature May 2018: 14.7°C ± 4.0, mean temperature May 2019: 11.6°C ± 2.9, $p = 0.0019$, $t = 3.27$, df = 54) and August (mean air temperature August 2018: 23.0°C ± 1.5, mean air temperature August 2019: 21.6°C ± 1.7, $p = 0.0018$, $t = 3.28$, df = 55), and significantly lower than 2021 in June (mean air temperature June

2019: 17.3°C ± 3.3, mean air temperature June 2021: 20.0°C ± 3.2, $p = 0.0012$, $t = 2.657$, df = 40) and August (mean air temperature August 2019: 21.6°C ± 1.7, mean air temperature August 2021: 24.1°C ± 2.7, $p < 0.0001$, $t = 4.27$, df = 57). The average water temperature in the mesocosms was also colder in 2019 being significantly colder than 2018 in May (mean water temperature May 2018: 23.9°C ± 0.5, mean water temperature May 2019: 16.0°C ± 2.1, $p < 0.0001$, $t = 14.95$, df = 138) and July (mean water temperature July 2018: 27.9°C ± 11.5, mean water temperature July 2019: 21.4°C ± 11.8, $p < 0.0001$, $t = 4.12$, df =1099), and significantly colder than 2021 in May (mean water temperature May 2019: 16.0°C ± 2.1, mean water temperature May 2021: 17.1°C ± 4.3, $p = 0.007$, $t = 2.71$, df = 406).

In 2018 there was a heat wave and intense sun in July and August. The average air temperature in 2018 was significantly higher than 2021 in July (mean air temperature July 2018: 23.2°C ± 1.9, mean air temperature July 2021: 20.6°C ± 2.0, $p > 0.0001$, $t = 5.12$, df = 57). This heat wave further caused a spike in mesocosm water temperature to nearly 30 °C in the months of July (average mesocosm water temperature 27.9°C ± 11.5) and August (average mesocosm water temperature: 28.8°C ± 0.7). In 2018, mesocosm water temperature was significantly higher than 2019 (*see above*) and 2021 in May (mean water temperature May 2018: 23.9°C ± 0.5, mean water temperature May 2021: 17.1 ± 4.3, $p > 0.0001$, $t = 6.31$, df = 298), July (mean water temperature July 2018: 27.9°C ± 11.5, mean water temperature July 2021: 23.3°C ± 1.7 $p > 0.0001$, $t = 7.68$, df = 988), and August (mean water temperature August 2018: 28.8°C ± 0.7, mean water temperature August 2021°C: 24.4 ± 0.7, $p > 0.0001$, $t = 24.0$, df = 67).

In the field sites, temperature surprisingly did not vary much with pond location in the same year (**Figure 4.1, Figure 4.2**). Natural pond surface temperature did vary between years, with 2019 being significantly colder than 2021in May (average surface temperature May 2019: 19.2^{o}C ± 2.1, average surface temperature May 2021: 26.3^{o}C ± 1.8, $p > 0.0001$, $t = 9.19$, df = 38), but significantly warmer than 2021in July (average surface temperature July 2019: 28.3^{o}C ± 0.5, average surface temperature July 2021: 25.8^{o}C ± 1.5 $p = 0.0361$, $t = 2.36$, df = 12).

Only 3 field sites were measured in both 2019 and 2021. When considering only those sites, we once again saw that 2019 was colder than 2021 in May (average surface water temperature May 2019: 20.3^{o}C ± 0.27, average surface water temperature May 2021: 25.0^{o}C ± 0.5), and water surface temperature was relatively consistent between ponds in the same year (**Figure 4.3**).

Nutrients

Mesocosms

In 2018, total nitrogen concentration trended downwards regardless of tadpole presence between May and August, but this observed trend did not hold true in 2019 or 2021 (**Figure 4.4**). In all years, there was no notable difference in total nitrogen concentration in mesocosms that had toad tadpoles, and those that did not. There was, however, a notable difference in the amount of variation of phosphorus when tadpoles were present or absent, as there was significantly more variation in control mesocosms than single species American toad mesocosms (Levene's test of homogeneity: F = 7.32, df = 43, $p < 0.01$). This trend did not hold true in 2019 or 2021, however, with no significant differences in the variation of total phosphorus concentration when tadpoles were present or absent in those years. Between May and August in 2021, regardless of tadpole presence or absence, there was greater variation in nutrient concentration observed between mesocosms than in 2018 or 2019 (**Figure 4.5**).

Field Sites

There were notable differences in the concentration of total nitrogen and total phosphorus in the same ponds between 2019 and 2021 (**Figure 4.6**). Both total nitrogen and total phosphorus were observed higher at the initial time point, collected in May, in 2021 than they were in 2019. The concentrations of total nitrogen and total phosphorus began higher in the Port Rowan Wetlands in 2021 (Port Rowan Wetland Pond 1: TN = 856.9μg/L, TP = 58.6μg/L; Port Rowan Wetland Pond 2: TN = 1119.2μg/L, TP = 85.5μg/L) than in 2019 (Port Rowan Wetland Pond 1: TN = 559.3μg/L, TP = 34.8μg/L; Port Rowan Wetland Pond 2: TN = 530.8μg/L, TP = 57.3μg/L). In the Crown Marsh, where the Winston site is located, total nitrogen and total phosphorus concentration began higher in 2021 (TN = 2571.0μg/L, TP = 48.6μg/L) than in 2019 (TN = 962.8μg/L, TP = 37/0μg/L), but after the Initial time point, by Sample 1 (approximately a two week time period), the observed nutrient concentration decreased to the level it was in 2019 at Sample 1, or dipped below the 2019 Sample 1 value (2019 Sample 1: TN = 1110.4μg/L, TP = 53.4μg/L, 2021 Sample 1: TN = 951/6μg/L, TP = 28.6μg/L) (**Figure 4.6**).

Notably, total nitrogen and phosphorus concentration also varied greatly within years. Despite being within visual distance of one another, the nutrient concentration of natural ponds varied greatly, as did the trends they exhibited through time (**Figure 4.7, Figure 4.8, and Figure 4.9**). In both 2019 and 2021, the observed concentration of total nitrogen and total phosphorus behaved differently depending on which pond it was in, regardless of location, tadpole presence or absence, or which species of tadpole was present or absent. For example, between Sample 1 and Sample 2 in 2019, Total Phosphorus decreased at the Winston site (Sample 1 TP = 53.4µg/L, Sample 2 TP = 8.2µg/L)and slightly decreased at Port Rowan Wetlands Pond 2 (Sample 1 TP = 80.6 µg/L, Sample 2 TP = 73.2µg/L), but increased at Port Rowan Wetlands Site 1 (Sample 1 TP 59.4µg/L = , Sample 2 TP =95.3µg/L). In 2021, however, between Sample 1 and Sample 2, Total Phosphorus decreased slightly at the Winston site (Sample 1 TP = 28.6µg/L , Sample 2 TP = 18.5µg/L), but increased at Port Rowan Wetlands Site 2 (Sample 1 TP = 117.5µg/L, Sample 2 TP = 162.2µg/L) and Port Rowan Wetlands Site 1 (Sample 1 TP = 53.2µg/L , Sample 2 TP = 139.4µg/L). These changes were not driven by tadpole presence, as Port Rowan Wetlands Pond 1 and Winston had toad tadpoles, and Port Rowan Wetlands Pond 2 did not.

Algal Biomass

Mesocosms

In 2018 and 2021, the observed chlorophyll *a* concentration of both phytoplankton and periphyton was higher in control mesocosms, where tadpoles were absent, for the majority of sample times when compared with mesocosms that had 100 American toad tadpoles, with only the Initial time point for phytoplankton in 2018, and the Initial and Sample 2 time points in 2021 being exceptions to this trend (**Figure 4.10**). While this observed trend held true for phytoplankton in 2019, it did not hold true for periphyton, where chlorophyll concentration was higher in mesocosms with 100 American toad tadpoles than in mesocosms with no tadpoles at the Initial, Sample 2, and Sample 3 time points. Notably, at Sample 1, collected approximately 2 weeks after tadpoles were added to the mesocosms, algal biomass was always higher when tadpoles were absent in 2018 (Phytoplankton chl *a* concentration: American toads: 3.7µg/L ± 1.6, Control: 4.2µg/L ± 1.1, Periphyton chl *a* concentration: American = 28.0µg/L ± 4.6, Control

=79.0μg/L ± 17.5), 2019 (Phytoplankton chl *a* concentration: American toads: 5.9μg/L ± 8.4, Control: 10.1μg/L ± 16.3, Periphyton chl *a* concentration: American = 72.5μg/L ± 163.5, Control =99.4μg/L ± 78.8), and 2021 (Phytoplankton chl *a* concentration: American toads: 7.4μg/L ± 9.0, Control: 19.0μg/L ± 12.0, Periphyton chl *a* concentration: American = 6.4μg/L ± 4.0, Control =144.8μg/L ± 194.4)

In 2018, algal biomass increased through time whether tadpoles were present or absent, but increased to a greater extent when tadpoles were absent. This observed trend did not hold true across years, however, as phytoplankton biomass decreased through time in 2019 and 2021 regardless of tadpole presence or absence. Periphyton biomass varied between increasing and decreasing between sample points in 2019 and 2021 (**Figure 4.10**).

Field sites

Algal biomass varied dramatically across geographically similar ponds within the same year, and between years, with no obvious trends. The field sites also did not follow the same trends as the mesocosms within the same year. Instead, algal biomass concentration, and the observed trends of algal biomass through time, varied dramatically depending on which pond they were in (**Figure 4.11, 4.12, and 4.13**). In 2021, there was always a smaller and lower range of algal biomass in Site 1, 1.5, 3, and 3.5 (min chl *a* concentration = 1.3μg, max chl *a* concentration = 63.1μg), which had sandy substrate, than any other, non-sandy sites (min chl *a* concentration = 4.3μg, max chl *a* concentration = 228.4μg).

Algal biomass and nutrients

The concentration of algal biomass and nutrients was consistently more highly correlated, as measured by a higher R^2 value, in control mesocosms, where tadpoles were absent, than in mesocosms with 100 American toad tadpoles present (**Table 4.2**). The correlation between algal biomass and nutrient concentration was higher in control mesocosms than mesocosms with tadpoles 65% of the time (31/64 correlations). While generally, the R^2 value between total phosphorus and total nitrogen concentration was higher when tadpoles were absent, the R^2 values were often low regardless.

Zooplankton diversity

Mesocosms

In 2018, zooplankton diversity was notably higher when American toad tadpoles were present, than when they were absent at the Sample 1 and Sample 2 time points (mean zooplankton Shannon diversity at Sample 1: American = 0.67 bits ± 0.40 Control = 0.27 ± 0.40; mean Shannon diversity at Sample 2: American = 0.50 bits ± 0.30 , Control: 0.17 ± 0.31) (**Figure 4.14**). In 2019, no such trend emerged, with little difference in zooplankton Shannon diversity between mesocosms with tadpoles and those without (mean zooplankton Shannon diversity at Sample 1: American = 0.52 bits ± 0.30 Control = 0.68 ± 0.21; mean Shannon diversity at Sample 2: American = 0.81 bits ± 0.50 , Control = 0.77 ± 0.52) (**Figure 4.14**). In 2021, there was greater Shannon diversity when American tadpoles were present in mesocosms during the Initial and Sample 1 time points, as there was no zooplankton diversity in the mesocosms without tadpoles, with only 1 cyclopoid being recorded in the control mesocosms at the Initial time point, and only 10 cyclopoids at Sample 1 (mean zooplankton Shannon diversity at Sample 1: American = 0.21 bits ± 0.37 Control = 0 bits ± 0; mean Shannon diversity at

Sample 2: American = 0.88 bits ± 0.23 , Control = 0 bits ± 0). However, zooplankton Shannon diversity was higher in control mesocosms at the Sample 2 time point, before again dropping below the Shannon diversity of the mesocosms with American toad tadpoles (mean zooplankton Shannon diversity at Sample 2: American = 0.70 bits ± 0.56 Control = 0.81 bits ± 047; mean Shannon diversity Post Tads: American = 0.97 bits ± 0.40 , Control = 0.45 bits ± 0.64) (**Figure 4.14**). We also noted differences in the dominant families in the zooplankton communities between years. In 2018 and 2021, the zooplankton communities were dominated by Bosminid cladocerans, regardless of tadpole presence or absence. In 2019, however, the zooplankton communities in the mesocosm were dominated by Daphniid cladocerans, specifically *Scapholeberis mucronata.*

Field sites

In 2019, there were very few, if any, zooplankton found in the samples from the field sites. Only two field sites, Port Rowan Wetland Ponds 3 and 4, had any zooplankton diversity, with Port Rowan Wetland Pond 3 containing the 1 individual zooplankton in the family Bosminidae, 3 Chrydoridae, and 3 Cyclopoida, and Port Rowan Wetland Pond 4 containing 1 Bosminidae, 3 Chrydoridae, 3 Cyclopoida, 1 Daphniidae, and 1 Leptodoridae during the Sample 2 time point. Many more species of zooplankton in the families Bosminidae, Chrydoridae, Cyclopoida, Daphniidae, and Sididae were found in the field sites in 2021, generating higher Shannon diversities. Zooplankton Shannon diversity varied widely between field sites in 2021 between May and August (American: minimum zooplankton Shannon Diversity = 0.21 bits, maximum zooplankton Shannon Diversity = 0.97 bits; Control: minimum zooplankton Shannon Diversity = 0 bits, maximum zooplankton Shannon Diversity = 0.82 bits) with no consistent patterns, regardless of tadpole presence or absence (**Figure 4.15**).

Discussion

We found strong evidence for inter-annual variation in our semi-controlled mesocosm experiment, and in local natural ponds, between 2018, 2019, and 2021. Intense rain in 2019 and wet periods in 2021 may have been responsible for the decrease in phytoplankton through time, as there was little sun on rainy days, which phytoplankton require to grow (Marra et al, 1982), and the rainstorms in Long Point often lasted for days at a time. 2019 was also colder than other years, and while the high specific heat capacity of water tends to temper the effects of air temperature on ponds, the still cooler water temperatures may have reduced algal growth. Although phytoplankton communities tend to be more heavily influenced by nutrient concentrations than temperature (Moss, 1992; Salmaso, 2010), we did not find algal biomass to be consistently correlated with nutrient concentration in the mesocosms, even when no tadpoles were present. Likewise, increased nutrients should increase algal biomass (Salmaso, 2010), but this trend was not evident in 2018. In 2021, there was a stronger correlation between algal biomass and nutrients when tadpoles were absent than when tadpoles were present. This lack of correlation between algal biomass and nutrients could be explained by a strong top-down effect of tadpole grazing in 2021, which would interfere with the bottom-up effect of nutrient changes. The lack of a correlation between nutrients and algal biomass in 2018 and 2019, however, indicates that there may be another driver of algal biomass that we did not measure in our experiment. Meanwhile, zooplankton diversity was higher when tadpoles were present in 2018 and 2021, but not in 2019. The record amounts of rain in 2019 may have diluted out many of the effects of tadpoles, and made all the mesocosms more similar to one another regardless of tadpole presence or absence.

The expected drivers, which were temperature, precipitation, and nutrient concentration, did not consistently explain the large amount of variation we observed in our mesocosms and field sites both within and across years. As many ponds establish anew each year, which species dominate may change due to what Dakos et al. (2009) called "seasonally entrained chaos" even without an obvious year effect as a driver. Ponds in seasonal environments establish again each spring, influenced by which species were present in autumn, and those species of algae and zooplankton that are first to establish due purely to chance (Dakos et al, 2009). The relative proportions of different species of algae and zooplankton establishing by chance early in the season can result in interannual variation, even without any changes in weather (Dakos et al, 2009). Of course, there *were* still changes in weather conditions, primarily temperature and precipitation, across our three years, which are both year effects that cause inter-annual variation. In addition, we know that the presence of American toad and Fowler's toad tadpoles influences aquatic environments (Chapter 1*, this thesis*), and thus whether tadpoles are present in an ecosystem may further amplify inter-annual variation and seasonally entrained chaos.

The combined effects of seasonally entrained chaos, year effects, and tadpole presence or absence, logically, ensure that inter-annual variation will be an inevitable property of these pond ecosystems. Such inter-annual variation is often treated as noise in long-term ecosystem studies (Werner et al, 2020), but this variation is just as likely a feature, and not a flaw, in a system of small ponds across a landscape. Variation in nutrient concentration, algal biomass, and zooplankton diversity between individual ponds across a landscape would increase habitat heterogeneity, thus creating more habitats that may be more suitable to more species and therefore, in theory, increasing overall biodiversity (MacArthur and MacArthur, 1961; Cramer and Willig, 2005; Douda et al, 2012; Hamm and Drossel, 2017). The increase in possible niches with habitat heterogeneity would increase the number of species that would find the habitat suitable (Hamm and Drossel, 2017). If one pond were inaccessible, occupied, or indeed too different due to inter-annual variation, another may still be adequate on the same landscape. Because ponds hold such a high proportion of regional biodiversity and are often ephemeral (Biggs et al, 2005; Williams et al, 2004; Reinhardt et al, 2015; Hill et al, 2021), inter-annual changes in biodiversity may be even more pronounced.

Because of these same features of interannual variation and biodiversity, such that individual ponds may change significantly even between successive years (Winder and Schindler, 2004), a particular pond may be assigned a higher or lower conservation value depending merely on the year it was evaluated (Hassall et al, 2012) and ponds that are important for conservation may thus be overlooked in surveys conducted during only a single year. As such, the number of ponds necessary for a fully functioning ecosystem, considering different yearly conditions, may be underestimated. Additionally, with changing climates and extreme weather events becoming more common, it is no longer tenable to simply attribute different results in species interactions between years to noise (Rudolf, 2019). Instead, it is necessary to apply insights on how species respond to changes in yearly conditions towards understanding changes in species interactions, biodiversity patterns, and potential responses to future climatic shifts (Rudolf, 2019).

Ecological studies that do not replicate their experiments across years or within a season risk showing only a “snapshot” of that ecosystem during that single year or time point (McMeans et al, 2015). Not replicating an ecological study limits our ability to understand how communities change through time in response to external or climactic-driven changes, which is fundamental to our understanding of ecosystem dynamics (Yin and Rudolf, 2024). Yet, despite the clear importance of understanding yearly variation in ecology, field experiments are rarely repeated (Rudolf, 2018; Rudolf, 2019; Vaughn and Young, 2010; Werner et al, 2020). Only 5% of field experiments are repeated across multiple years but, of those, 76% show significant inter-annual variation (Vaughn and Young, 2010). Field ecology has been considered to be in a “replication crisis” (Filazzola and Cahill, 2021), with an overall failure to repeat experiments within a study, or to repeat studies altogether. While ecologists generally consider the repetition of studies to be important (Fraser et al, 2020), challenges in conducting, obtaining funding for, and then publishing repeated studies limit replication in ecological research (Fraser et al, 2020). Originality is held in high regard in funding requests, publications, and graduate student projects, leading to little incentive for scientists to redo a study that has already been completed (Fraser et al, 2020). Due to the importance of inter-annual variation and the need for more studies to understand the full scope of species interactions (Rudolf, 2018; Rudolf, 2019; Vaughn and Young, 2010; Werner et al, 2020), this partiality towards originality should be re-evaluated. Databases could be created to deposit yearly data for researchers using the same field site to compile data across years. Additionally, a general framework for studies assessing temporal changes would allow data to be more comparable between studies (Yin and Rudolf, 2024). Both these factors would increase our ability to study inter-annual variation, even within the current context of a lack of resources for repeated studies.

Our results can serve as an important case study highlighting how much variation can occur across years, even in semi-controlled mesocosms, and especially in natural ecosystems. In only three years, we captured three very different states of pond ecosystems. In 2018, we showed that tadpoles can have profound influences on the surrounding aquatic communities, but if we had conducted that same study in only 2019, we would have concluded that tadpoles do not have much of an influence on their aquatic environment, and if we had only conducted the study in 2021 we would have obtained mixed results. Our current understanding of ecological communities relies on one-off experiments that assume ecosystems are static, instead of recognizing the reality of their inherent dynamism (Yin and Rudolf, 2024). While non-repeated experiments are still useful, they provide only part of the full picture. Inter-annual variation can have such strong effects on ecological functions that the result of field studies can be strongly contingent on the year in which the study was conducted (Vaughn and Young, 2010). Knowing how ecosystems function across years, instead of only within a field season, is thus crucial for understanding the full scope of ecological interactions (Vaughn and Young, 2010). We show that replication is essential for beginning to understand the role of a species in an ecosystem, especially in highly dynamic systems such as freshwater ponds.

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Tables

Table 4.1: Average air temperature, mesocosm water temperature, and rainfall data for Long Point, Ontario, in 2018, 2019, and 2021. We collected water temperature ourselves in the mesocosms, air temperature was collected by the government of Canada, and rainfall data was collected by Agricorp. Standard deviation is not shown for rainfall data as only total rainfall values were collected by Agricorp and only the averages are available.

Year	Month	Average daily max air temp (ºC) ± SD	Average daily mean air temp (ºC) ± SD	Average daily min air temp (ºC) ± SD	Average daily mean water temp in mesocosms (ºC) ± SD	Total rainfall (mm)	Percent of normal rainfall (%)
2018	May	19.4 ± 4.7	14.7 ± 4.0	9.8 ± 4.0	23.9 ± 0.5	69.8	80
	June	21.5 ± 3.1	18.9 ± 2.6	16.4 ± 2.5	21.6 ± 2.9	93.4	120
	July	25.6 ± 2.2	23.2 ± 1.9	20.8 ± 2.0	27.9 ± 11.5	95.4	60
	August	24.8 ± 1.8	23.0 ± 1.5	21.2 ± 1.6	28.8 ± 0.7	83.0	120
2019	May	15.4 ± 4.3	11.6 ± 2.9	7.8 ± 2.2	16.0 ± 2.1	112.6	100
	June	20.5 ± 3.8	17.3 ± 3.3	14.0 ± 3.0	21.4 ± 11.8	135.8	120
	July	25.1 ± 1.7	22.7 ± 1.6	20.4 ± 1.7	25.6 ± 1.3	142.8	140
	August	23.7 ± 1.7	21.6 ± 1.7	19.5 ± 1.9	-	112.6	140
2021	May	18.3 ± 5.6	12.5 ± 5.1	6.6 ± 5.0	17.1 ± 4.3	40.2	60
	June	25.1 ± 3.3	20.0 ± 3.2	15.0 ± 3.7	22.4 ± 2.5	158.4	120
	July	24.8 ± 2.2	20.6 ± 2.0	16.4 ± 2.1	23.3 ± 1.7	156.4	160
	August	28.3 ± 2.7	24.1 ± 2.7	19.9 ± 3.5	24.4 ± 0.7	74	-

Table 4.2: Correlations (R^2) between nutrients and algal biomass in the mesocosms in 2018, 2019, and 2021 across sample times.

Year	Sample time	Tank composition	Phytoplankton and nitrogen	Phytoplankton and phosphorus	Periphyton and nitrogen	Periphyton and phosphorus
2018	Initial	Control	0.0776	0.0027	0.1074	0.0528
		American	0.0003	0.0169	0.0587	0.0419
	Sample 1	Control	0.4929	0.7885	0.2299	0.0082
		American	0.1346	0.2098	0.3319	0.2676
	Sample 2	Control	0.2171	0.4975	0.0199	0.172
		American	0.0778	0.3526	0.0025	0.3864
	Sample 3	Control	-0.9072	0.7558	0.4312	0.1079
		American	0.6826	0.8481	0.1746	0.2003
2019	Initial	Control	0.2815	0.3858	0.192	0.0172
		American	0.5829	0.546	0.0013	0.1014
	Sample 1	Control	0.5325	0.9699	0.5264	0.0545
		American	0.0014	0.0057	0.0004	0.0019
	Sample 2	Control	0.7899	0.3684	-0.0383	0.2253
		American	0.2084	0.1037	0.0728	0.0018
	Sample 3	Control	0.0009	0.1058	-0.5627	0.0934
		American	-0.0007	0.0294	-0.9156	0.3816
2021	Initial	Control	0.1633	0.1136	0.7194	0.5971
		American	-0.7725	-0.1667	-0.3358	0.0115
	Sample 1	Control	0.9305	0.7518	0.7538	0.9326
		American	0.8391	0.9283	-0.916	-0.7302
	Sample 2	Control	0.6505	0.1204	0.9951	0.619
		American	-0.0464	0.117	0.2646	0.0473
	Post tads	Control	0.9587	0.7027	NA	NA
		American	0.4943	0.1085	0.1928	0.5397

Figures

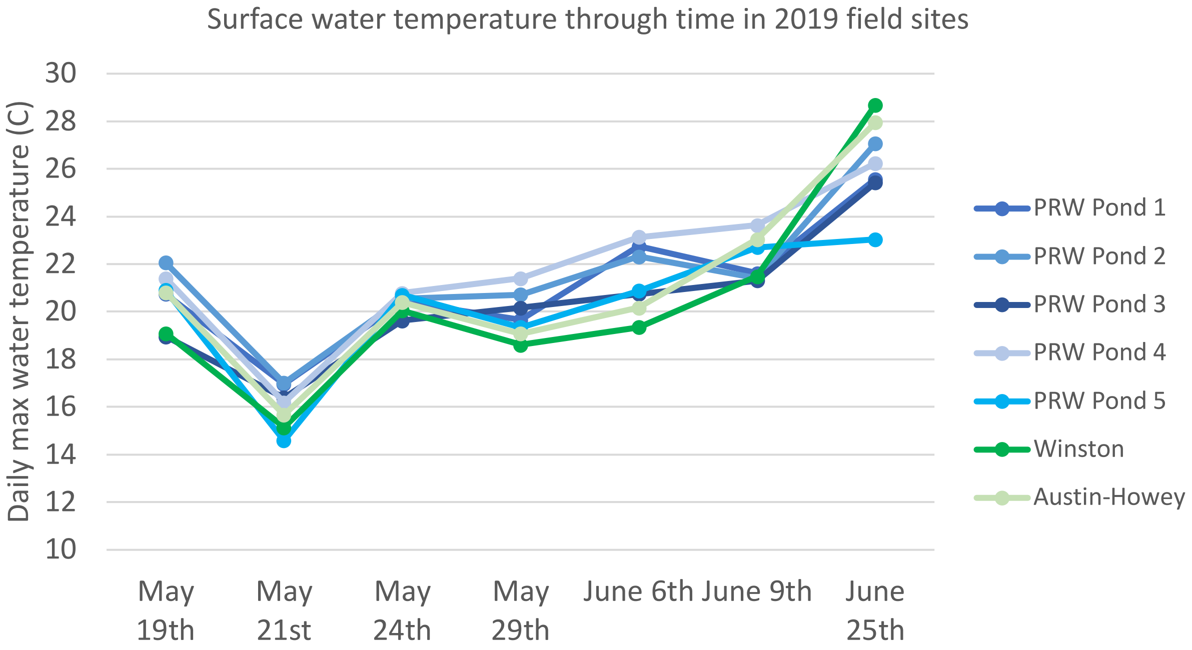


Figure 4.1: Variation in the surface water temperature (ºC) of natural ponds through time in Port Rowan and Long Point, Ontario in 2019. Shades of blue denote ponds that did not have tadpoles, shades of green denote ponds that had American toad tadpoles.

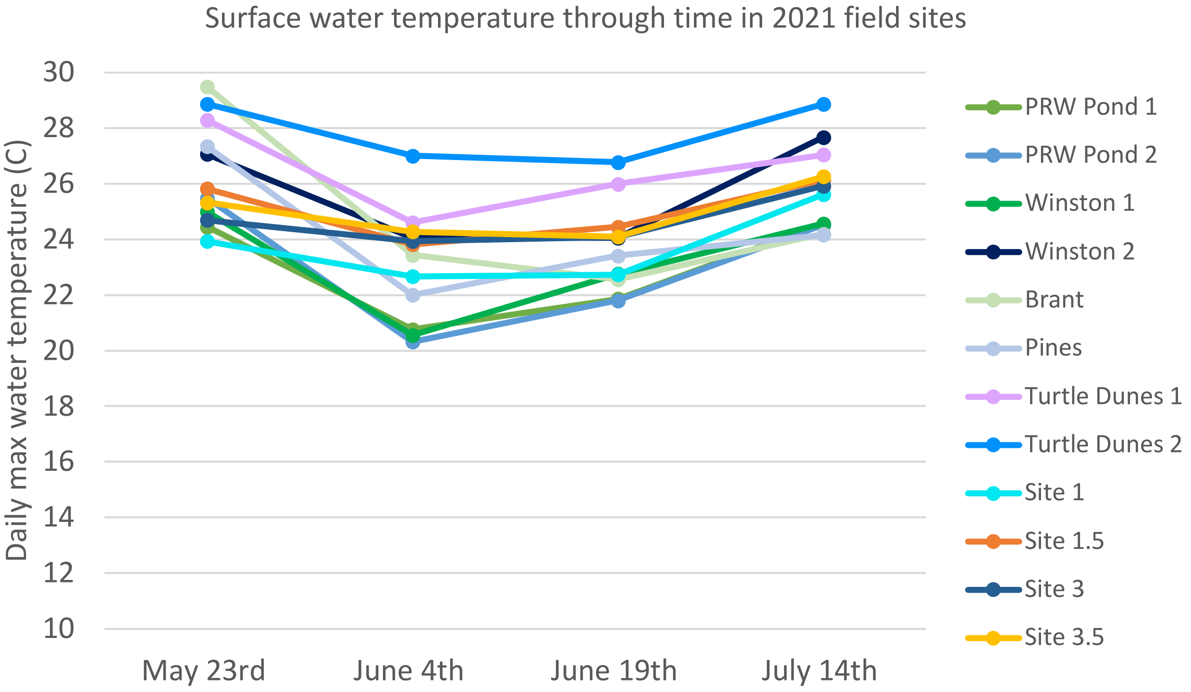


Figure 4.2: Variation in the surface water temperature (°C) of natural ponds through time in Port Rowan and Long Point, Ontario in 2021. Shades of blue denote ponds that did not have tadpoles, shades of green denote ponds that had American toad tadpoles, shades of pink denote ponds with both species of tadpoles, and shades of orange/yellow denote ponds with Fowler's toad tadpoles.

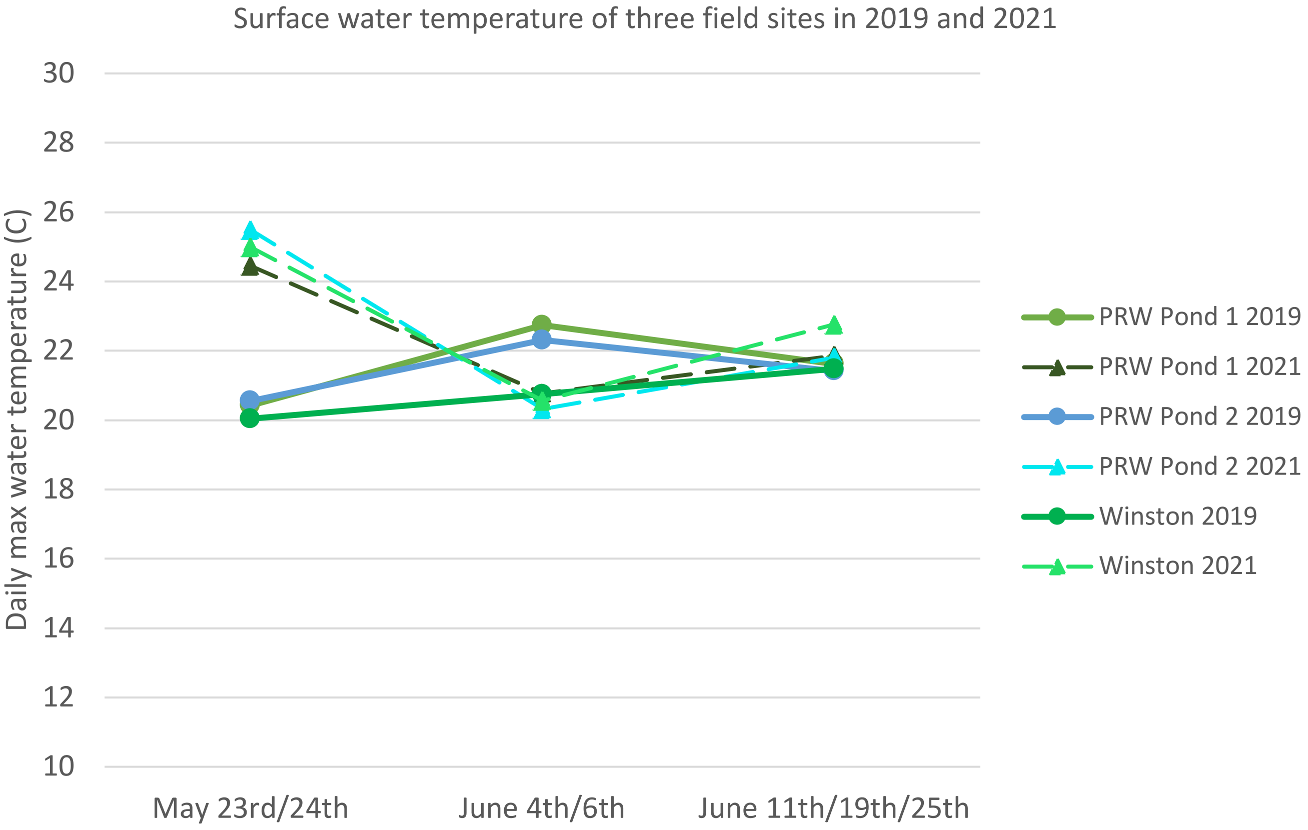


Figure 4.3: Surface water temperature (°C) of the same natural ponds through time in Port Rowan and Long Point, Ontario in 2019 and 2021. Shades of blue denote ponds that did not have tadpoles, shades of green denote ponds that had American toad tadpoles.

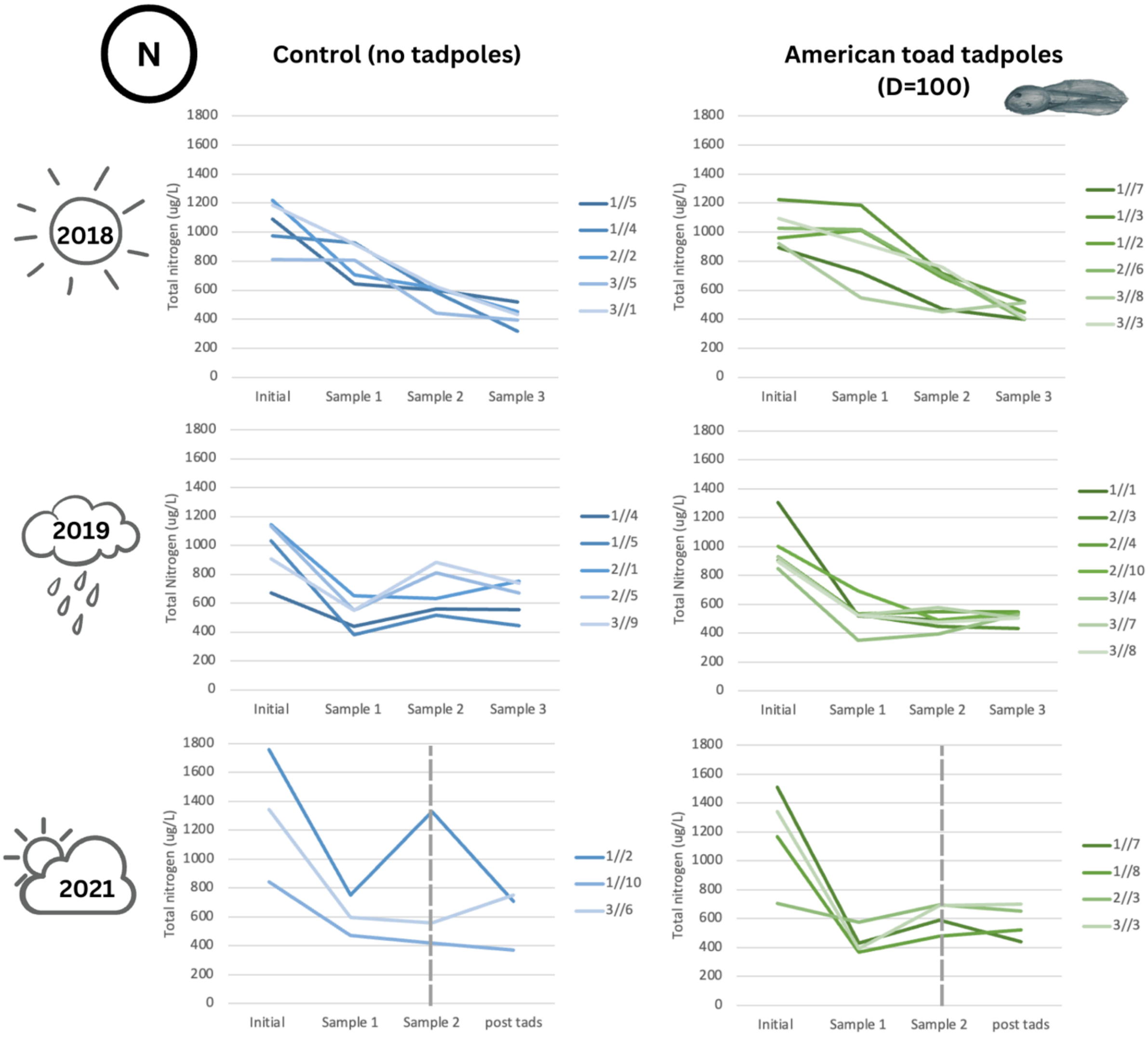


Figure 4.4: The concentration of total nitrogen per mesocosm through time in 2018, 2019, and 2021. Shades of blue denote control mesocosms with no tadpoles, and shades of green denote American toad mesocosms with a density of 100 American toad tadpoles. In 2021, the grey dashed lines represent the point after which tadpoles metamorphosed and left the mesocosms.

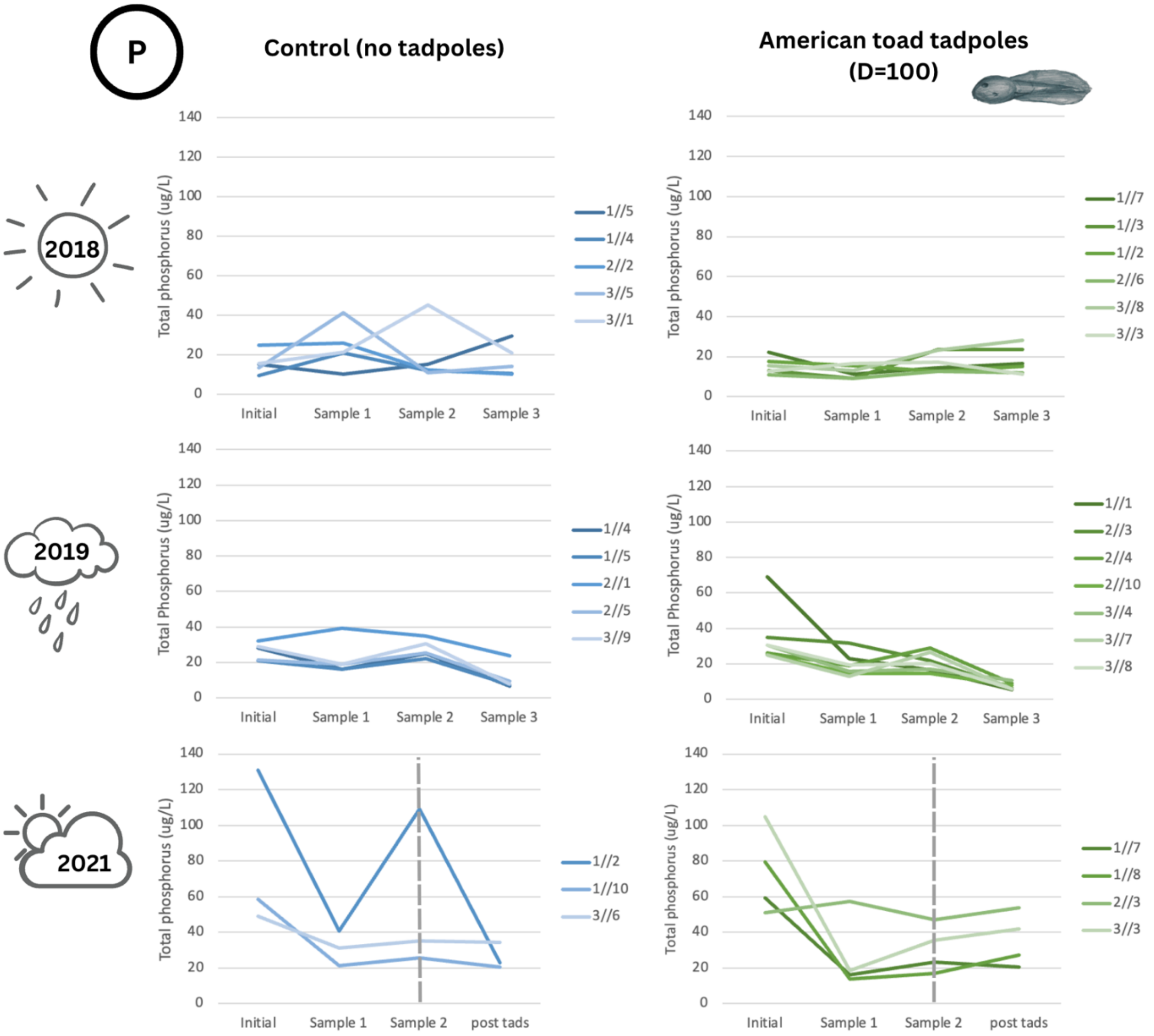
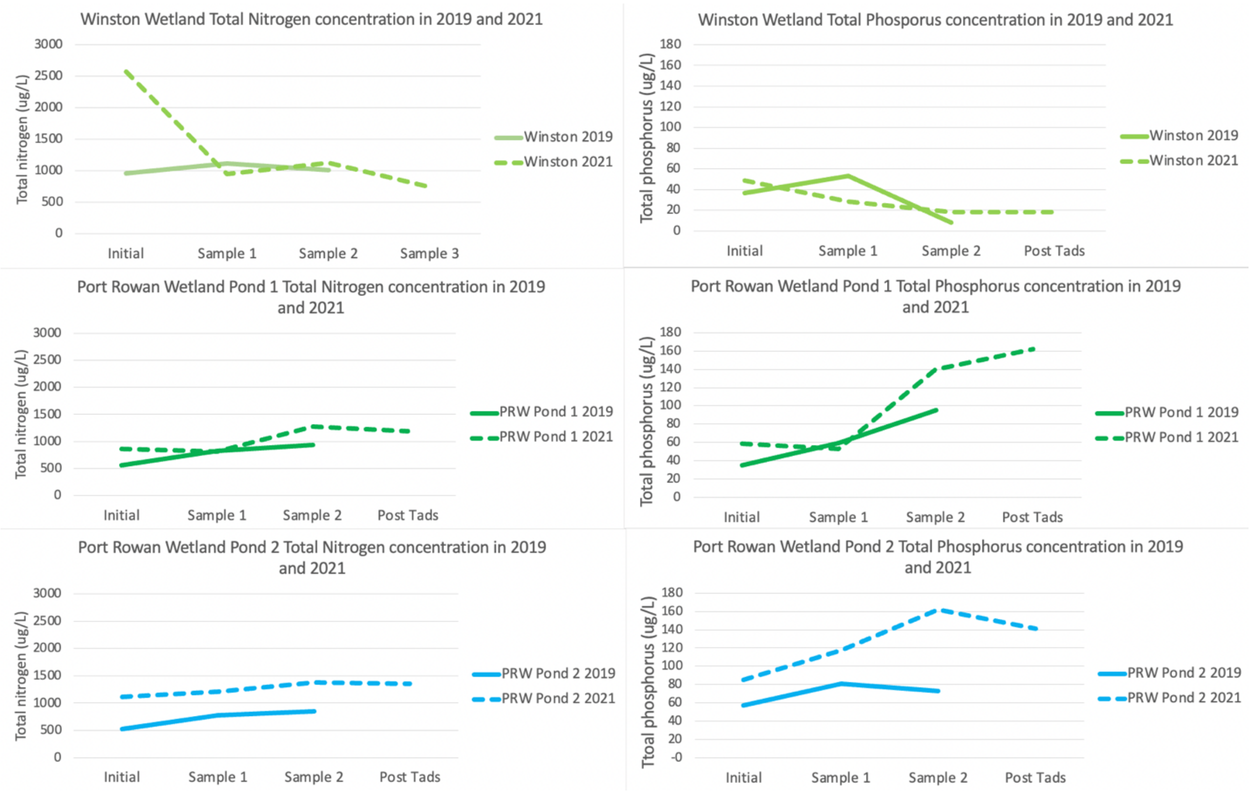


Figure 4.5: The concentration of total phosphorus per mesocosm through time in 2018, 2019, and 2021. Shades of blue denote control mesocosms with no tadpoles, and shades of green denote American toad mesocosms with a density of 100 American toad tadpoles. In 2021, the grey dashed lines represent the point after which tadpoles metamorphosed and left the mesocosms.



Winston Wetland Total Nitrogen concentration in 2019 and 2021

Winston Wetland Total Phosporus concentration in 2019 and 2021

Port Rowan Wetland Pond 1 Total Nitrogen concentration in 2019 and 2021

Port Rowan Wetland Pond 1 Total Phosphorus concentration in 2019 and 2021

Port Rowan Wetland Pond 2 Total Nitrogen concentration in 2019 and 2021

Port Rowan Wetland Pond 2 Total Phosphorus concentration in 2019 and 2021

Figure 4.6: The concentration of total nitrogen and total phosphorus through time in field sites in 2019 and 2021. At the post tads time point in 2021, tadpoles had undergone metamorphosis and left the natural pond. Blue lines indicate that tadpoles were absent from the site, and green lines indicate that American toad tadpoles were present at the site.

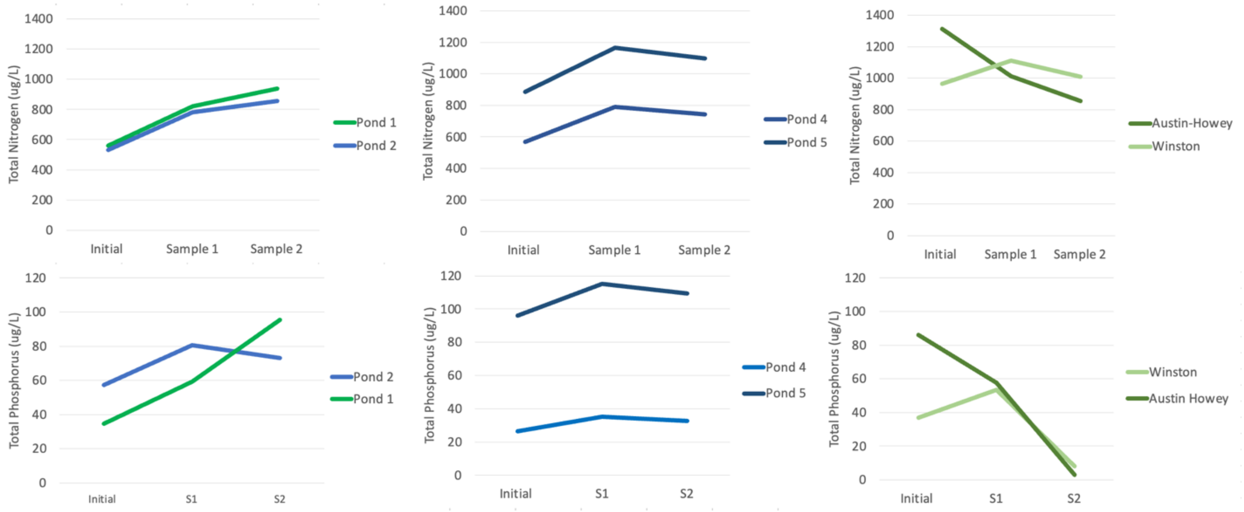


Figure 4.7: The concentration of total nitrogen and total phosphorus through time in field sites in 2019. Ponds are paired with their nearest neighbour geographically. Blue lines indicate that tadpoles were absent from the site, and green lines indicate that American toad tadpoles were present at the site.

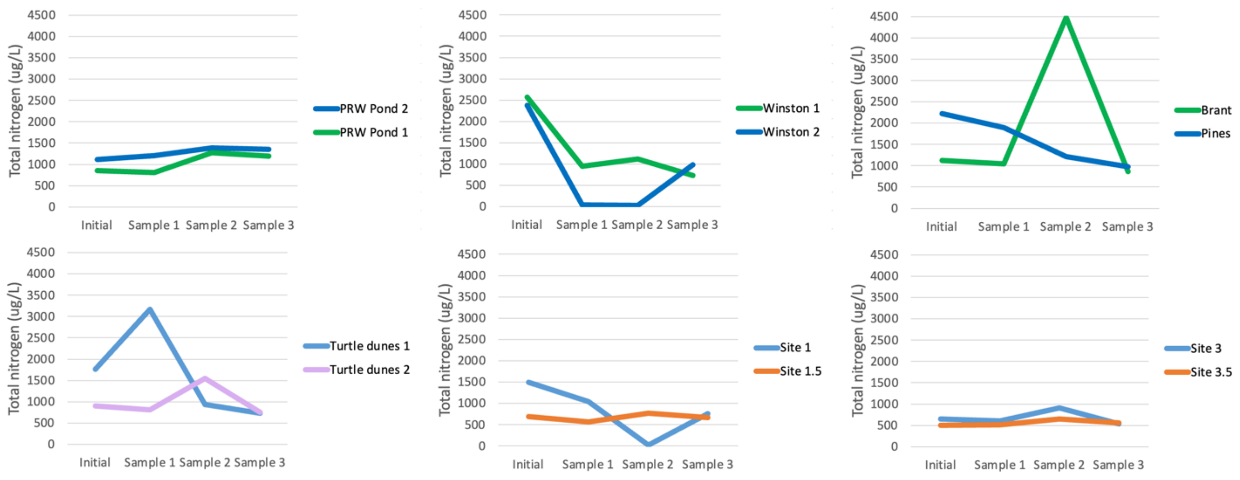


Figure 4.8: The concentration of total nitrogen through time in field sites in 2021. Ponds are paired with their nearest neighbour geographically. Blue lines indicate that tadpoles were absent from the site, green lines indicate that American toad tadpoles were present at the site, purple lines indicate that both American and Fowler's toad tadpoles were present at the site, and orange lines indicate that Fowler's toad tadpoles were present at the site.

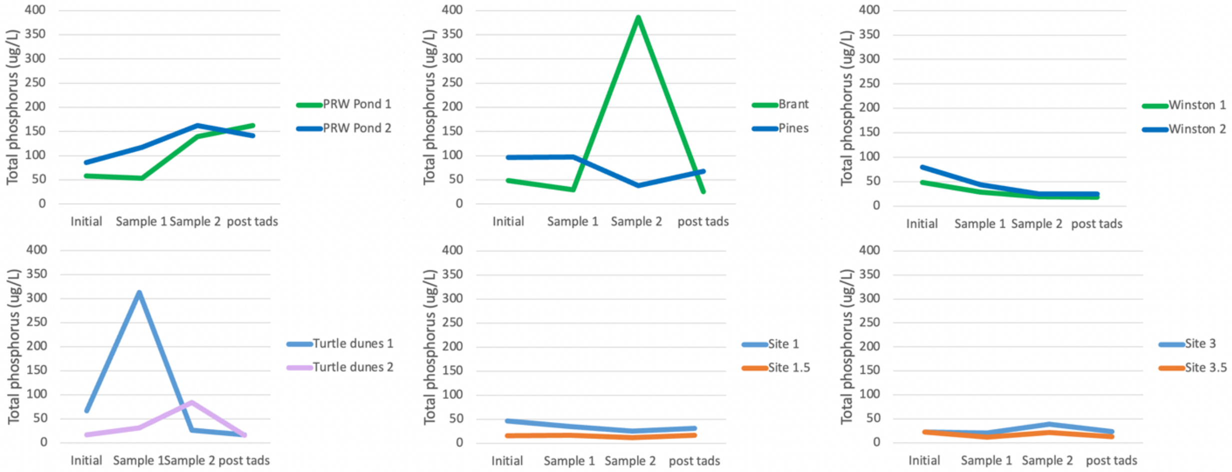


Figure 4.9: The concentration of total phosphorus through time in field sites in 2021. Ponds are paired with their nearest neighbour geographically. Blue lines indicate that tadpoles were absent from the site, green lines indicate that American toad tadpoles were present at the site, purple lines indicate that both American and Fowler’s toad tadpoles were present at the site, and orange lines indicate that Fowler’s toad tadpoles were present at the site.

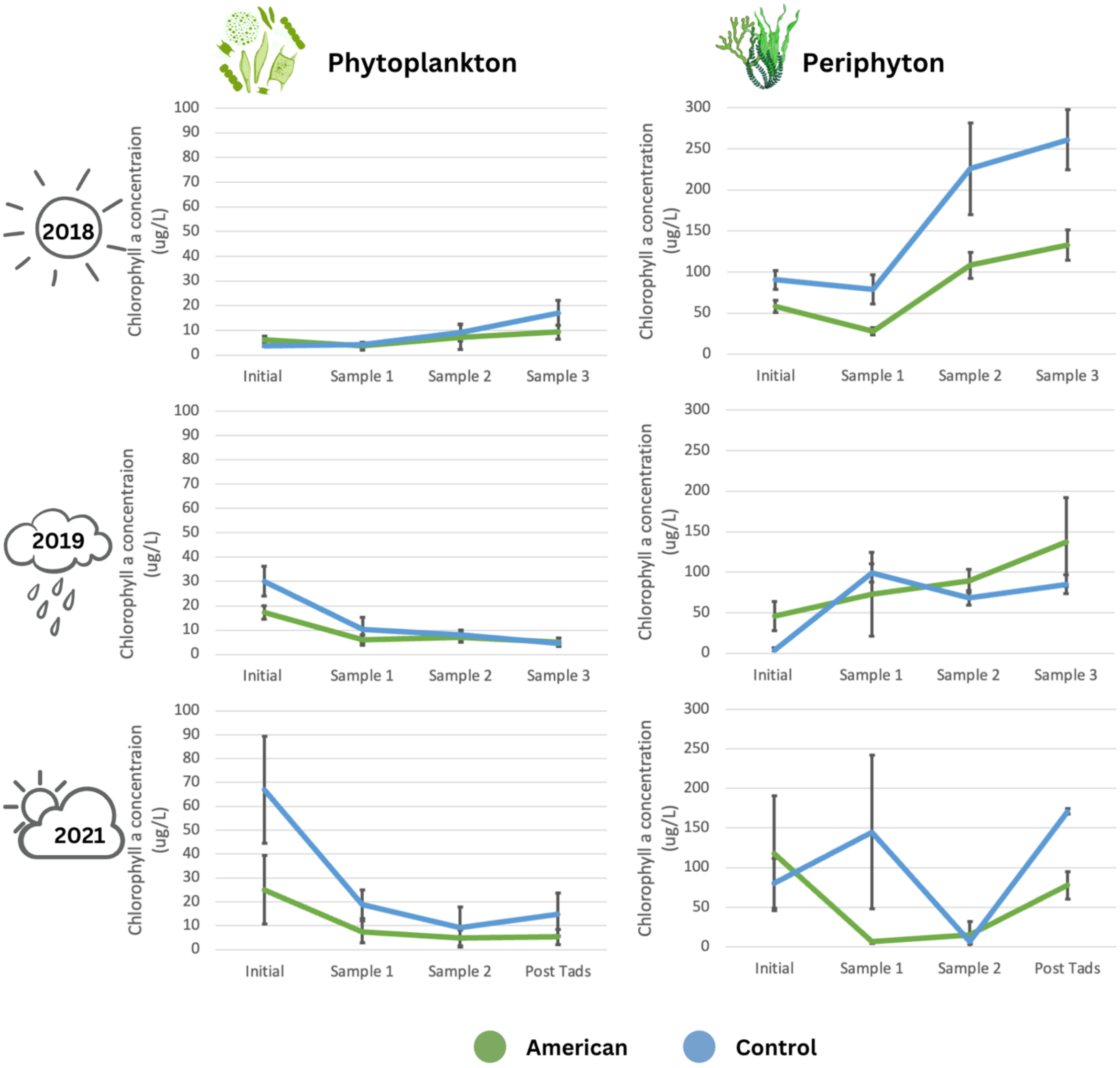


Figure 4.10: Chlorophyll a concentrations of phytoplankton and periphyton in the mesocosms in 2018, 2019, and 2021 when no tadpoles were present, and when 100 American toad tadpoles were present.

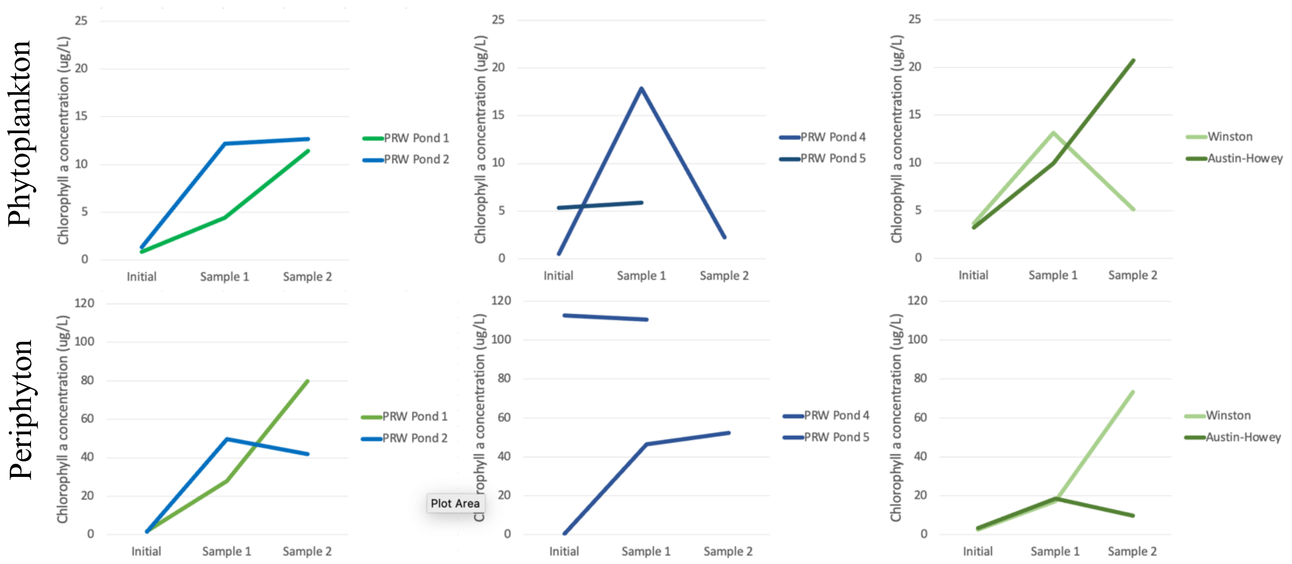


Figure 4.11: The chlorophyll *a* concentration of phytoplankton and periphyton through time in field sites in 2019. Ponds are paired with their nearest neighbour geographically. Blue lines indicate that tadpoles were absent from the site, and green lines indicate that American toad tadpoles were present at the site.

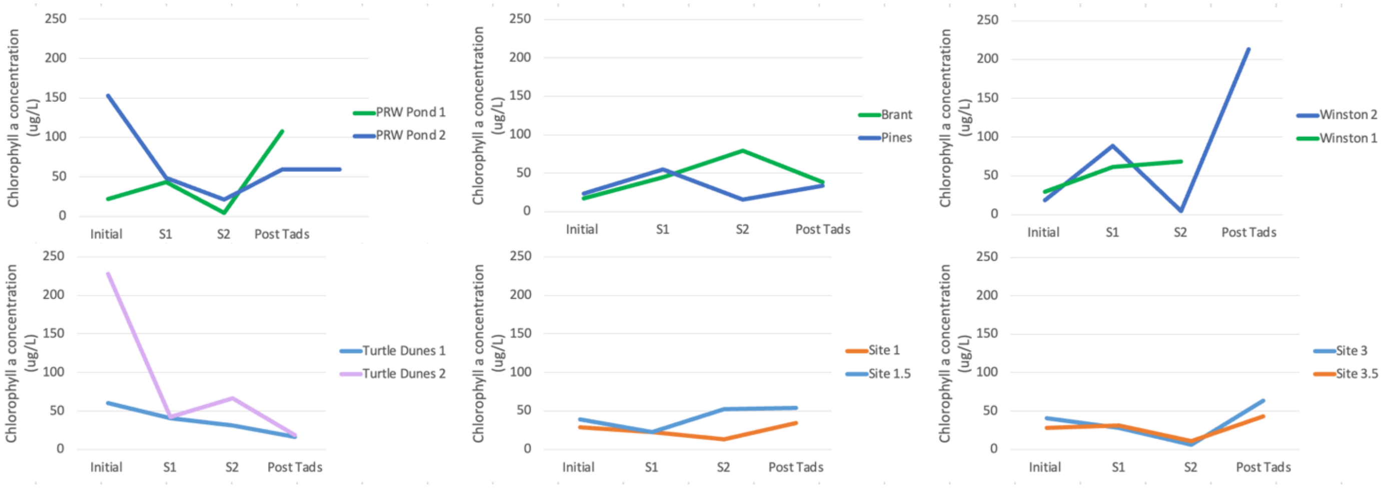


Figure 4.12: The chlorophyll *a* concentration of phytoplankton through time in field sites in 2021. Ponds are paired with their nearest neighbour geographically. Blue lines indicate that tadpoles were absent from the site, green lines indicate that American toad tadpoles were present at the site, purple lines indicate that both American and Fowler's toad tadpoles were present at the site, and orange lines indicate that Fowler's toad tadpoles were present at the site.

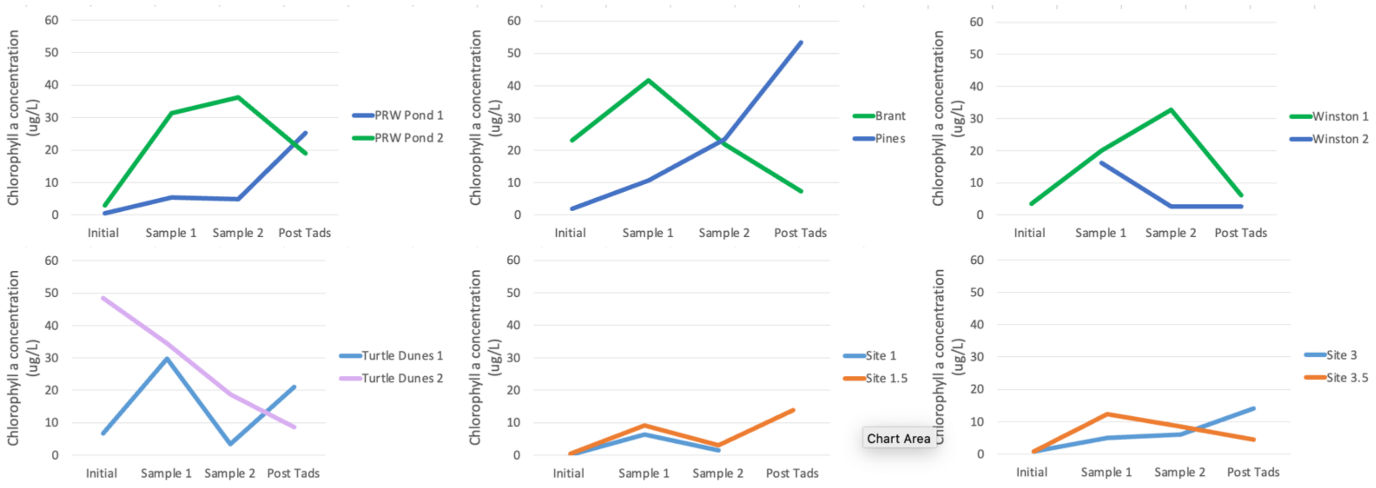


Figure 4.13: The chlorophyll *a* concentration of periphyton through time in field sites in 2021. Ponds are paired with their nearest neighbour geographically. Blue lines indicate that tadpoles were absent from the site, green lines indicate that American toad tadpoles were present at the site, purple lines indicate that both American and Fowler’s toad tadpoles were present at the site, and orange lines indicate that Fowler’s toad tadpoles were present at the site.

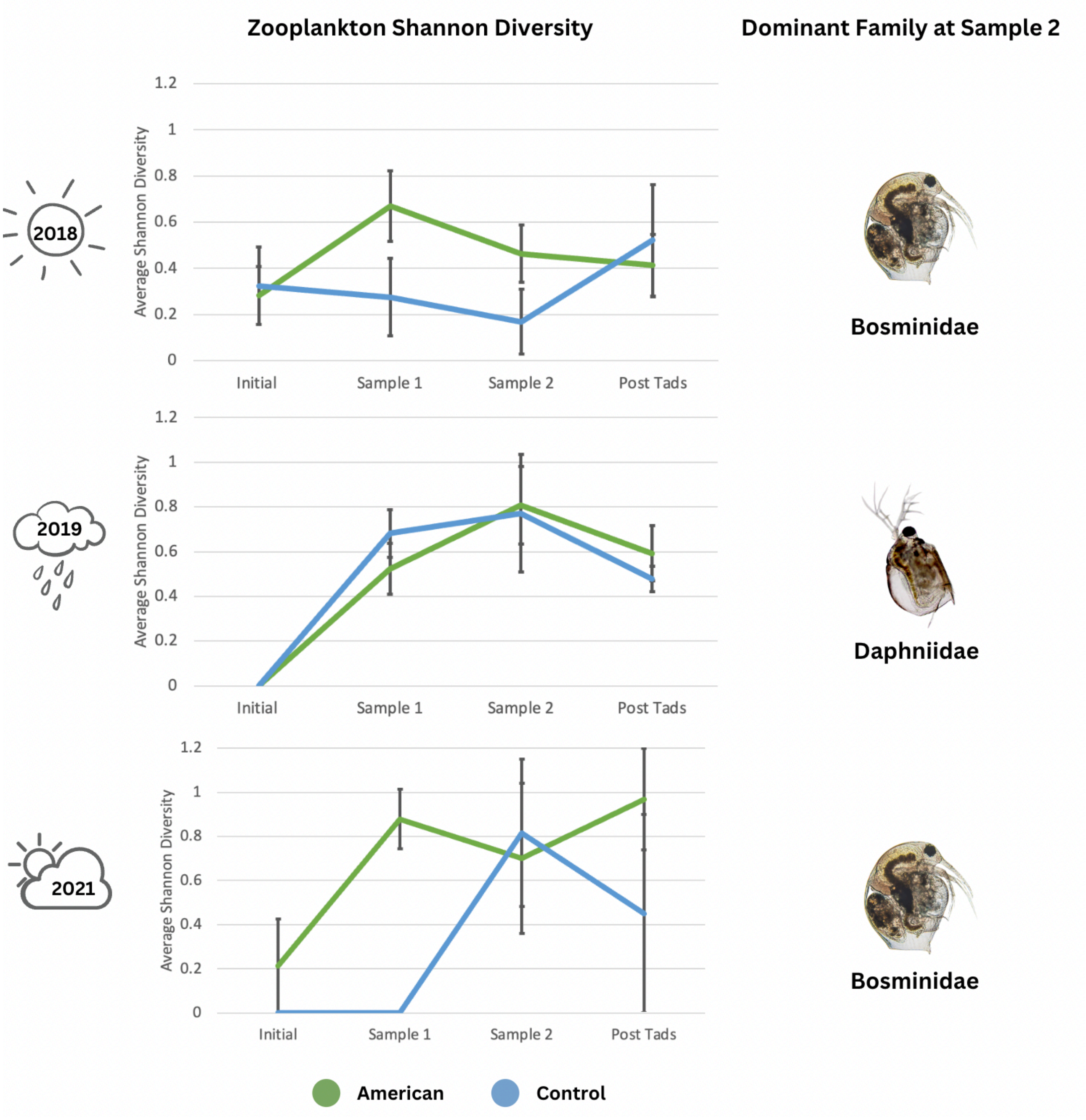


Figure 4.14: Average Shannon-Wiener diversity through time in mesocosms with and without American toad tadpoles in 2018, 2019, and 2021.

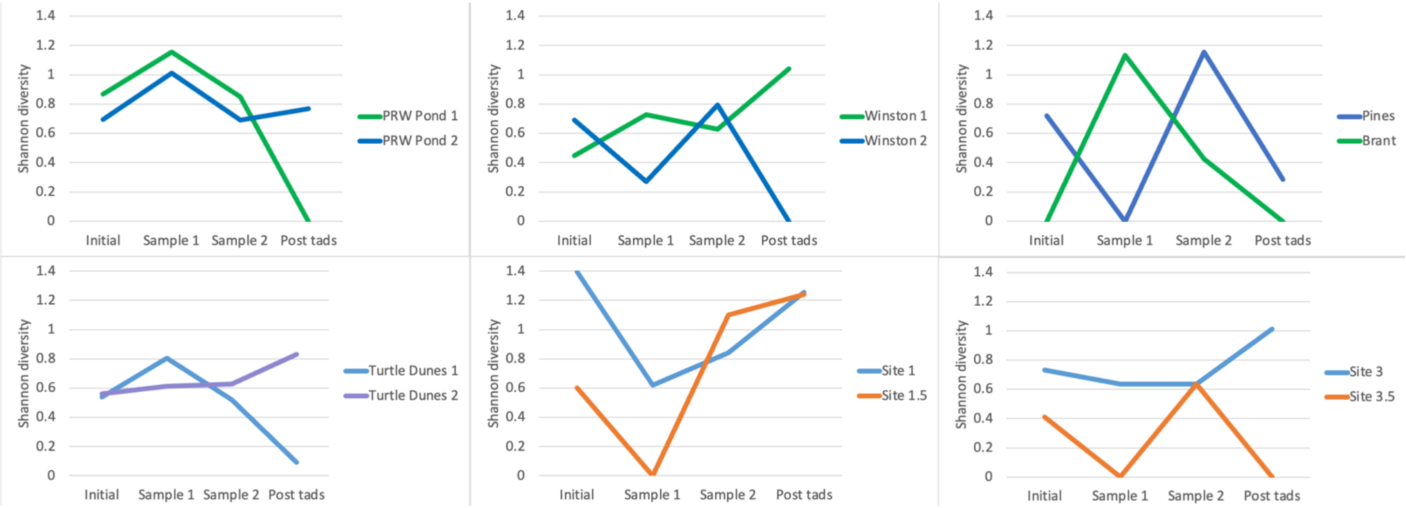


Figure 4.15: The Shannon diversity of zooplankton through time in field sites in 2021. Ponds are paired with their nearest neighbour geographically. Blue lines indicate that tadpoles were absent from the site, green lines indicate that American toad tadpoles were present at the site, purple lines indicate that both American and Fowler's toad tadpoles were present at the site, and orange lines indicate that Fowler's toad tadpoles were present at the site.

Supplemental

Supplemental Table S4.1: Breakdown of experimental mesocosm allocation in 2018, 2019, and 2021. A total of 30 mesocosms were used each year, with the additional, non-experimental mesocosms being used as top up, nursery, or breeding tanks.

	American (D=100)	Fowler's (D=100)	Both (D=100)	Control (D=0)	Half density American (D=50)	Half Density Fowler's (D=50)	Total
2018	6	6	5	5	-	-	22
2019	14	-	-	9	-	-	23
2021	4	4	3	4	5	4	24

Supplemental Table S2: Latitude and longitude location of field sites in 2019 and 2021, and the species of tadpoles present at each.

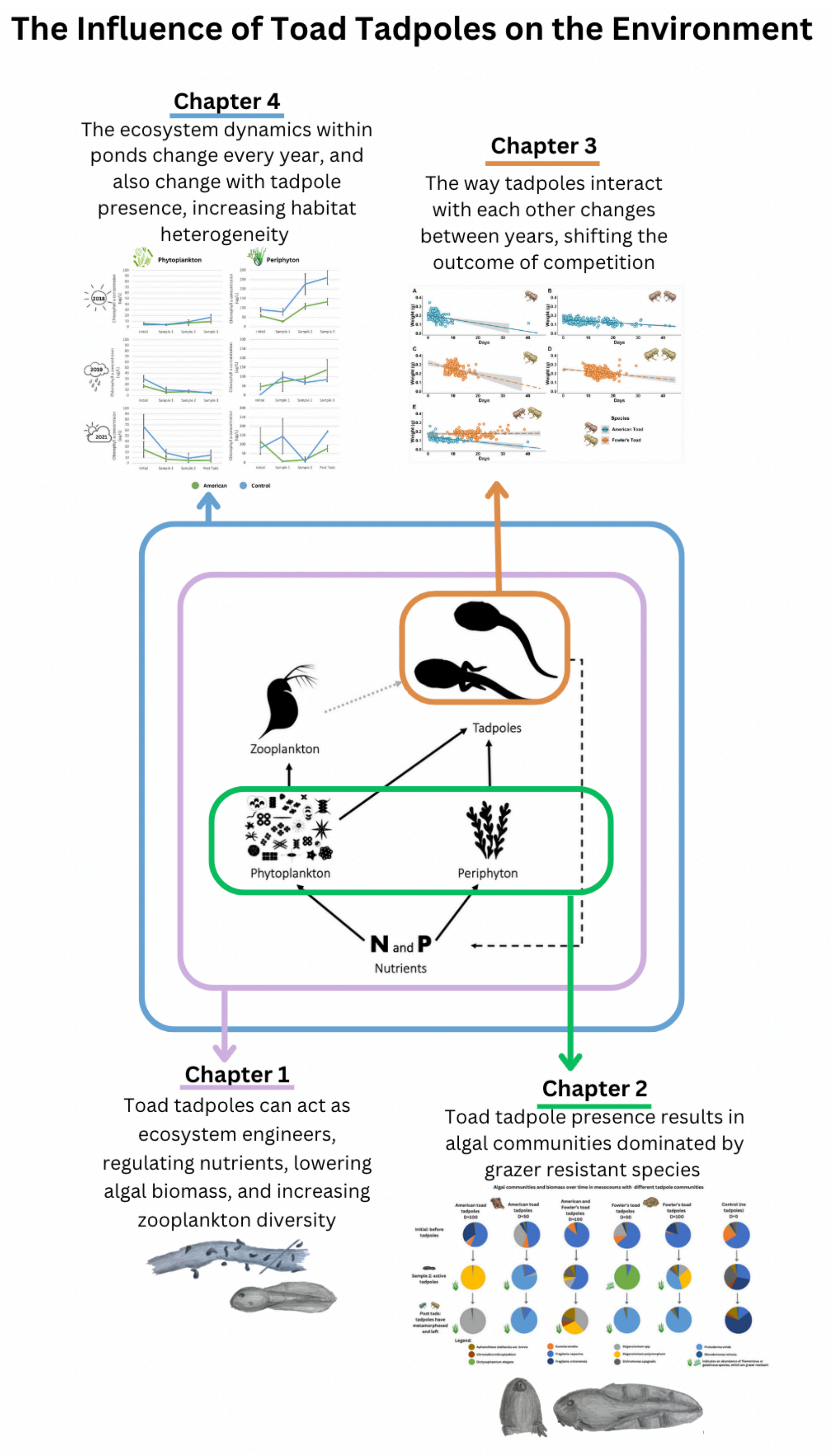
Year	Site name	Latitude	Longitude	Species Present
2019	Port Rowan Wetlands Pond 1	42º37’19.37” N	80º27’38.57” W	American
	Port Rowan Wetlands Pond 2	42º37’21.35” N	80º27’37.81” W	None
	Port Rowan Wetlands Pond 3	42º37’22.59” N	80º27’43.56” W	None
	Port Rowan Wetlands Pond 4	42º37’28.82” N	80º27’39.58” W	None
	Port Rowan Wetlands Pond 5	42º37’30.19” N	80º27’38.35” W	None
	Winston	42º34’50.72” N	80º25’23.91” W	American
	Austin-Howey	42º34’53.05” N	80º24’40.29” W	American
2021	Port Rowan Wetlands Pond 1	42º37’19.37” N	80º27’38.57” W	American
	Port Rowan Wetlands Pond 2	42º37’21.35” N	80º27’37.81” W	None
	Winston 1	42º34’50.72” N	80º25’23.91” W	American
	Winston 2	42º34’50.79” N	80º25’20.47” W	None
	Brant	42º34’50.72” N	80º25’12.61” W	American
	Pines	42º34’51.22” N	80º25’02.16” W	None
	Turtle Dunes 1	42º34’41.66” N	80º22’31.25” W	American and Fowler’s
	Turtle Dunes 2	42º34’39.52” N	80º22’31.00” W	None
	Site 1	42º34’34.19” N	80º22’12.98” W	None
	Site 1.5	42º34’33.71” N	80º22’08.46” W	Fowler’s
	Site 3	42º34’31.40” N	80º21’53.22” W	None
	Site 3.5	42º34’29.76” N	80º21’46.67” W	Fowler’s

Discussion and Final Conclusion

Understanding the ecological role of a species in an ecosystem, and how that role changes with time and shifting conditions, is of key importance when determining how ecosystems function (Rudolf, 2018). In this thesis, I assess the impacts of Fowler's toad and American toad tadpoles through time, both within a season and across years, to determine the impact of these grazers on their surrounding communities, and how aquatic ecosystems would respond to tadpole presence or absence. Throughout, my thesis exemplifies the complexity of seemingly simple, relatively low diversity pond ecosystems (**Figure 1**). Additionally, I demonstrated the need to re-evaluate our assumptions surrounding the ecological similarities of closely related species, and highlight the dynamic nature of ecosystems, even without obvious disturbance events.

Tadpoles as ecosystem engineers

Despite being ephemeral, small, and often overlooked, tadpoles may have profound impacts on their surrounding aquatic communities. There is evidence to suggest that frog tadpoles act as ecosystem engineers in tropical streams (Flecker et al, 1999; Ranvestel et al, 2004; Connelly et al, 2008; Colón-Gaud et al, 2010), and sparse evidence that toad tadpoles in temperate environments may do the same (Wood and Richardson, 2010). Overall, however, there is a lack of consensus on whether tadpoles influence their environment, and, if they do, in what way they are likely to impact surrounding communities (Whiles et al, 2006; Connelly et al, 2008, Wood and Richardson, 2010; Buck et al 2012). In Chapter 1, I help to fill this gap through determining the net effects of toad tadpoles on their environment using experimental mesocosms, from the time toad tadpoles were at Gosner stage 26 to Gosner stage 42.



The Influence of Toad Tadpoles on the Environment

Chapter 4

The ecosystem dynamics within ponds change every year, and also change with tadpole presence, increasing habitat heterogeneity

Chapter 3

The way tadpoles interact with each other changes between years, shifting the outcome of competition

Chapter 1

Toad tadpoles can act as ecosystem engineers, regulating nutrients, lowering algal biomass, and increasing zooplankton diversity

Chapter 2

Toad tadpole presence results in algal communities dominated by grazer resistant species

Figure 1: Summary of main findings from each chapter. Boxes indicate which part of the food web was focused on for that chapter.

I demonstrated that, through time, toad tadpoles regulated phosphorus concentration, decreased algal biomass, and increased zooplankton diversity, shaping the aquatic communities they occupied. In order to complete this study successfully, I developed novel methods to raise toad tadpoles in mesocosms with minimal human intervention (Appendix 1). Chapter 1 showed that:

1) The system of toad tadpoles in mesocosms with a sand substrate as an excellent study system to evaluate the role of a species in the environment

2) The question of what do tadpoles do in their environment is much more complex than we thought.

Which species of tadpole was present, and the composition of tadpoles (single or mixed species) influenced how the surrounding community responded to tadpole presence, such as how the algal biomass or zooplankton diversity changed through time with tadpole presence. Despite being closely related, Fowler's toad and American toad tadpoles did not have the same net effects on the aquatic environment, as explored further in Chapter 2. Additionally, when Fowler's toad tadpoles and American toad tadpoles were in the same mesocosm, even at the same density as single species mesocosms, there were different community responses than either species alone, likely because of the interactions between the two species, which we explored in Chapter 3.

Tadpoles shaping algal communities

After determining that American and Fowler's toad tadpoles had a large effect on the algal biomass in mesocosms (Chapter 1), I set out to determine if tadpoles were only influencing the *amount* of algae, or if tadpole grazing was also influencing the *kind* of algae that was present, in Chapter 2. I expected, due to the large amount of algae being consumed by tadpoles, that the remaining algal community may be altered as tadpoles grazed down competitors thus increasing certain algal populations (McCormick and Stevenson, 1991). I also suspected, due to the intense effect tadpoles had on algal biomass demonstrated in Chapter 1, that tadpoles may leave a legacy effect on the algal communities in the mesocosms even after metamorphosis. Research has shown that amphibian larvae can leave legacy effects on their environment (Blaustein et al, 1996; Rowland et al, 2017), but no study that we are aware of has been conducted with toad tadpoles.

In conducting this study, I was met with the assumption that American and Fowler's toad tadpoles would be functionally redundant, due to being closely related and having similar mouthparts. While I already challenged this assumption due to the two species having differing net effects in Chapter 1, I solidified this difference in Chapter 2, showing that Fowler's and American toad tadpole presence resulted in different algal communities. I also found, through examining literature from Miss Mary Hinkley, written in 1882, that Fowler's and American toad tadpoles did, indeed, have different mouthparts, a distinction that had since been overlooked. This reinforces that we cannot assume functional redundancy even when two species seem to look and act the same. I also demonstrated the importance of examining older, less prominent scientific literature, especially that written by traditionally excluded voices in science, and scientific literature written in languages that are not English, to find additional resources and viewpoints on ecological topics.

In Chapter 2, I also challenged the assumption that these tadpoles and pond ecosystems are simple, as I found different algal communities with different tadpole species, different tadpole densities, and again with mixed vs single species tadpole communities. While the communities were different in each different experimental group, showing how complex and dynamic these systems truly are, we did find similarities in the functional groups present. When tadpole communities of any kind, composition, or density, were present in the mesocosms, periphyton communities became dominated by grazer resistant species that were either filamentous or gelatinous. Additionally, differences in the communities and functional groups that tadpoles left behind after metamorphosis persisted in the mesocosms, confirming that tadpoles have a legacy effect in ponds that they occupy. The impact of toad tadpoles that I showed in Chapter 1 and Chapter 2 demonstrate how tadpoles shape their ecosystem through:

1. changing nutrient availability by regulating phosphorus concentration (Chapter1)
2. determining what species are present through shifting algal and zooplankton (Chapter 2)
3. community structure (Chapter 1 and 2)
4. the changing the structure of the habitat by grazing down periphyton biomass and transforming the remaining algal community (Chapter 1 and 2).

Through these two chapters, I show that tadpoles, despite being ephemeral and often being overlooked, are important members of pond communities, even acting as ecosystem engineers.

Tadpoles and community interactions across years

Chapter 1 and 2 were both single-year studies, but the legacy effects left by tadpoles at the end of the season could influence ponds in successive years as they establish after winter (Yin and Rudolf, 2023). As I discussed in Chapters 3 and 4, ecological studies are rarely repeated across years (Werner, 2020), but my thesis serves as an important case study showcasing why we must repeat ecological experiments to attempt to capture the full scope of ecosystem interactions. In Chapter 1 and 2, it became obvious that when American and Fowler's toad tadpoles were raised in mesocosms together, there were different cascading effects on the rest of the community than in single species mesocosms. In 2018, when the experiment for Chapter 1 was conducted, I observed that that Fowler's toad tadpoles had very low survivorship when raised in the presence of American toad tadpoles. Despite both species of tadpoles being considered herbivores, the lack of ammonia spike and sudden disappearance of Fowler's toad tadpoles over only two days indicated a possible predation or scavenging event by the American toad tadpoles.

When I repeated the 2018 experiment in 2021 with the intention of examining this possible predation event further, this interaction was not repeated. When I looked for the same net effects of tadpole presence or absence that we saw in 2018 in 2021, that was not repeated either. Between 2018, 2019, and 2021, few of the trends we observed in the mesocosms were consistent. We generally consider ecosystem interactions to be static, and the effect of a species presence or the outcome of competition to be consistent, but this is not typically the case in natural systems (Yin and Rudolf, 2023). In my three years of field experiments, I was able to observe interannual variation in how toad tadpoles interacted with their surrounding ecosystem

(Chapter 4), and how toad tadpoles interacted with each other (Chapter 3), demonstrating that the ecological role of toad tadpoles is far from static. Changes in yearly conditions, both with observable year effect drivers such as the extreme amount of precipitation in 2019, and without, as was the case in 2021, impacted tadpole interactions with their ecosystem. Shifts in yearly conditions from year effects or seasonally entrained chaos changed both the net effects of tadpole presence on their environments, and the outcome of interspecific interactions, between years. These findings were true in semi-controlled mesocosms, and in natural pond systems. This shows the immense complexity and unpredictability of these pond systems, as how the ecosystem responded to tadpole presence and absence depended on the species of tadpoles, the number of species present, tadpole density, and yearly conditions. If I had only conducted my study in 2019 or 2021, I would have missed the profound effects tadpoles had on their ecosystem in 2018, and would have drawn drastically different conclusions. Thus, my thesis highlights the need for replication in field experiments in order to fully capture the scope of species interactions, and joins recent papers calling for increased replication in ecology (Vaughn and Young, 2010; Rudolf, 2018; Rudolf, 2019; Werner, 2020).

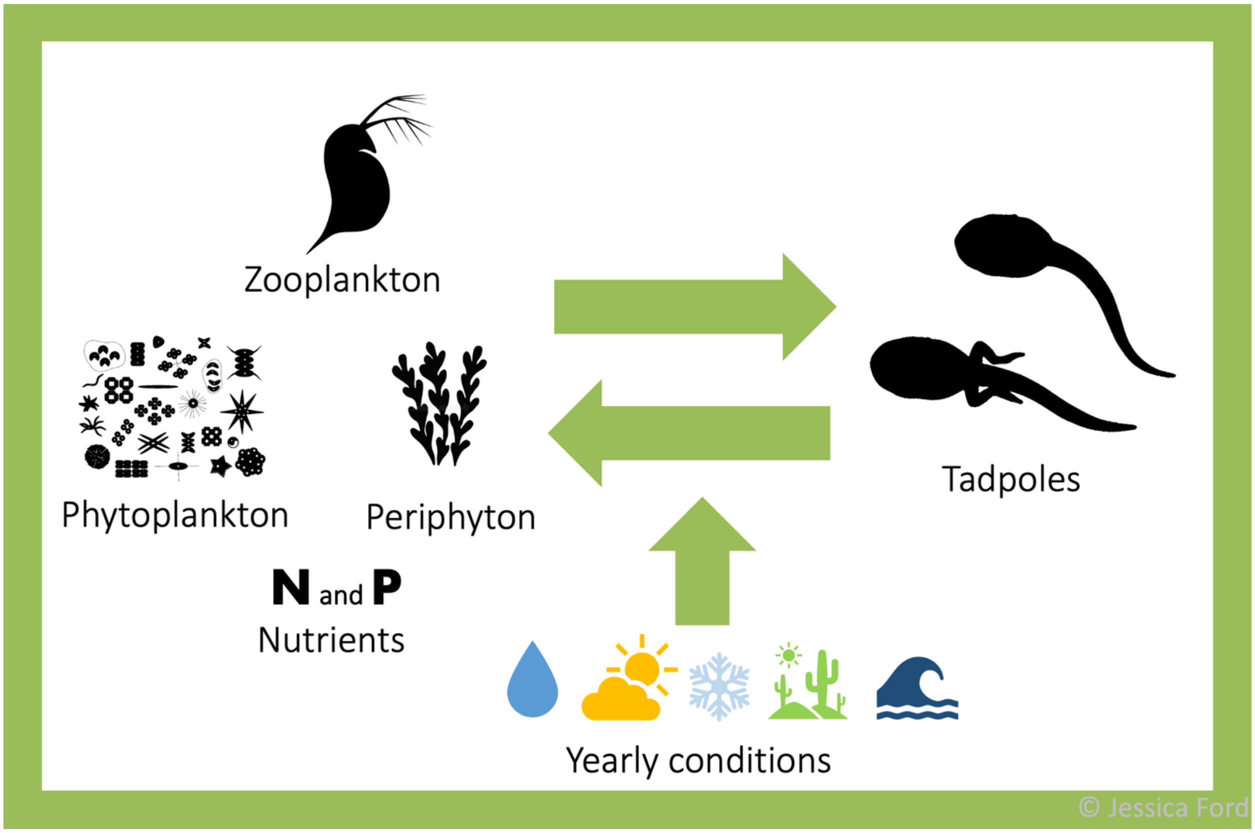


Figure 2: Showing the directions of interactions between tadpoles, their aquatic ecosystem, and yearly conditions.

Final Remarks

Throughout my thesis, I have shown that there is not only one answer to the question of what tadpoles do in their environment, and perhaps there should not be. The variability of how toad tadpoles shape their ecosystem when there are different kinds, densities, and compositions of tadpoles, and how the net effect of tadpole presence changes within a season and throughout years, provides a massive amount of habitat heterogeneity. With the multitude of different ways tadpoles can shape their environment (Chapter 1, 2, 3 and 4), tadpole presence could create or amplify habitat heterogeneity, creating many different habitats for other species and increasing biodiversity across a landscape. When I began my thesis, we were unsure if tadpoles would impact their environment at all, and I have now shown that tadpoles impact surrounding aquatic communities (Chapter 1 and 2). These surrounding aquatic communities, in turn, impact tadpole interactions (Chapter 3), and all of this is impacted by the conditions in the year when the study was conducted (Chapter 4). Throughout this thesis I have shown that "simple" ecological questions are not so simple, that tadpoles and the small ponds they occupy are far more complex than we thought, and that tadpoles play a crucial role in these small water bodies, even and especially if that role is not consistent across species or years.

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Appendices

Appendix 1 – Mesocosm Methodology

Published in Herpetological Review, 2021

Captive Rearing Oligotrophic-Adapted Toad Tadpoles in Mesocosms

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Mesocosms are valuable tools for studying the evolutionary ecology, ecotoxicology, and community interactions of aquatic amphibians and their larvae (Wilbur 1987; Rowe and Dunson 1994; Hamilton et al. 2012). Following Semlitsch and Boone (2009), most mesocosms used in amphibian ecology studies consist of deep metal or fiberglass containers (usually cattle tanks) that may be partially buried in the ground, furnished with a leaf litter substrate and provisioned with a nutritional supplement, creating a deep, cool, and eutrophic aquatic environment. These mesocosms have been used successfully with tadpoles of many species, particularly ranid frogs (Wilbur 1987; Liebold and Wilbur 1992; Hamilton et all 2012; Melvin and Houlahan 2012). They may not, however, be as effective for tadpoles of all species, including many bufonid toads (Caut et al. 2012; Arribas et al. 2014, 2015) that preferentially breed in shallow, warm, oligotrophic environments, and require higher temperatures and dissolved oxygen levels (Noland and Ultsch 1981). As we postulated that mesocosms that more closely mimic the natural environments of particular amphibian larvae are likely to yield better growth rates and higher survivorship, we established aquatic mesocosms specifically meant for tadpoles adapted to oligotrophic conditions. The species we were interested in was the locally endangered Fowler's Toad (*Anaxyrus fowleri*; COSEWIC 2010), which preferentially breeds in shallow, sandy-bottomed, sparsely vegetated pools (Dickerson 1906; Wright and Wright 1949). Accordingly, we investigated the use of oligotrophic mesocosm environments established without the addition of supplemental food or nutrients to raise *A. fowleri* tadpoles through metamorphosis. To determine if this protocol may also be suitable for a more generalist species, we also included American Toad (*A. americanus*) tadpoles, in separate mesocosms of the same design.

We established 30 above-ground mesocosms (Fig. 1A) in 378.6 L (100 US gal.) structural foam cattle tanks (Rubbermaid®) with dimensions of 63.50 cm L × 78.74 cm W × 134.6 cm H. We established the mesocosms in early spring each year, 2017–2019, at Long Point Provincial Park in southern Ontario, Canada, during the first week of May. We placed 5 cm of locally obtained sand at the bottom of each tank and filled the tanks with treated (chlorinated, mechanically filtered, and ultraviolet irradiated) drinking water to achieve a depth of 120 cm. We draped 70% shade cloth over each mesocosm and secured it with a bungee cord to act as a lid. We allowed the mesocosms to sit undisturbed for 5 d to allow the sand to settle, the water to clear, and allow chlorine in the water to evaporate. We then inoculated each mesocosm with 1 L of local, surface-collected pond water from a nearby wetland known to be a breeding site for *A. americanus*. Healthy biotic communities developed in the mesocosms after one week and were identifiable by the presence of a thin film of periphyton on the walls, clear water, a pH between 8.0 and 9.0, dissolved oxygen approaching 100% or higher, ammonium (NH4+) and nitrate (NO2-) at 0 ppm, and nitrite (NO3-) between 0–5 ppm.

We designated each mesocosm into one of four categories: four nursery mesocosms where tadpoles would hatch from eggs and begin to grow, 24 rearing mesocosms where post

Gosner stage 26 (Gosner 1960) tadpoles would be raised, one breeding mesocosm where breeding pairs of toads could lay eggs, and two top-up mesocosms where standing water was held in reserve in case of a water change. Nursery, rearing, and top-up mesocosms were all established in the same manner. We collected eggs from naturally breeding pairs of toads, both *A. americanus* and *A. fowleri*, and placed them into one of four nursery mesocosms within 24 h, separated by clutch, species, and collection site. Nursery mesocosms were mixed into the mesocosm array. *Anaxyrus americanus* eggs were collected from nearby wetlands. One breeding pair of *A. fowleri* in amplexus was collected from a nearby puddle in a parking lot. This breeding pair was placed in a breeding mesocosm, which had 5–10 cm of water, and additional sand piled to one side to create an incline. Floating debris and live plants were added to these mesocosms as well (Fig. 1B). Once eggs were deposited in the mesocosm (within 24 h) the breeding pair was released. We draped the strings of toad eggs over sticks floating in the mesocosms to keep them in the warmest, most highly oxygenated, topmost layer of water during their early development (Fig. 1C). Once tadpoles reached developmental stage 26 (Gosner 1960), we transferred them to rearing mesocosms and reduced the density to 100 tadpoles per mesocosm, or one tadpole per 3.7 L (Melvin and Houlahan 2012) and released the rest at point of origin.

We monitored all mesocosms for ammonium, nitrate, and nitrite levels at least weekly using an API® Freshwater Master Test Kit. If ammonium, nitrate, or nitrite levels were above 0 ppm, we conducted a partial water change by siphoning out ca. 10–15% of the water from the bottom of the mesocosm and replacing it with aged, chlorine-free water from designated top-up mesocosms held in reserve. We covered the inlet of the siphon with a 70 µm mesh screen to prevent siphoning out tadpoles or zooplankton. We monitored dissolved oxygen levels daily between 1200 and 1400 h using a Multivariate Probe (HANNA® Instruments Inc.). Any mesocosm found to have less than 60.0% dissolved oxygen was aerated for 24 h using an air stone connected to a Marina® 300 air pump. Spikes in ammonium, nitrate, and nitrite and low oxygen conditions occurred rarely in these mesocosms, with water changes only needing to be conducted in mesocosms with a density of 100 tadpoles twice in 2017 and 2018, and once in 2019. Aeration was sparsely needed as well, being necessary only two or three times a season, and affected tanks seemed to be random.

As tadpoles reached Gosner stage 42 (Gosner 1960), we added floating cork platforms to the mesocosms for transforming toadlets to climb onto. These platforms were 14 × 15 × 0.6 cm with a 15 cm portion of a foam pool noodle hot glued to one edge to keep the cork on an incline in the water (Fig. 1D). Upon completion of their larval development, which averaged 40.3 days for *A. fowleri* tadpoles among six mesocosms in 2018 (Table 1), we collected all metamorphosing toadlets from the mesocosms and released them at point of origin.
Using these methods, we obtained an average of 91% survivorship through metamorphosis in 2017 among *A. fowleri* tadpoles reared at a density of one tadpole per 3.7 L (Table 1) in mesocosms established with only a sand substrate and a single inoculation of pond water. We had less success the following year (45% average survivorship among six mesocosms in 2018; Table 1), which we attributed to an intense heat wave while the tadpoles were at an early stage of development, around Gosner stage 30 (Gosner 1960).

The mesocosms appeared to successfully replicate the low nutrient environment in the natural ponds used by *A. fowleri*. Based on the success of our inoculated mesocosms, we infer that food supplementation was unnecessary as the algal, zooplankton, and bacterial communities established in the initial inoculation were sufficient to support the tadpoles (Table 1). By not adding leaf litter or food supplements such as rabbit chow to the mesocosms, we reduced buildup of excess nutrients or spikes in ammonium levels. Both dissolved nitrogen and dissolved phosphorous levels remained between oligotrophic and mesotrophic levels (Nurnberg and Shaw 1999) throughout the period of the tadpoles' development. The concentrations of total dissolved nitrogen and total dissolved phosphorus averaged 713 µg/L (± 271 SD) and 18 µg/L (± 14.8 SD), respectively, among 23 mesocosms in 2018 and averaged 657 µg/L (±224.9 SD) and 21 µg/L (± 11.5 SD) among 11 mesocosms in 2019. These values compare favourably to the averages of total dissolved nitrogen and total dissolved phosphorus in two nearby natural ponds that we recorded in 2019, which averaged 748 µg/L (± 158.5 SD) and 67 µg/L (± 20.4 SD), respectively. To test if our methods could be applicable to other anuran larvae with similar or more broadly tolerant ecological requirements, we also raised tadpoles of sympatric *A. americanus*, and obtained survivorship rates of 75%, 70%, and 91% during 2017, 2018 and 2019, respectively, at densities of one tadpole per 3.7 L. A trial using *A. americanus* at a density of two tadpoles per 3.7 L (200 tadpoles per mesocosm) was less successful. This density yielded only 52.7% average survivorship among six mesocosms and required more water changes and oxygen supplementation.

We conclude that raising oligotrophic adapted tadpoles in mesocosms that mimic their natural environment, without additional food and nutrient supplements is a viable technique for studying amphibian larval ecology and raising these tadpoles with high survivorship. Our methods should be applicable to other anuran larvae with similar ecological requirements. Using toad tadpoles in mesocosm studies may have advantages over the use of other anuran species, as in addition to being relatively low maintenance, by not adding leaf litter or food supplements such as rabbit chow to the mesocosms, we avoided the buildup of excess nutrients that could lead to detrimental spikes in ammonium levels which would necessitate more frequent water changes, allowing our mesocosms to resemble oligotrophic natural ponds more closely.

Acknowledgments.—Support for this study came from the Ontario Ministy of Natural Resources and Forestry (OMNRF) and Environment Canada. We thank Bruce Pauli from Environment and Climate Change Canada for his thoughtful advice on tadpole rearing. We thank J. Benbahtane, N. Jreidini, A. Zerafa, V. Tawa, E. Forget-Klein, D. Shaban, T. Pulciano, A. Botsko, M. Martini, K. Kwaku, A. Brimacombe, C. Berruezo i Llacuna, S. Thivierge, J. Purdy, A. Malet, L. Meehan, J. Cuffaro, E. Jaeger, P. Harindranath, G. Rimok and R. Tremblay for their assistance in tadpole rearing and mesocosm maintance, as well as Ontario Parks, OMNRF and the staff at Long Point Provincial Park for logistical support. This research was carried out in accordance with McGill University Animal Use Protocol 4569 and permits from OMNRF and Environment Canada. We also acknowledge the traditional owners of the land on which we work; Long Point Provincial Park is situated on the traditional territories of the Mississauga and Haudenosaunee nations, and McGill University is located on the traditional territories of the Haudenosaunee and Anishinabeg nations.

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TABLE 1: Average survivorship, time to metamorphosis, and weight at metamorphosis of *Anaxyrus americanus* and *Anaxyrus fowleri* tadpoles in the mesocosms. In 2017*, Anaxyrus fowleri* tadpoles were collected as tadpoles, not eggs, so time to metamorphosis was not recorded. In 2019, *Anaxyrus fowleri* only emerged and bred in June, too late to be included in the mesocosm study.

Species	Year	Average % Survivorship ± S.D. (N)	Average Time to First Emergence (days) ± S.D. (N)	Average weight at metamorphosis (g) ± S.D. (N)
A. americanus	2017	75.3 ± 22.2 (6)	40.3 ± 0.82 (6)	0.119 ± 0.016 (628)
	2018	70 ± 23.2 (6)	46.1 ± 2.93 (6)	0.092 ± 0.040 (413)
	2019	90.6 ± 22.5 (15)	53.6 ± 1.5 (15)	0.108 ± 0.128 (1357)
A. fowleri	2017	90.8 ± 9.75 (4)		0.159 ± 0.015 (358)
	2018	44.7 ± 11.7 (6)	40.3 ± 2.5 (6)	0.118 ± 0.030 (232)

FIGURE 1: Mesocosms for rearing oligotrophic-adapted toad tadpoles. A) Mesocosm set-up at Long Point Provincial Park, Ontario, with shade screen covers. B) Breeding mesocosm set up for Fowler's toad, *Anaxyrus fowleri*, pair. C) American Toad, *Anaxyrus americanus*, eggs developing in a nursery mesocosm. D) American toadlet that crawled onto a floating platform after completing metamorphosis.