Density-dependence and Dispersal Mechanisms in a Pond Breeding Amphibian

Katharine T. Yagi

Department of Natural Resource Science & Redpath Museum

Faculty of Agricultural and Environmental Sciences

McGill University

Montréal, Quebec, Canada

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Abstract

In this thesis, I attempt to quantify the effect of density on toad dispersal in a series of steps that coincide with the amphibian’s notable life stages, which are outlined by the five chapters of my thesis. Chapter 2 examines the direct effect of density on tadpole survival, growth and size at metamorphosis using a unique technique of density manipulation. Here, I discuss potential downfalls of the traditional method of tadpole density-manipulations whereby raising them in crowded conditions might confound results as tadpoles are known to respond differently to chemical cues left in the water by conspecifics. My method of manipulating density by volume resulted in a strong negative relationship with tadpole survival, growth and size at metamorphosis, as predicted, while the traditional method showed similar but less striking trends.

Chapter 3 addresses whether carry-over effects are present in the new metamorphs (i.e. toadlets) that emerge from a range of density treatments, by monitoring changes in behaviour between the tadpole and toadlet life stages. I discovered a significant change in activity levels in those grown under high densities as tadpoles, where their small post-metamorph body size coincided with a notable decrease in activity. My results support the concept of density-dependent carry-over effects being present in these amphibians, and that their post-metamorphic mobility may be impacted by their early-life growth conditions. Due to the complexities of identifying interacting effects of density and temperature on behaviours in tadpoles using field enclosures, Chapter 4 examines the behavioural plasticity of tadpoles under these effects using a controlled factorial experiment. My results showed that larger sized tadpoles had high activity levels under all temperature regimes only when they came from high density treatments. This suggests that the size of the animal is important, as it becomes relevant under more stressful

conditions and that the behavioural plasticity of tadpoles is governed by an interaction among individual body size, water temperature and density.

Chapter 5 follows the size-dependent movement capabilities of individual toadlets from density-treatments in a controlled performance test, and their subsequent free-ranging movement behaviour in the field. I discovered that the body size of toadlets predicted dispersal in a quadratic relationship, where intermediate sized toads, regardless of their tadpole density, move greater distances and had the highest probability for dispersal. Interestingly, these intermediate-sized toads became the largest in their cohort as adults, resulting in a positive logistic relationship between adult size and dispersal probability. Finally, Chapter 6 uses the information gathered for size-dependent dispersal probabilities, and calculates the populations density-dependent dispersal rates using historical data, to inform model simulations to predict the extinction risk of this Fowler's toad population. I discovered that this population has a quadratic relationship between dispersal probability and population density, where very low and very high densities coincided with the highest dispersal rates. Additionally, I was able to predict a low extinction risk for the population if carrying capacity was kept at a minimum of 16 toads, however if continuous habitat management, which translates into continuous habitat disturbances, were to be employed on a short 7-year cycle, extinction risk could be as high as 78%.

Human activities, including fragmentation and restoration, continue to impact the animals living in the surrounding environment. As dispersal is a key process that can save many populations from local extinctions it is important to understand how movement is restricted on a species-specific level. My thesis provides a detailed examination of density-dependence and

movement capabilities across multiple life stages in a pond breeding amphibian and advances our understanding of how density itself can impact dispersal under different mechanisms.

Résumé

Dans cette thèse, je tente de quantifier l'effet de la densité sur la dispersion des crapauds dans une série d'étapes qui coïncident avec les stages de vie remarquables des amphibiens, qui sont décrites par les cinq chapitres de ma thèse. Le chapitre 2 examine l'effet direct de la densité sur la survie, la croissance et la taille des têtards à leur métamorphose en utilisant une technique unique de manipulation des densités. Ici, je discute des problèmes potentiels des méthodes traditionnelles des manipulations de densité des têtards, où leur élevage dans des conditions encombrées qui pourraient confondre les résultats, vu que les têtards sont connus pour répondre différemment aux indices chimiques laissés dans l'eau par leurs conspécifiques. Ma méthode de manipulation de la densité en utilisant des volumes différents a entraîné une relation négative forte avec la survie, la croissance et la taille du têtard à la métamorphose, comme prévu, alors que la méthode traditionnelle présentait des tendances similaires mais moins frappantes.

Le chapitre 3 adresse si les effets de report sont présents dans les nouveaux métamorphes (c'est-à-dire les jetons) qui émergent d'une gamme des traitements de densité, en surveillant les changements de comportement entre le têtard et les stades de vie des allaches. J'ai découvert un changement important dans les niveaux d'activité des metamorphes cultivés sous les densités élevées des têtards, où leur petite taille de corps post-métamorphisme coïncidait avec une diminution notable d’activité. Mes résultats appuient la notion que les effets de report dépendants de la densité sont présent dans ces amphibiens et que leur mobilité post-métamorphique peut être significativement affectée par des conditions de croissance précoces. En raison de la complexité

de l’identification des effets interagissant de la densité et de la température sur les comportements des têtards à l'aide d'enceintes de terrain, le chapitre 4 examine la plasticité comportementale des têtards sous ces effets en utilisant une expérience factorielle contrôlée. Cependant, mes résultats ont montré que les plus grands têtards avaient des niveaux d'activité élevés dans tous les régimes de température seulement lorsqu'ils provenaient de traitements de haute densité. Cela suggère que la taille de l'animal est importante, car elle devient pertinente dans des conditions plus stressantes et que la plasticité comportementale des têtards est régie par une interaction significative entre la taille corporelle, la température de l’eau et la densité des individus.

Le chapitre 5 suit les capacités de mouvement dépendantes de la taille des juvéniles individuels des traitements de densité dans un test contrôlé de performance et leur comportement de mouvement détaillé sur le terrain. J'ai découvert que la taille du corps des juvéniles prévoyait leur dispersion selon une relation quadratique, où les juvéniles de taille intermédiaire, quelle qu’étaient la densité des têtards pendant leur croissance, se déplacent sur de plus grandes distances et ont une probabilité plus forte de dispersion. Ce qui était plus intéressant, est que les juvéniles de taille intermédiaire sont devenus les plus grands de leur cohorte en tant qu'adulte, ce qui a effectué une relation logistique positive entre la taille des adultes et la probabilité de dispersion. Enfin, le chapitre 6 utilise les informations recueillies des probabilités de dispersion dépendantes de la taille et calcule le taux de dispersion dépendant de la densité de la population en utilisant des données historiques, afin de préciser les prédictions des simulations de risque d'extinction des populations des crapauds Fowler. J'ai découvert que cette population avait une relation quadratique entre la probabilité de dispersion et la densité de la population, où des densités très faibles et très élevées coïncidaient avec les taux de dispersion les plus élevés. En outre, j'ai pu prédire un risque d'extinction faible pour la population si la capacité de charge était maintenue à au moins 16 crapauds, mais si la gestion continuelle de l'habitat, qui ferrait des perturbations continuelles de l'habitat, sont employée sur un cycle court de 7 ans, le risque d'extinction pourrait atteindre 78%.

Les activités humaines, y compris la fragmentation et la restauration, continuent d'avoir un impact sur les animaux vivant dans les alentours. Vu que la dispersion est un processus important qui peut sauver des nombreuses populations de l’extinction locale, il est important de comprendre comment le mouvement est restreint d’une façon spécifique à l'espèce. Ma thèse fournit un examen détaillé de l’impact de la densité sur les capacités de mouvement d’un amphibien des étangs à travers plusieurs étapes de sa vie et avance notre compréhension de diffèrent mécanismes sous lesquelles la densité elle-même peut avoir un impact sur la dispersion.

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Preface

Thesis Format

This thesis is manuscript based, and adheres to the guidelines of McGill online thesis submission. Each chapter has been written to stand alone as a unique publication in an academic journal, with linking statements between each of the five chapters to help explain the flow of concepts. A general introduction is given to cover concepts that are relevant to the understanding of the whole thesis. A summary of the results outlines my contributions to knowledge, and directions for future research. The references have been formatted to match the specification for the journal *Copeia*.

Author Contributions

I am the first author on all five chapters. I was responsible for the development of the conceptual framework, experimental design, field work and data collection, statistical analyses, synthesis of results, and the writing of all papers. During the field work between 2012 and 2016, I was responsible for the management of six research assistants and twenty student volunteers, who aided in various data collection tasks. David Green provided supervision and input over the course of the project, including substantial editing support for the manuscripts, and is a co-author on all publications from this thesis.

Chapter 2: In this chapter I quantify the effects of larval density on tadpole growth, survival and metamorph size and compare these outcomes between two methods; a unique technique of density manipulation, and the traditional one. **This chapter has been published in the journal *Copeia*.** Yagi, K.T., and D.M. Green. 2016. Mechanisms of density-dependent growth and development in tadpoles of Fowler's Toad, *Anaxyrus fowleri*: volume vs. abundance. Copeia 104: 942-951. doi: http://dx.doi.org/10.1643/CE-16-438

Chapter 3: This chapter investigates whether carry-over effects are present in the density-manipulated tadpoles by examining their activity behaviour between the tadpoles and toadlet life stage. **This chapter is in press in the journal *Copeia*** [CE-17-593].

Chapter 4: I examine behavioural plasticity of tadpoles in this chapter, using a controlled multifactorial experiment with density and temperature.

Chapter 5: In this chapter, I follow size-dependent movement capabilities of individual toadlets from both wild-caught and density-treatment origins. **This chapter is published in the *Journal of Herpetology*** [DOI: 10.1670/17-058].

Chapter 6: The final chapter compiles information obtained from past publications on this population, and my own results from chapter 4, to inform a predictive model that simulates its extinction risk in the context of a metapopulation. **This chapter is in preparation for submission to the *Journal of Applied Ecology*.**

Statement of Originality

This thesis contains the following original contributions to knowledge:

While there is much research on the effects of density on amphibian larval growth, development, survival and metamorph size, my study was unique in my method of density manipulation. To my knowledge manipulating density by changing the volume of a mesh enclosure while keeping the number of individual's constant, has not been done before. In Chapter 2, I carry out this new method, which allows for the elimination of the potential confounding effect of chemical signalling between tadpoles, and to focus solely on the physical competitive interactions among individuals. I was able to demonstrate some differences between the new and traditional method by examining their effects on tadpole growth, survival and metamorph size. I believe this method will become useful in future studies aiming to determine the underlying mechanisms of density-dependence in larval amphibians.

It is understood that carry-over effects exist in various systems, as shown by a variety of studies comparing early-life and later-life conditions in animals who are more sensitive to habitat quality. This concept is not new to the amphibian taxa, however in my Chapter 3 I was able to demonstrate the presence of carry-over effects in terms changes in their behaviour rather than body condition. In addition, while it was challenging, the results of this chapter were relevant in the subsequent three as I could identify individuals and track their movement and behaviour in subsequent years. The consistent information on individuals I collected is rare, but valuable to understanding the long-term impacts of carry-over effects.

Plasticity is also a well-studied concept, particularly in tadpoles where their morphology is easily manipulated by external conditions. However, in my Chapter 4 I examined potential underlying mechanisms of behavioural plasticity in tadpoles, as it is often overlooked since their behaviour does not result in population-level responses like adult reproduction. My examination of such behavioural responses to density, temperature and body size are unique because the interaction these effects is rarely studied, and the results from this chapter provide useful insight to potential contributing factors to post-metamorphic traits.

Monitoring of individuals across all life stages is a knowledge gap in many taxa due to the difficulties in accurately tracking individual’s over large distances and/or extended time periods. With amphibians, this issue is even more challenging as tracking devices and marking techniques cater more to larger, or less vulnerable taxa. In my Chapter 5, I employed the use of photo recognition software created in my supervisor’s lab to enable the monitoring of toads as small as 15 mm through to adulthood. With this technique, I could monitor a large number of individual toads and examine their movement rates through recapture data and relate it back to their known tadpole growth conditions. To my knowledge, this type of continuous data has not been achieved before, therefore the information presented is not only informative of how carry-over effects can impact individuals in the long term, but how it is possible to evaluate such long-term effects in small, vulnerable animals.

Finally, my Chapter 6 is novel because the updated population estimates, results from past publications, and my own calculations have not been incorporated together in any models previously. Using the endangered Fowler’s toads in Long Point in a case study, I took past published predictions of the populations growth, habitat loss, dispersal rates and combined them with my own estimations for density- and condition-dependent dispersal in a simulation model to predict the populations extinction risk. The results from this chapter highlight the importance of having calculated conservation methods and are invaluable to the ongoing initiatives to improve wetland quality at this location. More generally, this kind of study demonstrates the importance of long-term monitoring and provides a modern example of how scientific research can inform management decisions.

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The preparation of this thesis could not be completed without the support and guidance from many people in my life. I'd like to first acknowledge my supervisor, David Green, for his academic support over the last five years. Your easy-going nature made it possible for me to discover my own potential as a researcher and teacher in the academic world. I enjoyed being a teaching assistant to your courses, Herpetology and Conservation Biology, which were right up my alley! Second, I want to thank my mother, Anne Yagi, for her inspiration and support over the last three decades. Growing up with a mother as a biologist inspired my love of biology and academia. You inspire me every day and it was your passion for conservation that inspired my project in the first place. I'm so proud of you for pursuing your own graduate degree in snake ecology and physiology!

Next, I could not get through an entire PhD without the help my lab mates. I'd like to thank Flavia Papini, Nicholas Gervais, Daniel Greenberg, David O'Connor, Elizabeth McCurry, Pablo Menendez, Brandon Varela and Jim Barnett for their help with stats, moral support and lengthy amphibian-related discussions. I greatly appreciated Dan's assistance in explaining R code in the early stages of my project as well as Nick's continual support and help in translating various abstracts into French over the years, thank you! I especially need to thank Flavia for the past five years; you were an awesome lab mate and partner-in-crime during our time in Long Point! You helped me organize four seasons-worth of student volunteers, kept the cottage in tip-top shape, helped me with my field work as my assistant and still helped me in following years even when you had your own Master's research going on … Thank you so much!

In addition to my lab mates, I need to also thank all the student volunteers and field assistants that were involved in the Long Point population survey. I was fortunate to get to know all of you at some point during my time as “Leader of the Toad People”, so thank you to: Annie Tseng, Meghan McNiel, Stephanie Yung, Meghan McGee, Marianne Coquilleau, Bronwyn Lira Dyson, Elisabeth Belanzaran, Edith Shum, Stephen Lee, Adam Verillo, Stefan Gallini, Luke Currin, Nadia Dalili, Kirsten Crandall, Andrea Cherry, Owen Tao, Karel Cantelar-Ramos, Jihane Benbahtane, Clara Del Degan, Jeremie Maranda, and Manuela Tomic. I wish you all the best in your future endeavors.

My roommates, who were also my peers, also deserve a big thank you! I truly enjoyed our friendships and your continual support over the years, thank you to Dorothy Maguire, Olivia Leung and Kay Min for always believing in me and listening to my practice talks whenever I asked.

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Finally, I’d like to thank my funding sources; OMNRF Species at Risk Stewardship Fund, OMNRF Species at Risk Research Fund, Wildlife Preservation Canada, and NSERC for providing the means for me to conduct this study with Fowler’s toads in Long Point Ontario. The funding made everything possible and I hope the results of my study aid in the management and recovery of Fowler’s toads, and other species at risk, in Canada.

General Introduction

Dispersal is ultimately a process by which populations exchange genetic material, escape unfavorable environmental conditions, and rescue each other from local extinction. Due to its apparent impact on behaviour and physical condition of individuals, a major contributing factor to their probability of dispersal is the level of density-dependence. This phenomenon may affect organisms at multiple life stages, therefore the ultimate effect of density may not become apparent until the life stage where dispersal occurs.

Because amphibians are known for their global declines, they have been a focus when examining human-derived changes to the landscape. Therefore, the mechanism of density-dependence has been well studied in amphibians at their larval and terrestrial stages separately, yet the continuity of the effect across all life stages is rarely documented, as well as the potential impact to their dispersal ability. Therefore, I focused my efforts into examining density-dependent dispersal using empirical data, and overall population dynamics of a well-studied population of Fowler's toad (*Anaxyrus fowleri*).

Chapter 1: Literature Review

In ecological terms, dispersal is the unidirectional movement of individuals or propagules away from the natal habitat to a new breeding habitat (Semlitsch, 2008). It is an important population level process in facilitating gene flow, species range expansion and maintaining connections within a metapopulation (Gadgil, 1971; Ronce, 2007). A metapopulation is a collection of subpopulations, where local extinctions are minimized by dispersal-mediated rescue events (Hanski and Gilpin, 1991; Caley et al., 1996; Hastings and Botsford, 2006; Bell and Gonzalez, 2011). Although sometimes used interchangeably with dispersal, migration is a substantially different process where individuals exhibit navigation behaviour that ultimately returns them to their starting point, also called philopatry (Semlitsch, 2008).

From an individual's perspective, dispersal can be viewed as a multi-stage process; *emigration*, the departure from the natal or source habitat, *transfer*, the movement through the landscape, and *immigration*, or settlement in a new habitat (Clobert et al., 2009; Bonte et al., 2012; Martin and Fahrig, 2016). However, measuring this process among natural populations is often challenging due to the difficulties in following individuals over large spatial- and time scales, as well as the high mortality risk associated with it (Bonte et al., 2012). Thus, many biologists tend to use models to estimate dispersal probabilities rather than use measures of actual dispersal to predict the long-term persistence of populations (Poethke and Hovestadt, 2002; Travis et al., 2009; Ponchon et al., 2014).

Dispersal can be considered a passive or active process. As a passive process, it is a result small individuals or propagules moving by external forces, such as by wind, currents or when carried off by animals (Frisch et al., 2006; Bruckerhoff et al., 2014). This kind of movement is found mainly in plants, microbes and small sessile invertebrates (Clobert, 2012), and can generally be predicted by simple Brownian motion governed by normal Gaussian statistics (Viswanathan et al., 2011). Active dispersal occurs when the organism controls its own movement (Clobert, 2012), and involves complex integrated mechanisms that contribute to a decision-making process (Bowler and Benton, 2005; Poethke et al., 2016) based on environmental, social, physical and genetically-based cues (Bowler and Benton, 2005; Poethke et al., 2016).

There are also many costs and risks associated with dispersal, such as the energy investment required to develop dispersal-related phenotypes, like wings or larger body size (Dingle, 1996; Jenkins et al. 2007), the risk of predation during the transfer stage, or the risk of being unsuccessful in finding an appropriate habitat patch to settle in (Yoder et al., 2004; Benard and McCauley, 2008; Bonte et al., 2012). However, it is thought that dispersal will be selected for when environmental change alters these costs (Bonte et al., 2012).

There are two basic patterns of animal movement; correlated and uncorrelated. Correlated movements describe how an animal moves in a directed manner, and the direction of a movement step is dependent on the immediately previous one (Byers, 2001; Bartumeus and Levin, 2008; Barton et al., 2009). The initiation of correlated movement may often be caused by external triggers, like predation, competition, or change in habitat quality (Bowler and Benton, 2005). There is also some evidence of correlated movement based on the individual's genetic predisposition for dispersal (Clobert et al., 2001). Uncorrelated movements, however, follow a random-walk pattern, typical of searching behavior in a homogenous landscape (Turchin, 1998; Mårell et al., 2002; Codling et al., 2008). Both movement patterns can occur at different spatial scale throughout an individual's lifetime (Nathan et al., 2008). Also, such movement patterns can result in dispersal as an end-product secondarily, if dispersal was not the original intention of their movements (Van Dyck and Baguette, 2005; Conradt and Roper, 2006). Movement behavior with both correlated and uncorrelated patterns has been associated with “fat-tailed” probability distance curves (Nathan et al., 2008), also called a Lévy walk (Viswanathan et al., 2000). This movement pattern includes random walks, with occasional long-distance directed movements, used to define foraging behavior in animals living within a patchy resource landscape (Bartumeus et al., 2005; Benhamou, 2007; Reynolds et al., 2009; Focardi et al., 2009).

Density-dependent dispersal, in the context of animal movement, can most easily be described by the ideal free distribution model, which assumes that animal fitness decreases as crowding increases (Fretwell, 1972). Therefore, it is understood that we should expect dispersal rates to increase when environmental conditions become unfavorable (Travis et al., 1999; Innocent et al., 2010; Hovestadt et al. 2010) leading to the prediction that dispersal should increase with density (i.e. positive density-dependent dispersal; Matthysen, 2005; Clobert et al., 2009; De Meester and Bonte, 2010; Innocent et al., 2010; Altwegg et al., 2013). Dispersal can also decrease with density (i.e. negative density-dependent dispersal; Matthysen, 2005; Ims and Andreassen, 2005; Meylan et al., 2007), where dispersal is mediated by Allee effects or conspecific attraction (Roland et al., 2000; Fauvergue, 2013), the seasonality of resources (Matthysen, 2005; Baguette et al., 2011; Rodrigues and Johnstone 2014), or by density-dependent carry-over effects (Harrison et al., 2011; O’Connor et al., 2014; Betini et al., 2015).

There has been increasing evidence suggesting that not all dispersers are equal in body condition (Meylan et al., 2002; Clobert et al., 2009; Clausen et al., 2015), a trait understood to influence dispersal decisions (Anholt, 1990; Bowler and Benton, 2005; Clobert, 2012). For example, it may be expected that large individuals are better competitors, and would out-compete the small, weak competitors, therefore increasing the likelihood of small-sized dispersers (Léna et al., 1998; Hanski et al., 1991). However, one might also observe the opposite trend, where individuals of good condition or large size have better energy stores (Scott et al., 2007) and large step lengths, suggesting a higher dispersal propensity, compared to their smaller, slower counterparts (Anholt, 1990; Léna et al., 1998; Barbraud et al., 2003). This phenomenon has been studied across various taxa, under the term "condition-dependent dispersal" (Ims and Hjermann, 2001; Bonte and de la Peña, 2009; Dubois et al., 2010). Therefore, condition-dependent dispersal is another dispersal-related phenomenon that defines the intrinsic capacity of an individual impacting their immigration and survival (Bowler and Benton, 2005; Bates et al., 2006; Clobert et al., 2009; Bonte and De La Peña, 2009; Harrison et al., 2011).

A major factor influencing the body condition of individuals is the quality of the local habitat (Pettorelli et al., 2002; Brown and Sherry, 2006; Burton et al., 2006; Janin et al., 2011). This relationship has been observed to occur across generations through maternal-effects, given the condition of the maternal parent is transferred to her offspring (Mosseau and Fox, 1998). There is also much evidence that poor body condition is the result poor early life growth that becomes apparent later in adult life stages (Benard and McCauley, 2008), in the form of "carry-over effects" (Harrison et al., 2011; O'Connor et al., 2014). This may add a level of temporal- to the otherwise spatial understanding of dispersal predictions in populations with degraded habitat. Therefore, the impacts of reduced habitat quality are important to consider when measuring the effects of habitat loss in populations.

Carry-over effects have been detected in a wide variety of taxa, including birds (Drake et al., 2013; Clausen et al., 2015), mammals (Perryman et al., 2002; Cook et al., 2004), reptiles (Goodman, 2008), fish (Kennedy et al., 2008), invertebrates (Hettinger et al., 2013; Van Allen and Rudolf, 2013), and plants (Krepkowski et al., 2013), and may be particularly strong in animals with complex life histories, such as many marine invertebrates (Hettinger et al., 2013; Fischer and Phillips, 2014) and amphibians (Earl and Semlitsch, 2013; Tarvin et al., 2015). The larvae of these organisms are exposed to very different environmental conditions than the adults, which can have lasting effects on the animals after metamophosis (Goater, 1994; Álvarez and Nicieza, 2002; Green and Bailey, 2015).

Understanding density-dependent dispersal in amphibians is complex due to common traits like pond fidelity (Reading et al., 1991; Smith and Green, 2006; Gamble et al., 2007), their dependency on habitat connectivity (Cushman, 2006; Semlitsch, 2008) and relatively slow terrestrial movement rates (Smith and Green, 2006). However, some amphibians have been documented to move large distances (Smith and Green, 2006; Semlitsch, 2008) allowing their probability-distance curve to be "fat-tailed" (Nathan et al. 2008). Amphibians also show various degrees of density-dependence at different life stages (Altwegg, 2003), for example as larvae, density negatively correlates with survival, growth and metamorph size (Goater, 1994; Yagi and Green, 2016). Yet, it has been documented that juvenile frogs are attracted to areas inhabited by conspecifics (Pizzatto et al., 2016), and in many species, males aggregate to produce choruses, a cue to attract females during the breeding season (Bee, 2007; Swanson et al., 2007). These behaviours suggest there is a preference in adult amphibians, for living at higher densities (Courchamp et al., 2008), however, carry-over effects from their larval stage may prohibit advantageous dispersal behaviour when environmental change occurs.

With the understanding that density-dependence and condition-dependence are likely interrelated mechanisms of dispersal, and our gap in empirical evidence showing how these mechanisms correlate to affect dispersal in a metapopulation (Bonte and de la Peña, 2009), it is necessary to apply this theory in a conservation framework where habitat loss is evident. For populations at risk of local extinction, these mechanisms become necessary to understand in order to determine the best methods of conservation intervention and habitat management.

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Chapter 2: Mechanisms of Density-dependent Growth and Survival in Tadpoles of Fowler's Toad, *Anaxyrus* (*Bufo*) *fowleri*: Volume *vs.* Abundance

Katharine T. Yagi and David M. Green. Published in *Copeia*.

2.1 Abstract

Density dependent growth has ordinarily been studied in aquatic ecosystems by varying the abundance of animals in mesocosms of equal volume. Aside from the unequal sample sizes involved with using this abundance-limited method, confounding factors potentially associated with levels of social interactions may also be introduced. The alternative, volume-limited method, *i.e*. varying the volume while maintaining equal numbers of animals, can provide a test for the presence of potentially confounding factors. Using tadpoles of Fowler's Toad, *Anaxyrus fowleri,* we examined the effect of density on growth rate, timing of metamorphosis and size at metamorphosis in both abundance-limited and volume-limited experiments. We found no difference in tadpole growth rate or timing of metamorphosis between these two methods, but the metamorphs emerging from abundance-limited low density treatments were significantly smaller when compared to those in volume-limited low density treatments. Because toad tadpoles may naturally form social aggregations and schools, this suggests that the actual number of animals present may be important for normal social behaviour and optimizing feeding rate. If volume-limited and abundance-limited methods of manipulating density are not equivalent, treatment method may itself be a factor that can differentially affect growth variables.

2.2 Introduction

Conspecific density is a fundamental factor influencing somatic growth and survival of plants and animals (Watkinson, 1980; Goater, 1994; Goldberg et al. 2001; Lorenzen and Enberg, 2002; Browne et al. 2003). At high densities, increased competition among individuals for mates and resources is usually associated with negative consequences, such as reduced growth rate (Wilbur 1977; Sedinger et al., 2001; Lorenzen and Enberg, 2002), prolonged development rate (Warner et al., 1991), and lower survivorship (Dash and Hota, 1980; Warner et al., 1991; Browne et al. 2003). However, it is possible to have positive effects resulting from high larval densities, such that reduced survival in early life stages can increase survival in later life stages and ultimately increase recruitment (Vonesh and De la Cruz, 2002; Karraker et al., 2008). At low densities, reduced competition rates usually allow for positive responses among individuals, like faster development rate or increased somatic growth rate (Wilbur, 1977; Persson, 1986). Yet, at low densities social interactions and mating opportunities should be less frequent or impeded altogether, which will negatively affect individual fitness and reproduction (Courchamp et al., 1999).

The phenomenon of density-dependence has been examined thoroughly in aquatic organisms (Post et al. 1999; Lorenzen and Enberg, 2002; Gimnig et al., 2002; Hildrew et al., 2004), particularly in amphibians with bi-phasic life histories (Dash and Hota, 1980; Wilbur, 1980; Loman, 2004). The complex life history of amphibians includes a dependency on both aquatic and terrestrial habitats at different life stages. Tadpoles occupy aquatic habitats that can be unpredictable in its permanency and quality, therefore tadpoles may experience very high local densities (Crump, 1989; Skelly, 1996; Amburgey et al., 2016). The timing of metamorphosis, a process known to incur high mortality rates (Dash and Hota, 1980), is dependent on both biotic (*i.e.* predators and competitors) and abiotic (*e.g.* pond drying) factors (Wilbur and Collins, 1973; Werner, 1986; Newman, 1998). The size at metamorphosis depends on the amount of resources tadpoles were able to store during their larval growth period (Tejedo and Reques, 1994; Newman, 1998), but is only initiated once a minimum threshold size has been reached (Wilbur and Collins, 1973). In addition, it has been shown that metamorphs incurred higher survival rates when emerging from their ponds at larger sizes, and earlier in the season (Altwegg, 2003).

The classic experimental method for examining density dependence in aquatic organisms is to raise them in aquaria or mesocosms of equal volume and to vary animal density by controlling their abundance, an *abundance-limited* method (Wilbur, 1977; Newman, 1998; McCoy and Bolker, 2008). There are numerous reasons to take this approach. For example, containers, whether mesocosms, aquaria or cattle tanks, that are all the same size and all placed in the same location can be expected to maintain closely similar conditions of water quality, temperature and community composition (Skelly and Kiesecker, 2001). Also, there has been extensive research using abundance-limited mesocosms, including examining ecological interactions affecting food web structure (Gauzens et al., 2015), measuring responses to environmental toxins (Rowe and Dunson, 1994; Boone and James, 2003; Egea-Serrano and Van Buskirk, 2016), and quantifying behaviour and phenotypic plasticity in response to predator-prey interactions (Relyea, 2001, 2002, 2004; Van Buskirk, 2002). Therefore, a large body of literature exists on the success in using such methods.

Although the abundance-limited method has been used for decades to examine density-dependent effects, there are inherent issues associated with comparing animals raised in different group-sizes. Aquatic animals living in high densities, like tadpoles, may exhibit social behaviours to enhance their collective survival, like forming schools or aggregations (Wilbur 1980; Spieler and Linsenmair, 1999). There are many benefits to this behaviour, such as; an enhanced ability to avoid and detect predators (Watt et al., 1997; Spieler and Linsenmair, 1999; Spieler, 2003); enhanced foraging (Eterovick, 2000; Sontag et al., 2006) and thermoregulation efficiency (Brattstrom, 1962). The major cost associated with aggregation behaviour is an increased degree of competition, such as local resource exploitation (Kuzmin, 1995; Griffiths and Foster, 1998), behavioural interference (Steinwascher, 1978), and allelopathic interference via fecal matter (Petranka, 1989; Beebee, 1991; Griffiths et al. 1991). If animals are raised in different density levels, there would be potential bias in variables such as growth rate and survival due to the supposed influence of living in a group of a particular size. Furthermore, when density is manipulated by changing the abundance of individuals, the larvae of many species of anurans may naturally aggregate to form schools (Wassersug and Hessler, 1971; Beiswenger, 1978; Griffiths and Foster, 1998), and can be expected to be able to do so effectively only when their abundance is sufficiently high. Therefore, because most mesocosm experiments are inherently abundance-limited and each mesocosm is an isolated aquatic system, they may not be ideal to address all questions concerning the effects of density-dependence, particularly the potential influence of social behaviours.

To test for the potential influence of social behaviour on density dependent growth and development in tadpoles, we made a comparison of responses in growth rate, timing of- and size at metamorphosis, between the *abundance-limited* method and an alternative method of varying density, called *volume-limited*. Using Fowler’s toad (*Anaxyrus fowleri*) tadpoles, a species from a genus known to naturally form aggregations (Wassersug, 1973; Breden et al., 1982) and are typically exposed to various larval densities due to the stochastic nature of their breeding habitat

(Wilbur and Collins, 1973; Smith 1983; Semlitsch, 2002), we created a volume-limited system where the number of animals per treatment is kept constant and the size of the container is made variable. We implemented this scheme using free-floating mesh pens of various sizes deployed in the same semi-natural pond. This arrangement was designed to eliminate variance in environmental conditions between pens, including food availability, temperature and any negative effects associated with tadpole feces (Licht 1967; BeeBee, 1991; Griffiths et al., 1991) because the mesh sides and bottom of the pens allowed particles to fall through into the open pond water. Therefore, we hypothesized that if tadpole aggregation behaviour greatly influences the magnitude of their density-dependent response, then there should be differences in tadpole growth rate, timing of metamorphosis and size at metamorphosis between abundance-limited and volume-limited trials, especially at low abundances when aggregations cannot form.

2.3 Materials and Methods

2.3.1 Study species.— *Anaxyrus* (formally *Bufo*) *fowleri*, Fowler's Toad, is a relatively small and common toad ranging widely throughout the eastern United States reaching as far north as the northern shore of Lake Erie in Ontario, Canada. In Canada, this toad is classified as Endangered both provincially and federally (COSEWIC, 2010) and is considered to be a beach-dune specialist, isolated within three remnant populations. The population we focus on has been monitored for nearly 30 years (Green, 1989), and its breeding habitat is currently being limited by the invasion of the Common Reed, *Phragmites australis* (Greenberg and Green, 2013).

2.3.2 Study site.—The study was conducted in the Thoroughfare Point Unit of the Long Point National Wildlife Area (NWA) (between 42°34'33" N – 42°35'3" N and 80° 22'15" W – 80°28'24" W) in the Carolinian ecoregion of southern Ontario. In collaboration with the

Canadian Wildlife Service, twelve ponds were dug out in the marshes, north of the beach-facing dunes in November 2012. These ponds were left to naturalize, and were designed to be permanent sources of open water habitat for amphibian use. The twelve ponds, labeled 'pond 1' through 'pond 12', were situated in pairs separated by 500 m increments in an east-to-west transect bordering the edge of extensive spans of the invasive reeds. Paired ponds were placed approximately 3 m apart from each other. Individual ponds measured on average to be 13 m long, 7 m wide, 0.9 m at its deepest point, and were lined with a mixture of organic and sandy substrate.

Although they were in early succession, we recorded the presence of emergent vegetation within and around the ponds aside from *Phragmites*. This included bladderwort, *Ultricularia sp*., milfoil, *Myriophyllum sp*., horsetail, *Equisetum sp.*, Canada rush, *Juncus canadensis*, cattail, *Typha sp.*, and green algae, *Spirogrya sp*. We also documented larger vegetation in the marsh area, which included dogwood shrubs, *Cornus sp.*, and large cottonwood trees, *Populus sp*., but they were sparsely distributed and provided little-to-no canopy cover or shade. Other taxa documented using these ponds comprised of all local amphibian species; the majority being Green frog, *Lithobates clamitans*, Northern leopard frog, *Lithobates pipiens*, Bullfrog, *Lithobates catesbianus*, and American toad, *Anaxyrus americanus*, some reptiles such as Snapping turtle, *Chelydra serpentina*, Eastern garter snake, *Thamnophis s. sirtalis*, and Northern watersnake, *Nerodia s. sipedon*, three fish species; Mud minnow, *Umbra limi*, Northern pike, *Esox lucius*, and Pumpkinseed, *Lepomis gibbosus*, and many unidentified aquatic invertebrates, including dragonfly larvae, aquatic beetles, and crayfish. Fowler's toads were not found using the ponds in both 2013 and 2014 breeding seasons.

2.3.3 Experimental set-up.— We collected egg masses from a total of four amplectant pairs of Fowler's toads in the wild, one collected on 20 May, 2013 and three collected between 24 May and 26 May, 2014. We raised the larvae in floating mesh pens within 'pond 1' until they reached stage 25 of development (Gosner, 1960). All tadpoles were randomly chosen and deployed into their density trials at the same stage and time (12 June 2013, and 2 June 2014), except for the group of tadpoles deployed into 'pond 5' in 2013. Due to the relatively cool spring conditions in 2013, some toads delayed breeding up to two weeks, resulting in stage 25 tadpoles being found and deployed into their density trials 13 days later, on 25 June 2013, than the rest of the tadpoles. In 2014 the stage 25 larvae from three egg masses were mixed before being separated into their density trials.

The floating mesh pens were custom-built aquatic enclosures (John Radford, Ajax, Ontario) designed to hang from water-proofed, floating plywood and Styrofoam frames. To allow free passage of water and materials in and out of the enclosures, the sides were constructed of 0.7 mm fine nylon mesh whereas the bottoms were made of coarser 1 mm fiberglass mesh. Each enclosure also had a 1/8-inch aluminum frame inside the mesh enclosure as a weight and a lid with 1-inch wire mesh to help protect from large predators. Eight sizes of enclosures were constructed: 1,200 L, 800 L, 400 L, 200 L, 100 L, 60 L, 30 L and 20 L. All enclosures were 60 cm in depth and were placed in the ponds for a minimum of five days prior to initiating density treatments, to allow periphyton growth for tadpole consumption (Fig. 1).

We monitored pond temperature by deploying a total of 28 temperature data loggers (iButton, model DS1922L), waterproofed with Plastidip®, two into each of two floating mesh pens in three ponds in 2013, and four ponds in 2014 for the duration of the tadpole density trials. In each pond, two loggers were set to record surface temperatures by being inserted into a small sponge to allow the logger to float with changing water levels, and fixed with tape and fishing line to the lid of two floating pens. Meanwhile two more loggers were attached to the bottom of the same two pens to record the temperatures at a depth of 60 cm.

Density of adult Fowler's toads in this study site fluctuates annually, but has ranged from 4 to 93 toads per kilometer between 1989 and 2011 (Greenberg and Green, 2013). Natural Fowler's toad tadpole densities have not yet been estimated in Long Point. However, Fowler's toad tadpoles tend to aggregate and their habitat is ephemeral, it would be very difficult to make any precise measurements of natural tadpole densities to compare to. Therefore, we raised tadpoles in the enclosures through to metamorphosis at a range of densities based on previous and similar studies (Wilbur, 1977; Goater, 1994; Altwegg, 2003; Relyea, 2004), while attempting to cover a broad enough range in order to detect suitable density-dependent relationships. For *volume-limited* experiments, all eight sizes of enclosures held 100 tadpoles each, creating initial densities of 0.08, 0.13, 0.25, 0.50, 1.00, 1.67, 3.33 and 5 tadpoles/L. Two sets of eight different sized enclosures were installed in separate ponds ('pond 4' and 'pond 5') in 2013, and three sets of eight in 2014 ('pond 4', 'pond 5' and 'pond 7'). We executed an a*bundance-limited* experiment in one pond in 2013 ('pond 10'), using four 216 L enclosures stocked with; 17, 28, 216 and 719 tadpoles, producing densities of 0.08, 0.13, 1.00 and 3.33 tadpoles/L respectively. In order to determine if manipulating density by using various sized enclosures had no effect on the variance of the measured variables, we executed a *density-controlled* experiment in one pond in 2014 ('pond 6'), using one set of the eight different sized enclosures. Each enclosure held a different number of tadpoles; 300, 200, 100, 50, 25, 15, 8 and 5 so all eight enclosures held the same density, 0.25 tadpoles/L.

2.3.4 Data collection.—We measured the average total length, TL, of tadpoles, equal to the distance from the tip of the rostrum to tip of the tail (Scott, 1990; Van Buskirk 2002; Ukuwela and Ranawana, 2011), from 20 randomly chosen individuals per enclosure at the beginning of each experiment and every four to seven days thereafter over a maximum of 74 days in 2013, and 80 days in 2014. Digital photographs of the tadpoles were taken *in situ*, and were immediately returned to their density group afterwards. We recorded number of metamorphs emerging per treatment to estimate survival percentages. Upon the first sight of a metamorph, the pens were checked daily thereafter for new metamorphs. Initial Growth Rate, GR, of tadpoles per enclosure was quantified as TL at time t (i.e. number of days until the growth curve plateaus) minus TL at $t = 0$, divided by t. The value for t varied between ponds in 2013; ‘pond 4’ was 20 days, ‘pond 5’ was 23 days, except density level 0.08 and 0.25 tadpoles/L had a t value of 17 days, and ‘pond 10’ was 28 days for all density treatments. In 2014, t value was consistent at 22 days for all ponds and density treatments.

We defined size at metamorphosis, SM, as Total Length at stage 42 (Walsh 2010), when the animals had four developed limbs yet the entire tail was still present. This was done to estimate size at metamorphosis while making sure to avoid complications in measuring the animals during the subsequent period of drastic physical change (*i.e.* tail resorption). Time to metamorphosis, TM, for each enclosure was the mean number of treatment days, t, until stage 42 metamorphs were detected. All length measurements of tadpoles were made by placing them in a petri dish underlain by 6 mm grid graph paper, photographing them, and analyzing the photographs with ImageJ (vers.1.46r, Schneider et al., 2012). All photographs were taken in the field using a small level stool and a tripod to keep the petri dish and camera level, after which the tadpoles were immediately returned to their assigned density trial.

2.3.5 Analysis.— We compared temperatures between ponds and years, using mean daily temperatures collected in all ponds and analyzed with a one-way analysis of variance (ANOVA). We ran the analysis under the assumption that each pond was independent from others, within and across years, and used a variable called ‘pondyear’ to accomplish this statistically.

Survival proportions were calculated using the number of metamorphs emerging from each density trial from each pond, divided by the initial total number. If tadpoles did not reach metamorphosis, they were not counted as a survivor for their density group even if they were still alive by the end of the season. We kept calculations for the 2013 ponds separate to avoid potential biases from the late-starting tadpoles in pond 5, and we combined the data from 2014 to create mean survival proportions. Due to the lack of replication at the pond level between density methods and years, we only made simple quantitative comparisons of survival proportions.

We compared the linear relationship between initial tadpole density and GR, SM, and TM of the abundance-limited pond to the same relationships, from the two volume-limited ponds in 2013. A regression line was fit to the data from each 2013 pond separately, again allowing us to isolate any differences observed from the late-starting tadpoles in pond 5. This was accomplished using independent t-test comparisons of paired linear regressions, using the lm function and summary command in R (R Core Team, 2015). We tested the effect of the enclosures (*i.e*. density-controlled trials) on GR, SM and TM using the non-parametric Mann-Whitney U Test to compare between the 2014 volume-limited ponds (N = 3) and the density-controlled enclosures in pond 6 (N = 8) set at 0.25 tadpoles/L. Data from enclosures that had been damaged during the experiment were removed from the analysis. All variables were tested for normality using QQ plots and Shapiro-Wilk’s test, and tested for homogeneity of variance using Levene’s test. All density and TM values were transformed by natural logarithm to allow for linear comparisons. All statistical tests were performed using R Studio (version 0.99.491), with α = 0.05.

2.4 Results

All temperature data was found to have equal variance (*F* = 0.92, *p* = 0.48). In 2013, mean seasonal temperature for the ponds was 24.6˚C, ranging from 19.8˚C to 31.5˚C, which was warmer compared to the mean seasonal temperatures in 2014, being 23.7˚C, ranging from 19˚C to 28.7˚C. The pond temperatures were found to be different between years ($F_{1,418} = 23.4$, $p < 0.001$), but not within years (2013: $F_{2,177} = 0.07$, $p = 0.94$; 2014: $F_{3,236} = 2.55$, $p = 0.06$). In addition, aggregation behaviour was never observed during our density trials, however anecdotal observations showed tadpoles having a tendency to gather at the surface or the sides of the mesh pens, usually where the sunlight was more direct.

All GR and SM data were found to be normal with homogeneous variance. The TM data from 2013 were found to be normal once we used the natural-log transformation. A total of ten data points were removed from the 2013 volume-limited ‘pond 4’ dataset due to two damaged enclosures; density levels 1.00 and 3.33 tadpoles/L, and an outlier from density level 5.00. This included two survival measures, two GR data points from density levels 1.00 and 3.33 tadpoles/L, and three data points from each of SM and TM data, at density levels 1.00, 3.33 and 5.00 tadpoles/L. Also, no data was collected from the smallest enclosure size for the density-control dataset due to high mortality rates and the fact that we started with only five tadpoles.

2.4.1 Survival.— There was a high degree of variation in survival between density groups within *volume-limited* ponds. For example, the highest survival percentage came from pond 5 at 58% in the second lowest density group, 0.13 tadpoles/L, which was a 65% increase from the survival percentage in the lowest density group (Table 2.1). Similarly, pond 4 showed a 38% increase from 26% in density group 0.13 to 16% in group 0.08 tadpoles/L (Table 2.1). In both ponds however, the highest density group, 5.00 tadpoles/L, showed the lowest level of survival at 2% in pond 4 and 5% in pond 5 (Table 2.1). For abundance-limited groups, the lowest density level, 0.08 tadpoles/L had the highest survival, at 35%, which was a 67% increase from survival at 0.13 tadpoles/L. From density level 1.00 tadpoles/L decreasing to 0.13 tadpoles/L, there was a 200% increase in survival (Table 2.1). The highest density level had the lowest survival, at 4%, a 75% decrease from density level 1.00 tadpoles/L (Table 2.1). Interestingly, for the density-controlled experiment, survival percentages ranged from 40% at 0.25 tadpoles/L to 72% at 1.00 tadpoles/L, with the exception of the smallest enclosure size having zero survivors (Table 2.1).

2.4.2 Abundance-limited density.—The lowest density level (0.08 tadpoles/L) produced the fastest GR (0.76 mm/day), the largest mean SM (23.98 mm ± 0.48 SE) and the shortest mean TM (17 days ± 1.63 SE; Table 2.2). Density had a significant ln-linear relationship with all three variables; GR ($R^2 = 0.97$, $P = 0.01$), SM ($R^2 = 0.98$, $P = 0.008$) and TM ($R^2 = 0.93$, $P = 0.02$; Table 2.3).

2.4.3 Volume-limited density.—Overall, the lowest density level (0.08 tadpoles/L) from the volume-limited density ponds in 2013 produced the fastest mean GR (1.24 mm/day ± 0.03 SE), largest mean SM (29.97 mm ± 1.68 SE), and the shortest mean TM (17 days ± 0 SE; Table 2.2). Conversely, the highest density level from the volume-limited density ponds 5 in 2013 generated the slowest GR (0.17 mm/day, at 5 tadpoles/L; Table 2.2). The highest density level from volume-limited density pond 5 in 2013 showed the smallest mean SM (20.31 mm ± 0.50 SE at 5 tadpoles/L; Table 2.2). The longest mean TM came from the highest density level in the volume-limited density ponds in 2014 (66 days ± 2.34 SE at 5 tadpoles/L; Table 2.2). Density appeared to have significant ln-linear relationship with all three variables in all five *pondyears* (Table 2.3).

2.4.4 Abundance-limited vs. Volume-limited.—The independent comparisons of the paired linear regressions revealed that the regression slopes for the relationship between Density and GR is not differ between abundance-limited pond 10 and volume-limited pond 4 ($t = -1.18$, $df = 12$, $P = 0.26$) , but did differ with volume-limited pond 5 ($t = -3.20$, $df = 12$, $P = 0.01$; Fig. 2.2). Similarly, the same relationships were found for the TM dataset, where the slope for pond 10 did not differ from the slope for pond 4 ($t = 0.69$, $df = 11$, $P = 0.51$), but did differ for pond 5 ($t = 2.29$, $df = 11$, $P = 0.04$; Fig. 2.2). Interestingly, the slopes for the SM data were found to differ from pond 10 in both pond 4 ($t = -4.99$, $df = 11$, $P < 0.001$), and pond 5 ($t = -2.92$, $df = 11$, $P = 0.01$; Fig. 2.2).

2.4.5 Density control.—Tadpoles that were reared under the density-control method did not show a large difference in mean GR, SM and TM across enclosure sizes (Table 2.4). These measurements produced a mean GR of 0.64 mm/day ± 0.02 SE, which was not significantly different from the mean GR of the volume-limited enclosures at the same density level (0.59 mm/day ± 0.09 SE; $U = 13$, $P = 0.65$). The mean SM for the density-control enclosures was 24.04 mm ± 0.12 SE, which was not significantly different from the mean SM of the volume-limited enclosures at the same density level (23.30 mm ± 0.98 SE; $U = 13$, $P = 0.67$). Finally, the mean TM for the density-control pens ranged from 28 to 41 days, with a mean of 33 days ± 1.9 SE (Table 2.3). This was not found to be significantly different from the mean TM for the volume-controlled pens at the same density level (33 days ± 0.66 SE; $U = 11.5$, $P = 0.91$).

2.5 Discussion

In terms of the general effect of density on the growth rate and size at metamorphosis, our results show a clear negative trend. Looking closely at the time to metamorphosis results for the *volume-limited* ponds, there appears to be a threshold, where above 1.00 tadpoles/L metamorphosis is delayed substantially. When we examine the survival data closely, a similar threshold is suggested by the values for the 2013 ponds, while the 2014 ponds appear to have above 30% survival up to 1.67 tadpoles/L. In all cases, certainly the highest two density levels we tested gave results showing tadpoles most negatively impacted by density. Considering our density-controlled pond, the only survival percentage that stood out was from the 100 L enclosure. This enclosure size was used in the 1.00 tadpoles/L density level in the *volume-limited* ponds, suggesting that tadpoles might do better in medium to small-sized pens (i.e. 30 to 100 L) compared to larger ones.

Looking at our methods comparison, our results suggest that there may be an effect of tadpole abundance level on metamorph size, due to the differences observed in the SM *vs* density regression slopes between *abundance-limited* and *volume-limited* ponds. Although we did find some differences between the two density methods for GR, these differences were only observed for pond 5, the group of late-starting tadpoles. Therefore, we are not confident that these differences are due to the degree of tadpole abundance alone. Since we expected to observe greater differences in all three variables, GR, SM and TM, our results are not completely consistent with the hypothesis.

In addition, the survival proportions showed a similar decrease with density, but the high degree of variation between density groups within a pond made it difficult to compare between ponds. After inspecting the results closely, we noticed a possible exponential decrease in survival for the *abundance-limited* pond, compared to a more-or-less linear relationship with the *volume-limited* ponds. It is possible this difference in relationship-type is caused by tadpoles living in low abundance and low density, however additional *abundance-limited* density trials are needed to confirm this.

In terms of social behaviour, it is at lower abundances where we might expect to see a difference in density-dependent effects. We are able to see this difference more clearly when looking at the SM regression lines (Fig. 2.2). Here, the individuals emerging from the low-abundance and low-density groups (*i.e.* pond10) emerged at much smaller sizes than their *volume-limited* counterparts, from both ponds. This may suggest that there could be a link between small metamorph size and the lack of social interaction or aggregation ability at this abundance level, although it is important to note that we did not directly observe aggregations in our density trials. On the contrary, Griffiths and Foster (1998) discovered that Bufonid tadpoles grew slower when raised in small groups under laboratory conditions, compared to isolated individuals.

Tadpoles benefit from aggregations in various ways. One is the enhancement of feeding efficiency, which can occur when the substrate is stirred by small swimming motions, allowing food particles to be exposed to more individuals in the group (Beiswenger, 1972; Wilbur, 1977). Continuous feeding in tadpoles during their development can allow individuals to reach metamorphosis at a larger body size (Eterovick, 2000). It is tadpoles with larger body size who have a higher chance of reaching their post-metamorphic stage (Goater, 1994; Chelgren et al., 2006; Scott et al., 2007). However, smaller metamorph size has been also shown to induce compensatory post-metamorphic growth and high overwintering survival rates (Boone, 2005).

Another benefit of aggregation is the facilitation of creating optimal thermal conditions (Lillywhite et al., 1973; Beiswenger, 1978; Guilford, 1988; Caldwell, 1989). Aggregations of black tadpoles, like Bufonids, are usually observed living as large black masses in shallow water, within which temperatures have been measured to be 2 - 3˚C warmer than surrounding water temperature (O'Hara, 1981). It is both the coloration and behaviour of such aggregations that appear to maximize their thermoregulatory ability by absorbing solar energy to heat up their microhabitat (Caldwell, 1989; Guilford, 1988). As shown by the density-controlled comparison, we did not find a difference in the measured variables and the enclosure size they were housed in. Since the enclosures were all built with the same depth, and we did not find strong differences in pond temperatures within the same year, all tadpoles likely had equal opportunities for thermoregulation. We also did not observe any noticeable differences in tadpole behaviour under these controlled conditions. If tadpoles were observed basking near the surface of the water, they were doing so in all eight various-sized enclosures.

Finally, aggregations can assist individuals in reducing the impact of predation (Watt et al. 1997; Spieler, 2003). For example, tadpoles can sense chemical cues released into the water, allowing for warning signals to be detected when predators are present (Stauffer and Semlitsch, 1993; Richardson, 2006; Fraker et al. 2009). Also, when living in large groups the impact of predation is diluted because there is equal chance for any other individual to get caught (Fraser and Keenleyside, 1995; Watt et al. 1997). A few studies have examined how predation-induced stress can cause changes in behaviour and phenotypic plasticity in amphibian tadpoles (McCollum and Leimberger, 1997; Relyea and Mills, 2001; Schoeppner and Relyea, 2009; Maher et al. 2013). More importantly, a reduction in body size as a result of increased predation pressure has been documented in some studies, where the effect is caused by; selection by predators favoring larger prey items (Werner, 1986; Blanckenhorn, 2000), a change in tadpole behavioural response (Laurila et al., 1997; Barry, 2014), or predation-induced stress (Relyea and Mills, 2001; Dahl et al. 2012). However, Hossie et al. (2010) reported predator-exposed *Lithobates pipiens* tadpoles to grow larger than their predator-exposed, stress-inhibited counterparts. Nonetheless, we postulate that when tadpoles exist in small group sizes, the effects of predation are not diluted, resulting in a reduced group metamorph size.

In general, our results showing the negative effects of increasing larval density on growth, metamorph size, days to metamorphosis and survival, are consistent with the literature (Wilbur 1977; Petranka, 1987; Altwegg, 2003; Relyea, 2004). It has been shown that larval density has a significant effect on growth and survival of metamorphs (Goater, 1994; Boone, 2005). Considering this, it is likely that the effects we observed in our Fowler's toad tadpoles will carry-over into post-metamorphic life stages. Boone (2005) discovered that some frog species were able to offset small metamorph size with terrestrial growth. In addition, John-Alder et al. (1990) discovered post metamorphic impacts on body size, and subsequently locomotor ability, in individual Fowler's toads when raised under high larval densities. If these impacts are similar to our own system, we may observe differences not only in toad growth and survival, but in dispersal ability as well.

Since the main finding of our study was that tadpoles do their best at higher abundances but at low density, these two factors must be considered together in order to properly assess the mechanisms of density-dependent growth in aquatic organisms. We recommend the use of the volume-limited density method in future studies that wish to control for potential confounding factors like social interaction, in addition to maintaining an equal sample size across treatments. We also recommend an optimal upper threshold density level for Fowler's tadpoles at 1.00

tadpoles/L, and an ideal mesocosm size between 30 L and 100 L. Further experiments are needed to delve deeper into examining tadpole social behaviour and its effects on individual fitness.

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2.8 Tables and Figures

Table 2.1 Proportions of survival through metamorphosis of Fowler's Toad tadpoles according to density (tadpoles/L) in volume-limited, abundance-limited and density-controlled experiments. The values for volume-limited treatments combine data from three ponds in 2014. The Density-controlled experiment had all eight various-sized enclosures run at 0.25 tadpoles/L. The size of each enclosure corresponds to all density-methods except Abundance-limited, and is shown in volume (L).

Density (tadpoles/L)	**Abundance-limited**	**Volume-limited**			**Density-controlled**	**Enclosure size (L)**
	2013 pond 10	2013 pond 4	2013 pond 5	2014 ponds	2014 pond 6	
0.08	0.35	0.16	0.20	0.40	0.42	1200
0.13	0.21	0.26	0.58	0.46	0.41	800
0.25	-	0.09	0.39	0.39	0.40	400
0.50	-	0.15	0.09	0.49	0.48	200
1.00	0.07	-	0.52	0.37	0.72	100
1.67	-	0.04	0.19	0.31	0.47	60
3.33	0.04	-	0.08	0.09	0.63	30
5.00	-	0.02	0.05	0.08	0.00	20

Table 2.2 Growth rate, mean size at metamorphosis and mean time to metamorphosis of Fowler’s Toad tadpoles according to density in volume-limited, abundance-limited and density-controlled experiments. The mean of three ponds are presented for 2014 data. All variance is measured in standard error.

		Abundance-limited	**Volume-limited**			**Density-controlled**
Density		2013 pond 10	2013 pond 4	2013 pond 5	2014 ponds	2014 pond 6
0.08	Growth Rate (mm per day)	0.76	1.21	1.27	0.68 ± 0.03	
0.13		0.68	1.02	0.81	0.65 ± 0.07	
0.25		-	0.90	0.73	0.59 ± 0.09	0.64 ± 0.02
0.50		-	0.83	0.50	0.51 ± 0.04	
1.00		0.57	-	0.41	0.36 ± 0.05	
1.67		-	0.68	0.42	0.32 ± 0.06	
3.33		0.47	-	0.33	0.32 ± 0.05	
5.00		-	0.64	0.17	0.19 ± 0.03	
0.08	Mean Size at Metamorphosis (mm)	23.98 ± 0.48	31.65 ± 0.71	28.28 ± 0.45	24.41 ± 1.28	
0.13		23.27 ± 0.57	27.75 ± 0.54	26.81 ± 0.17	23.94 ± 0.51	
0.25		-	26.17 ± 0.69	23.97 ± 0.29	23.30 ± 0.98	24.04 ± 0.12
0.50		-	25.82 ± 0.37	24.60 ± 0.53	22.71 ± 0.97	
1.00		21.99 ± 0.48	-	23.48 ± 0.58	22.62 ± 0.61	
1.67		-	21.39 ± 0.24	22.01 ± 0.20	21.54 ± 0.77	
3.33		21.21 ± 0.44	-	21.37 ± 0.23	22.35 ± 0.93	
5.00		-	-	20.31 ± 0.50	21.53 ± 0.59	
0.08	Mean Time to Metamorphosis (days)	17 ± 1.63	17 ± 0.00	17 ± 0.00	29 ± 0.48	
0.13		18 ± 1.29	20 ± 0.00	19 ± 0.46	30 ± 1.07	
0.25		-	20 ± 0.00	21 ± 0.45	33 ± 0.66	33 ± 1.92
0.50		-	30 ± 0.50	24 ± 1.58	39 ± 1.11	
1.00		33 ± 0.63	-	29 ± 0.69	45 ± 1.47	
1.67		-	36 ± 0.00	45 ± 0.86	56 ± 3.51	
3.33		35 ± 0.00	-	54 ± 0.87	64 ± 2.23	
5.00		-	-	60 ± 1.14	66 ± 2.34	

Table 2.3 The results of nine linear regressions examining the effect of density on three parameters; growth rate, GR; size at metamorphosis, SM; and time to metamorphosis, TM, under two methods of density manipulation; abundance-limited and volume-limited. Density and TM were transformed with the natural logarithm.

Method	**Model (y ~ x)**	**Year**	**Pond**	**slope**	**R^2**	***n***	**t value**	***SE***	***p* value**	
Abundance-limited	GR ~ Density	2013	pond 10	-0.07	0.96	4	-8.27	0.01	0.014	*
	SM ~ Density			-0.71	0.98	4	-11.32	0.06	0.008	**
	TM ~ Density			0.22	0.95	4	6.03	0.04	0.026	*
Volume-limited	GR ~ Density	2013	pond 4	-0.13	0.87	6	-5.97	0.02	0.004	**
			pond 5	-0.21	0.84	8	-6.24	0.03	0.001	**
	SM ~ Density		pond 4	-2.97	0.89	5	-5.82	0.51	0.01	*
			pond 5	-1.74	0.92	8	-9.17	0.19	<0.001	***
	TM ~ Density		pond 4	0.26	0.91	5	6.24	0.04	0.008	**
			pond 5	0.32	0.94	8	10.88	0.03	<0.001	***
Volume-limited	GR ~ Density	2014	pond 4	-0.09	0.74	8	-4.60	0.02	0.004	**
			pond 5	-0.11	0.95	8	-11.24	0.01	<0.001	***
			pond 7	-0.15	0.92	8	-9.17	0.02	<0.001	***
	SM ~ Density		pond 4	-0.58	0.54	8	-2.67	0.22	0.037	*
			pond 5	-0.75	0.76	8	-4.36	0.17	0.005	**
			pond 7	-0.60	0.18	8	-3.31	0.18	0.011	*
	TM ~ Density		pond 4	8.52	0.95	8	11.93	0.71	<0.001	***
			pond 5	11.09	0.91	8	8.56	1.30	<0.001	***
			pond 7	9.76	0.91	8	8.59	1.14	<0.001	***

Table 2.4 A breakdown of the density-controlled experiment design from 2014, with summarized results. Density was kept constant across the eight various-sized enclosures, at 0.25 tadpoles/L. The results were compared to those from the three volume-limited enclosures at the same density level in 2014. No data were collected from the highest density treatment due to mortality. Variance was measured as standard error.

Enclosure Volume (L)	***n***	**Growth Rate (mm per day)**	**Size at Metamorphosis (mm) ± SE**	**Time to Metamorphosis (days)**
1200	300	0.61	23.79 ± 0.21	41 ± 0.71
800	200	0.70	24.60 ± 0.29	36 ± 0.62
400	100	0.74	24.38 ± 0.29	37 ± 0.63
200	50	0.67	23.97 ± 0.44	34 ± 0.40
100	25	0.58	23.96 ± 0.42	28 ± 0.86
60	15	0.61	23.70 ± 0.35	28 ± 0.85
30	8	0.58	23.87 ± 0.52	29 ± 0.00
20	5	-	-	-

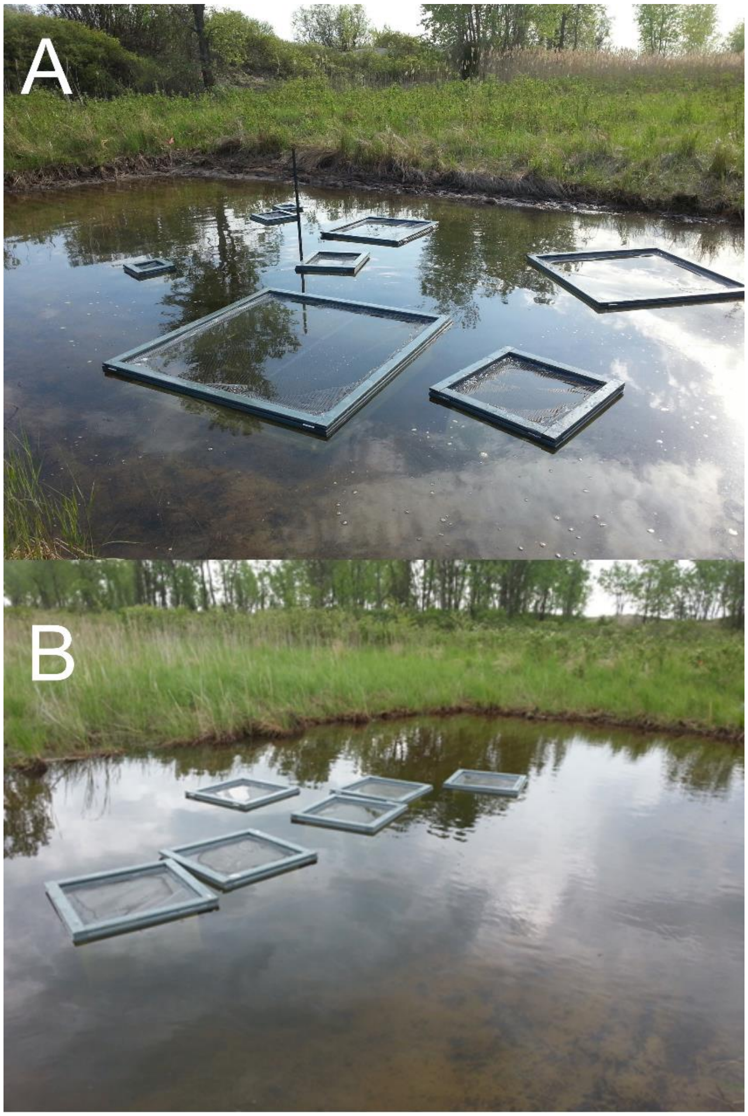

Fig. 2.1 Photo of pond with tadpole density enclosures; **A-** a pond with enclosures of various sizes, used for *volume-limited,* or *density-controlled* treatments; **B-** a pond with enclosures of the same size, used for *abundance-limited* treatments.

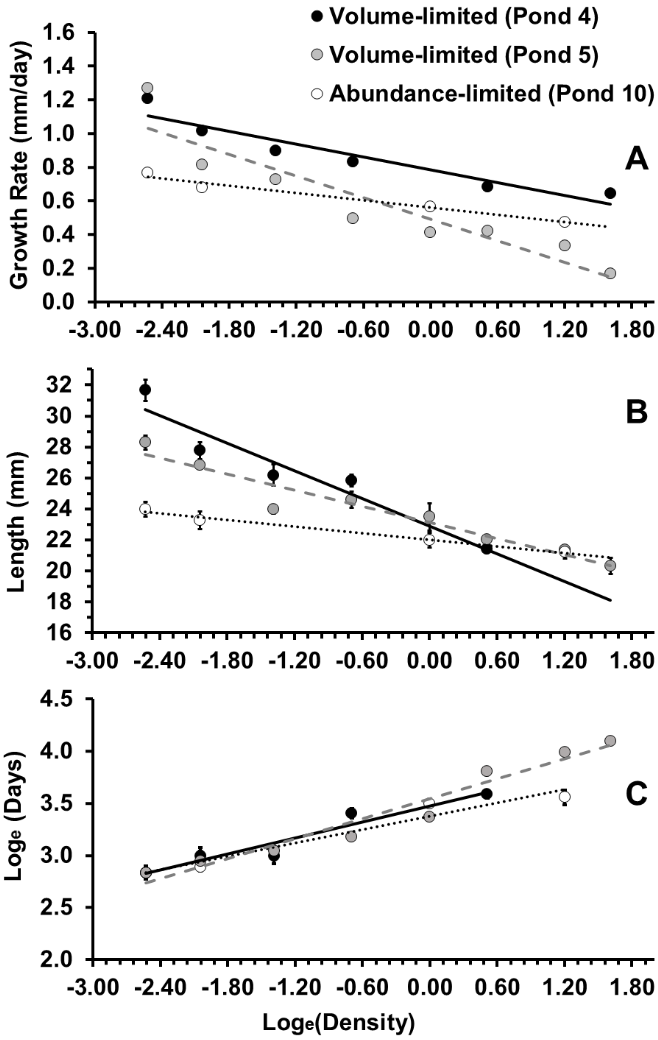

Fig. 2.2 Initial growth rate (A), mean size at metamorphosis (B), and mean days to metamorphosis (C), among Fowler's Toad tadpoles raised at various densities, where density is controlled either by limiting volume or by limiting abundance, in 2013. The dotted line is the best-fit line for the *abundance-limited* pond, the solid line is the best fit line for *volume-limited* density pond 4, and the grey dashed line is for *volume-limited* density pond 5. Density and TM were transformed with the natural logarithm, and error bars represent standard error.

2.9 Appendix

2.9.1 All results for tadpole growth experiments; initial growth rate, mean size at metamorphosis and mean time to metamorphosis of Fowler's Toad tadpoles according to specific pond, and density level in volume-limited, abundance-limited and density-controlled experiments. Variance is represented by standard error.

		Abundance-limited	**Volume-limited**				
Density (tadpoles per litre)		2013 pond 10	2013 pond 4	2013 pond 5	2014 pond 4	2014 pond 5	2014 pond 7
0.08	Growth Rate (mm per day)	0.76	1.21	1.27	0.61	0.71	0.72
0.13		0.68	1.02	0.81	0.51	0.77	0.67
0.25			0.90	0.73	0.43	0.60	0.73
0.50		-	0.83	0.50	0.44	0.53	0.56
1.00		0.57	-	0.41	0.27	0.45	0.36
1.67		-	0.68	0.42	0.23	0.43	0.31
3.33		0.47	-	0.33	0.37	0.35	0.22
5.00			0.64	0.17	0.17	0.26	0.14
0.08	Mean Size at Metamorphosis (mm)	23.98 ± 0.48	31.65 ± 0.71	28.28 ± 0.45	26.69 ± 0.28	23.06 ± 0.34	22.65 ± 0.49
0.13		23.27 ± 0.57	27.75 ± 0.54	26.81 ± 0.17	24.23 ± 0.27	24.65 ± 0.31	22.94 ± 0.66
0.25		-	26.17 ± 0.69	23.97 ± 0.29	24.22 ± 0.23	23.93 ± 0.25	21.15 ± 0.58
0.50		-	25.82 ± 0.37	24.60 ± 0.53	23.96 ± 0.24	22.98 ± 0.28	20.70 ± 0.46

1.00		21.99 ± 0.48	-	23.48 ± 0.58	23.56 ± 0.23	22.82 ± 0.24	21.49 ± 0.69
1.67		-	21.39 ± 0.24	22.01 ± 0.20	22.92 ± 0.25	21.41 ± 0.19	20.28 ± 0.26
3.33		21.21 ± 0.44	-	21.37 ± 0.23	24.22 ± 0.17	21.40 ± 0.34	21.44 ± 0.49
5.00		-	-	20.31 ± 0.50	22.99 ± 0.51	20.97 ± 0.31	21.62 ± 1.37
0.08	Mean Time to Metamorphosis (days)	17 ± 1.63	17 ± 0.00	17 ± 0.00	30 ± 0.43	28 ± 0.62	30 ± 1.10
0.13		18 ± 1.29	20 ± 0.00	19 ± 0.46	31 ± 0.46	28 ± 0.60	31 ± 0.38
0.25		-	20 ± 0.00	21 ± 0.45	34 ± 0.56	33 ± 0.43	32 ± 0.39
0.50		-	30 ± 0.50	24 ± 1.58	40 ± 0.50	36 ± 0.50	40 ± 0.78
1.00		33 ± 0.63	-	29 ± 0.69	45 ± 0.67	43 ± 0.60	48 ± 1.66
1.67		-	36 ± 0.00	45 ± 0.86	50 ± 0.60	58 ± 0.82	61 ± 0.62
3.33		35 ± 0.00	-	54 ± 0.87	60 ± 0.00	67 ± 0.81	66 ± 0.83
5.00		-	-	60 ± 1.14	65 ± 1.12	70 ± 0.82	63 ± 0.79

Preface to Chapter 3

In Chapter 2, I used a unique method to carry out a classic experiment of measuring density-dependent growth in amphibian tadpoles. The result of rearing tadpoles under a volume-limited method allowed the production of a more equal number of different-sized metamorphs to which the following chapters are based off. More specifically, the results from Chapter 2 give rise to the basis of Chapter 3, where using the knowledge of their larval growth, and development under various density conditions I could take the next step and examine their behaviour, and assess potential carry-over effects between two life stages.

Chapter 3: Post-metamorphic carry-over effects in a complex life history: behaviour and growth at two life stages in an amphibian, *Anaxyrus fowleri*

Katharine T. Yagi and David M. Green. Published in *Copeia*.

3.1 Abstract

Carry-over effects, which occur when an animal's early life experience has lasting effects on its later life, may be manifested in an animal's behaviour, growth and fitness, and are often overlooked as contributing factors to such aspects of animal ecology. Metamorphosis is a process which can designate such effects in animals with complex life histories, due to the significant energetic cost and physical changes undergone during this process. We explored the potential impact of tadpole density after metamorphosis in a pond-breeding amphibian, *Anaxyrus fowleri*. If larval density induces carry-over effects, then there should be a positive correlation in behaviour and/or relative growth rate between pre-metamorphic tadpoles and the same animals as post-metamorphic toadlets. We raised tadpoles at six density levels, ranging from 0.08 tadpoles/L to 1.67 tadpoles/L, designed to produce variation in growth rate and toadlet size, and quantified relative activity by monitoring movement per 5 second intervals in tadpoles and 1 min intervals in toadlets. Among tadpoles, activity varied with density as a quadratic curve, and best predicted growth rate as a negative loge-linear function. For toadlets, their density as tadpoles did not predict relative growth rate. Alternatively, we found a positive relationship between activity level and toadlet body size and, when considered in terms of density treatments as larvae, found evidence for body size predicting opposite trends in activity between the two life stages. Our results show evidence of density-dependent carry-over effects when comparing

relative growth rate, and indirectly when comparing size-dependent activity level between life stages, in this pond breeding amphibian. Further information is needed to determine if carry-over effects impact individual fitness, which can have implications at the population level.

3.2 Introduction

A carry-over effect can be defined as an impact on an individual's performance that can be explained by its previous life history or experience (O'Connor et al. 2014). Carry-over effects may impact various traits relating to survival and fitness, such as body condition (Marra et al. 1998; Bearhop et al. 2005) and reproductive output (Olive et al. 1997; Baker et al. 2004; Catry et al. 2013), and can have lasting effects across life stages (Van Allen et al. 2010; Green and Bailey 2015) and seasons (Norris 2005; Harrison et al. 2011). Long term impacts on individual fitness that can result from drastic environmental changes, such as habitat loss, can occur in the form of carry-over effects (Pechenik et al. 1998; Pechenik 2006; Harrison et al. 2011), and manifest in the next generation as maternal effects (O'Connor et al. 2014). Yet it is often difficult to amass empirical evidence for carry-over effects in wild animals since individual animals need to be tracked through successive life stages, juvenile mortality can be high in many taxa (Victor 1986; Gosselin and Qian 1997; Casale et al. 2015), and locating individuals over long distances or time periods can be logistically challenging.

Carry-over effects have been detected in a wide variety of taxa, including birds (Drake et al. 2013; Clausen et al. 2015), mammals (Perryman et al. 2002; Cook et al. 2004), reptiles (Goodman 2008), fish (Kennedy et al. 2008), invertebrates (Hettinger et al. 2013; Van Allen and Rudolf 2013), and plants (Krepkowski et al. 2013), and may be particularly strong in animals with complex life histories, such as amphibians (Earl and Semlitsch 2013; Tarvin et al. 2015) and many marine invertebrates (Hettinger et al. 2013; Fischer and Phillips 2014). The larvae of these organisms are exposed to very different environmental conditions than the adults, which can have lasting effects on the animals after metamorphosis (Goater 1994; Álvarez and Nicieza 2002; Green and Bailey 2015). Many amphibian tadpoles can be exposed to such stressful conditions as elevated temperatures (Kern et al. 2014), low oxygen levels or density-limited resources (Wilbur 1977; Smith 1983; Crespi and Denver, 2005) that are often related to the ephemeral nature of their aquatic habitat. Tadpoles trapped in shrinking aquatic habitats can become crowded to very high densities, which will negatively impact growth, size at metamorphosis, timing of metamorphosis, and foraging activity (Altwegg and Reyer 2003; Boone 2005; Gomez-Mestre et al. 2010). Furthermore, metamorphosis in these animals is a costly process that is frequently associated with high mortality (Wilbur 1980; Newman 1992) and is greatly affected by environmental conditions (Van Buskirk and Saxer 2001; Walsh et al. 2008; Touchon et al. 2013).

All animals must manage conflicting physiological demands and, ideally, optimize trade-offs in time and resource allocation (Kozłowski 1992; Takahashi and Pauley 2010). Because all their reproductive effort is deferred until after metamorphosis, anuran tadpoles manage their physiological resources only between growth, development, storage and maintenance (Fitzpatrick 1976; Perrin and Sibly 1993; Angilletta et al. 2003), and budget their time largely between foraging behavior and defensive behavior (Lima and Dill 1990). Tadpoles are constrained in the extent to which they can focus on these particular activities and still be able to reach metamorphosis at the proper time and body condition (Chelgren et al. 2006; Steiner and Pfeiffer 2007). In tadpoles, foraging *vs*. defensive behaviours can easily be quantified by monitoring activity level under various conditions (Anholt et al. 2000; Marshall et al. 2003) and their size immediately after metamorphosis is predicated by the conditions of their pre-metamorphic environment, particularly relative density (Scott 1994; Pechenik et al. 1998).

Thus, an anuran tadpole’s pre-metamorphic life could have a profound influence on its post-metamorphic fate, provided that its condition as a metamorph has a role in determining its subsequent success in the terrestrial landscape (Álvares and Nicieza 2002; Pechenik 2006). Larger body size following metamorphosis can confer a considerable survival advantage in a variety of anurans (Chelgren et al. 2006, Cabrera-Guzmán et al. 2013, Tarvin et al. 2015). Therefore, a carry-over effect of tadpoles living at high densities may be that they are of notably smaller metamorph size, and have a lower overall survivorship compared to tadpoles raised under lower densities (Yagi and Green, 2016). In contrast, smaller individuals that had suffered slow growth in early life can exhibit compensatory, or “catch-up”, growth as they mature in the terrestrial environment (Boone 2005, Tarvin 2015), a phenomenon observable in other sorts of animals as well (Radder et al. 2007).

We sought to examine how larval density, used as a proxy for varying larval growth conditions (Yagi and Green, 2016), can affect individual animals with complex life histories across the pre- to post-metamorphic transition. If the effect of larval density on growth rate and activity level directly carries over after metamorphosis in animals like Fowler’s Toads, *Anaxyrus fowleri*, then slow-growing or highly active larvae may remain smaller or relatively active as post-metamorphic juveniles. Alternatively, if larval density effects do not carry over after metamorphosis in these animals, then any effects relative to density may have had on individuals before metamorphosis should disappear after metamorphosis.

3.3 Materials and Methods

3.3.1 Study species and study site.— The Fowler's toad, *Anaxyrus fowleri,* is a common toad of eastern North America, noted for living in areas with sandy substrate and ranging as far north as the northern shore of Lake Erie in Ontario, Canada. In Canada, these toads exist in small, isolated populations. This study was conducted in the Thoroughfare Point Unit of the Long Point National Wildlife Area (NWA) in summer of 2014, (between 42°34'33" N – 42°35'3" N and 80°22'15" W – 80°28'24" W) in southern Ontario. This population is part of a long-term population monitoring study (Greenberg and Green, 2013; Middleton and Green, 2015), where the population estimate in 2014 was about 32 adults (unpubl. data), and natural tadpole densities are assumed to be very low (Yagi and Green, 2016). We used experimental ponds that were dug in the marshes in collaboration with the Canadian Wildlife Service in November 2012 (Yagi and Green 2016).

3.3.2 Experimental set-up.— As described by Yagi and Green (2016), we collected egg masses from three amplectant pairs of Fowler's toads between 24 May and 26 May, 2014, and raised the larvae in floating mesh pens until they resorbed their external gills (i.e. reached stage 25; Gosner, 1960). We then mixed all tadpoles together to reduce maternal and genetic bias, and counted out groups of 100 to grow in six density treatments varied by volume, in floating mesh pens placed in a pond: 0.08 tadpoles/L, 0.13 tadpoles/L, 0.25 tadpoles/L, 0.5 tadpoles/L, 1.0 tadpoles/L and 1.67 tadpoles/L (Yagi and Green 2016). Each density treatment was replicated three times (i.e. in three ponds). Density treatments were designed to vary by volume in order to produce equal number of metamorphs, and toadlets for further studies. The floating pens both physically protected the tadpoles from aquatic predators and ensured equal exposure of the tadpoles to any chemical cues in the water from either predators or other tadpoles (Yagi and Green 2016). All experiments occurring after the assignment of tadpoles to density treatments assume that the three egg masses are represented randomly in each test.

We monitored tadpole growth by photographing a sample of 20 individuals per floating pen, in a petri dish overlaid on 6 mm grid paper, repeated every 4 to 7 days through to metamorphosis. We measured total body length digitally, using the image processing software ImageJ (vers 1.46r, Schneider et al. 2012). Air and pond temperatures were measured hourly by deploying three temperature data loggers (iButton, model DS1922L) (i.e. one recording ambient air and two recording water temperature), waterproofed with Plastidip® at each pond location (Yagi and Green 2016). Upon reaching their developmental stage where four limbs and full tail are present (stage 42), metamorphs from each density treatment group were moved to semi-terrestrial, 2 m² fiberglass mesh enclosures with natural substrate and vegetation growth, and raised until complete tail resorption (stage 46), henceforth called toadlets. Toadlets were not fed, and instead sustained themselves on the naturally occurring insect community within the vegetated enclosures. We monitored toadlet growth every 4 to 7 days by sampling 10 individuals per group, measuring snout-vent length using dial calipers, and calculating the means. Once they had grown to > 13 mm snout-vent length toadlets were measured, weighed and photographed for later identification. This size was chosen based on the better visibility of their dorsal spot patterns, which were used for individual identification for further recapture studies.

3.3.3 Relative growth rates.— We calculated mean relative growth rates (mm/day/mm) of tadpoles groups from each density treatment in each pond as the mean maximum growth rate (Yagi and Green 2016) divided by the mean initial total length. For toadlets, growth rate for each density group from each pond was calculated as the difference in mean body length (i.e. snout-vent length) between the first and last mean body length measurements of each group, divided by the number of days between the two measurements. Relative growth rate was calculated by dividing this value by the mean initial toadlet body length.

3.3.4 Activity levels.— We measured activity level in 18 tadpoles, at developmental stage 39 (i.e. only hind legs present, just prior to forelimb bud formation; Gosner 1960), from each of the six density treatment groups, for a total of 108 tadpoles (Table 3.1). We used six tadpoles at a time, placing each tadpole individually in one of six 20 by 20 cm Pyrex® glass dish, in ca. 2 cm of pond water, under the shade of a large umbrella. All tadpoles were filmed continuously for 60 minutes between 12:00 PM and 4:00 PM, in their respective dishes using a digital camera (JVC, model: GZ-E300AU) mounted on a tripod. Tadpoles were only recorded on days when the weather was clear and sunny, to reduce variation in weather conditions between activity tests. Tadpole activity was scored at 5 sec intervals.

We measured activity levels in a total of 29 toadlets: seven from density levels 0.08, 0.13 and 0.25 tadpoles/L, five from density level 0.5 tadpoles/L and three from density level 1.0 tadpoles/L, each at least 13 mm in body length to ensure both visual detection on camera, and a decreased desiccation risk. Toadlets of appropriate size from the highest density level, 1.67 tadpoles/L, were not available. We placed toadlets individually into an arena measuring 90 cm in diameter, with ca. 2 cm of moist sand covering the bottom. All toadlet activity tests were conducted indoors, starting by 11:00 AM, and air temperature was recorded hourly using an iButton model DS1922L temperature data logger. We recorded the movements of the toadlets using an infrared, closed circuit television camera (3xLOGIC, model VX-3S-OD-I-VF, 3xLOGIC Inc., CO, USA; max resolution 2048 x 1536 px) and digital video recorder (VIGIL, model MVR-4IP-2TB, 3xLOGIC Inc., CO, USA). Recordings were processed with video recording software (v8.0, 3xLOGIC Inc.). The cameras were set to capture an image of the arena every 1 min. for up to 6 hrs.

The animals' activity was scored by recording "active" if the animal had moved during a 5 sec (tadpoles) or 1 min (toadlets) time period, and "inactive" if it had not. We counted the "active" time periods and "inactive" time periods scored to calculate percentages of the total number of time periods. For comparison, we then averaged individual % active scores per density treatment group and called the result "Activity Level". All toadlets were released to their natural terrestrial habitat once the activity test was complete.

3.3.5 Analysis.— Statistical analyses were conducted using appropriate packages in R v.3.3.2 (R Core Team, 2015). We tested for normality of relative growth rate, temperature and body length data using qq-plots, Shapiro Wilk's test, Levene's test or Bartlett's test as appropriate. We used $\log_e$ transformed density values ($\ln D$) for analysis. To assess relative growth rate in relation to $\ln D$ in R, we used linear mixed models, using the 'lme4' package and 'lmer' function. We set 'pond' as a random effect, and included mean pond temperature as an additional fixed predictor for tadpoles, and mean air temperature for toadlets. To assess the effect of $\ln D$ on activity in R, we used the binary ("active", "inactive") activity scores and conducted logistic regressions using generalized linear mixed models, with the 'glmer' function under a binomial distribution. We included body length and mean pond temperature (for tadpoles) and mean air temperature (for toadlets) as fixed predictors, while 'pond' (P) was included as a random effect variable, with "individual" (Ind) nested within it to account for repeated measures. We calculated mean temperatures for activity tests from temperatures recorded throughout the four-hour or six-hour time intervals used for tadpoles and toadlets, respectively. We employed model selection analysis (Akaike 1987) to determine whether linear or polynomial models best fit the data.

Where appropriate, model averaging was used using the ‘avg.model’ function from R’s ‘MuMIn’ package. The best fit model was examined using R’s ‘Anova’ function from the ‘car’ package.

To detect the presence of a carry-over effect in activity, we used tadpole Activity Level as the predictor and the corresponding toadlet Activity Level as the response in a linear regression, anticipating that a significant, positive relationship would be consistent with the presence of a carry-over effect. Similarly, to detect the presence of a carry-over effect in growth rate, we used mean tadpole growth rate as the predictor and toadlet growth rate as the response in a linear regression. As we did not have information on individual tadpole growth, we used density group means per pond to associate a tadpole growth rate measure per individual toadlet. To evaluate the influence of body size in our carry-over effect analyses, we carried out logistic regressions using Activity *versus* body length, separated into density groups for both life stages (tadpoles and toadlet). The significance of each regression was quantified in R using the ‘Anova’ function, or Wald test statistic. Differences in the Activity *versus* body length relationship between life stages (i.e. tadpole and toadlet) and density groups were assessed by creating a model with binary activity scores as the response variable, ‘length’ as a continuous predictor, and ‘density’ and ‘life stage’ as interacting categorical predictors, using R’s ‘glmer’ function. Again, ‘individual’ was nested within the categorical ‘pond’ variable and set as a random effect term. Multiple comparisons of means via a Tukey post hoc test was conducted on the model using the ‘glht’ function from the ‘multcomp’ package in R.

3.4 Results

3.4.1 Growth rate and body size.— Mean relative growth rate among tadpoles ranged from 0.048 ± 0.009 (SE) day^{-1} for those reared at a density of 1.67 tadpoles/L to 0.098 ± 0.005 (SE) day^{-1} for those reared a density of 0.08 tadpoles/L. Mean relative growth rate in tadpoles was negatively correlated with density ($R^2 = 0.83$; $P < 0.001$; Table 3.2, Fig. 3.1). Once these animals had become toadlets, however, mean relative growth rate ranged from 0.008 ± 0.002 (SE) day^{-1} for those reared at a density of 1.67 tadpoles/L to 0.016 ± 0.001 (SE) day^{-1} for those reared at a density of 0.25 tadpoles/L and was not correlated with density, or any other physical variable (Table 3.2; Fig. 3.1). Removing density from the equation, the relative growth rates of individuals as toadlets were positively correlated with the mean relative growth rates they exhibited in tadpole groups, but the relationship was not statistically significant (Slope = 0.04, $N = 18$, $R^2 = 0.09$, $P = 0.22$). Since trends can be observed, these non-significant results may be due to small sample size. Ponds did not differ from each other in mean daily temperatures over the course of the 2014 season (see Yagi and Green 2016).

Of the individuals used for the activity tests, the mean tadpole size ranged from a maximum of 31.6 ± 0.51 mm at density level 0.08 tadpoles/L, and a minimum of 25.98 ± 0.52 mm at density level 1.67 tadpoles/L. The size of tadpoles from each density group used for the activity level tests negatively correlated with density (Slope = -1.79, $R^2 = 0.37$, $F_{1,106} = 63.89$, $P < 0.001$), but there was high degree of overlap in the size range, in the highest four density groups. Tukey post hoc test revealed that only density levels, 0.08 and 0.13 tadpoles/L differed significantly from the rest ($P < 0.001$), and were not significantly different from each other ($P = 0.72$; Fig. 3.2). For the toadlets subjected to activity tests, the largest mean body length ranged from a maximum of 17.40 mm ± 0.87 (SE) at density level 0.25 tadpoles/L, to a minimum mean of 14.18 mm ± 0.33 (SE) at density level 0.50 tadpoles/L. The correlation, although a negative trend, between body length and rearing density, was not significant (Slope = -0.68, $R^2 = 0.10$, $F_{1,27} = 3.05$, $P = 0.09$; Fig. 3.2). However, toadlet initial mean body length (i.e. 4-7 days post emergence) ranged from 8.81 ± 0.43 (SE) mm in the 1.67 tadpoles/L treatment group to 12.52 ±

0.52 (SE) mm in the 0.08 tadpoles/L treatment group. There was a significant, negative, $\log_e$-linear relationship between their initial body length and density (Slope = -1.13, $N = 18$, $R^2 = 0.74$, $P < 0.001$).

3.4.2 Activity levels.— Tadpoles and toadlets showed different patterns of activity in relation to rearing density. Tadpole mean Activity Level increased with increasing density from 42 ± 10% (SE) at 0.08 tadpoles/L up to 73 ± 10% (SE) at 0.25 tadpoles/L, but then decreased to 51 ± 8% (SE) at 1.67 tadpoles/L. Mean air temperatures during the tadpole activity tests was 22.9˚C ± 0.30 (SE), ranging from 18.0˚C to 29.0˚C (Table 3.1) and, since each test was carried out on a different day, differed significantly among density groups ($F_{5,66} = 31.02$, $P < 0.001$). The top four explanatory models, all of which included quadratic density coefficients ($\ln D + \ln D^2$), were virtually indistinguishable in terms of information content (ΔAICc ≤ 1.1, Table 3.3).

Accordingly, we calculated an averaged model for analysis. But since Model 2 has Models 1, 3 and 4 all nested within it, we selected it for further analysis also. Both the averaged model and Model 2 define the relationship between tadpole activity and density in terms of a quadratic curve in which activity peaks at an intermediate density level (Fig. 3.3). The full averaged model consisted of four coefficients, where only the two density terms proved to be significant (lnD: Z = 4.98, $P < 0.001$, lnD^2: Z = 5.54, $P < 0.001$, T: Z = 0.94, $P = 0.35$, L: Z = 0.70, $P = 0.49$), with each coefficient's relative importance ranked as; $lnD^2 = 1.00$, $lnD = 1.00$, T = 0.62 and L = 0.49. Similarly, the only significant coefficients in Model 2 were the two density terms ($\ln D$: $\chi^2 = 30.07$, df = 1, $P < 0.001$, $\ln D^2$: $\chi^2 = 36.16$, df = 1, $P < 0.001$). Neither temperature, T ($\chi^2 = 3.11$, df = 1, $P = 0.08$), nor body length, L ($\chi^2 = 1.91$, df = 1, $P = 0.17$), were significant.

Among toadlets, Activity Levels were negatively correlated with body length when raised at higher densities (0.5 and 1.0 tadpoles/L) and positively correlated when raised at lower densities (0.08, 0.13, and 0.25 tadpoles/L), though only the result for density levels 0.25 tadpoles/L was significant (Fig. 3.4, Table 3.4). Mean air temperatures during the toadlet activity tests was 23.9˚C ± 0.17 (SE), ranged from 17.0˚C to 30.5˚C (Table 3.1) and did not differ significantly among density groups ($F_{4,170} = 1.86$, $P = 0.12$). As a result of model selection, the top four explanatory models, as observed with the tadpole data, were nearly indistinguishable in terms of information content ($\Delta\text{AICc} \leq 0.6$, Table 3.3). Therefore, we calculated an averaged model for analysis. The full averaged model included the three coefficients, where only body length showed significance (L: $Z = 4.97$, $P < 0.001$, *lnD*: $Z = 0.78$, $P = 0.43$, T: $Z = 0.77$, $P = 0.44$), and their relative importance was ranked as; L = 1.00, *lnD* = 0.54, and T = 0.53. Since the strongest correlate with toadlet Activity was body length, we chose to run Model 2 separately, which showed a significant relationship for toadlet activity as a function of body length (Table 3.3; $\chi^2 = 30.60$, df = 1, $P < 0.001$; Fig. 3.4).

The activity *versus* body length regressions proved to only be significant in both life stages at density level 0.25 tadpoles/L (tadpole: $\chi^2 = 5.34$, df = 1, $P = 0.02$, toadlet: $\chi^2 = 46.87$, df = 1, $P < 0.001$), where the log odds ratio was strongly negative for tadpoles (-0.23), but positive for toadlets (0.33; Table 3.4). The interaction of these regressions between density groups proved to be significant ($\chi^2 = 24.41$, df = 5, $P < 0.001$), as well as the interaction between life stages ($\chi^2 = 20.04$, df = 1, $P < 0.001$). The Activity Levels of tadpoles and their Activity Levels as toadlets (Appendix 3.9.1) were negatively correlated, but the relationship was not statistically significant (Slope = -0.13, $N = 29$, $R^2 = 0.02$, $P = 0.47$).

3.5 Discussion

Our results demonstrated that density during the tadpole stage in Fowler's toads produces some evidence of post-metamorphic carry-over effects among toadlets. Although increasing tadpole density is related to a $\log_e$-linear decline in tadpole growth rate (Yagi and Green, 2016) the relationship between toadlet relative growth rate and their density as tadpoles appears to be, more or less, a flat line (Fig. 3.1). This indicates that toadlets grow at the same relative rate whatever density they experienced as tadpoles. Evidently, once the density pressure imposed on them as tadpoles is released, they experience equal foraging opportunities in the terrestrial landscape (Werner and Anholt 1993; Stamps 2007). Since initial body size among toadlets is negatively correlated with their density during the tadpole stage, a uniform post metamorphic growth rate should ensure that smaller toadlets will remain small relative to their larger counterparts. This could be an important carry-over effect of the animals' density as tadpoles considering the survival advantage conferred by larger body size in anurans (Chelgren et al. 2006, Cabrera-Guzmán et al. 2013, Tarvin et al. 2015). Interestingly, our results are contrary to recent findings of carry-over effects causing compensatory growth in tree frogs (Bouchard et al. 2016), although morphology and digestion were examined here which is more specific than somatic growth rate. Body size in amphibians is a predictor of successful courtship (Houck 1988; Mathis 1991), female fecundity (Tejedo 1992; Gilbert et al. 1994; Camargo et al. 2005), and overwinter survival (Scott et al. 2007; Reading 2007).

If carry-over effects are significant drivers of adult fecundity and probability of survival, then there can be several implications, positive and negative, at the population level (Chelgren 2006; Touchon et al. 2013). A carry-over effect of tadpole density might be a potential component of density-dependent regulation of population size (Green and Middleton 2013;

Green 2015), where the ability for population size increase when at low density, and decrease when at high density is intrinsic, and does not necessarily require changes in external pressures, like predation. Nevertheless, body size in anuran populations may be highly variable (Galatti 1992) and body size among adults can be negatively correlated quite strongly with their density (Denton and Beebee 1993; Green and Middleton 2013), indicating that the carry-over effects of larval density on growth and adult body size may be variable and intricate.

The relationship of the animals' activity level pre- and post-metamorphosis may be even more complex. In tadpoles, the level of activity appears to peak at intermediate density, possibly as a three-way trade-off between increased competiveness for limited resources as density increases (Skelly and Werner 1990; Werner and Anholt 1996), increased susceptibility to predation as activity increases (Lawler 1989; Skelly 1994; Anholt and Werner 1995), and decreased resources for growth as energetic expenditure for movement increases (Scott et al. 2007). Tadpoles under stress, which may more likely occur under higher densities, may behave in a way such that smaller individuals do not have the energy reserves required to compete for resources possessed by their larger counterparts. Competition is known to induce stress responses in amphibians (Relyea 2004; Ledon-Rettig and Pfennig 2009; David and Maerz 2009; Crespi and Warne 2013). Among toadlets, though, the level of activity appears to be correlated predominantly with individual body size (Fig. 3.3), which, in turn, is negatively related to their density as tadpoles. There is thus some evidence of an indirect carry-over effect of rearing density on the toadlets' level of activity.

It is important to note that since our toadlets only came from three egg masses, which lead to our relatively small toadlet sample size, the representation of carry-over effects in this population may be limited. This issue was inevitable due to the difficulty in finding egg masses in a population of low abundance and the fact that amphibian mortality over multiple life stages is naturally very high. Another potential bias of using only three egg masses, is there may be some influence of maternal and genetic effects in our results. Another limitation of the tadpole activity tests was that each density level was tested on a different day. Although this could not be helped due to the effect density has on tadpole growth and development (Yagi and Green 2016), there is no way of determining if the observed effects were caused by density or daily weather conditions. Since the weather conditions during each tadpole test were generally clear and sunny, we are confident that temperature was the best indicator of the test conditions between days. Fortunately, this was less of an issue for the toadlets since their activity test dates overlapped with individuals from different density groups.

Our study provides empirical evidence pointing to potentially significant carry-over effects after metamorphosis, both directly and indirectly stemming from larval density. The consequences of growth conditions in early life stages has many implications regarding animal interactions with the environment. For metamorphosing individuals that undergo profound niche shifts and/or changes in habitat use, any latent effects on an individual may significantly affect how they prosper in the new food web especially, as with most anurans, if they have shifted to a different trophic level. Further investigation into carry-over effects and their impact on inter-specific interactions would be valuable for understanding the connections between small scale habitat conditions and broad scale ecosystem stability.

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3.8 Tables and Figures

Table 3.1 Samples, dates and temperatures for activity level tests of individuals as tadpoles and then as toadlets according their densities when reared as tadpoles. Variation is represented by standard error (SE).

Rearing Density (tadpoles/L)	**Tadpoles**			**Toadlets**		
	Sample Size (*n*)	Date of test	Mean Air Temperature (˚C)	Sample Size (*n*)	Dates of tests	Mean Air Temperature (˚C)
0.08	18	02-Jul-2014	25.75 ± 0.36	7	30-Jul – 05-Aug-2014	23.15 ± 0.62
0.13	18	03-Jul-2014	21.08 ± 0.59	7	30-Jul – 06-Aug-2014	23.48 ± 0.59
0.25	18	05-Jul-2014	22.25 ± 0.35	7	02-Aug – 12-Aug-2014	24.01 ± 0.30
0.50	18	09-Jul-2014	20.71 ± 0.42	5	09-Aug – 12-Aug-2014	25.00 ± 0.45
1.00	18	14-Jul-2014	25.79 ± 0.49	3	16-Aug – 17-Aug-2014	21.38 ± 1.18
1.67	18	15-Jul-2014	22.08 ± 0.18	-	-	-

Table 3.2 Most informative models, according to Akaike's information criterion for small sample sizes (AICc), for relative growth rate as a function of $\log_e$ transformed density, with "pond" set as a random effect, for both tadpole and toadlet life stages. ω = AICc weight.

Stage	**Model***	**AICc**	**ΔAICc**	***df***	**ω**
Tadpole	$\ln D + P$	-119.60	0.00	4	0.992
	$\ln D + T_p + P$	-109.80	9.80	5	0.007
	$\ln D + \ln D^2 + P$	-105.50	14.10	5	<0.001
	$\ln D + \ln D^2 + T_p + P$	-95.30	24.30	6	<0.001
	P	-88.00	31.60	3	<0.001
	$T_p + P$	-78.00	41.60	4	<0.001
Toadlet	P	-135.50	0.00	3	0.995
	$T_a + P$	-124.50	11.00	4	0.004
	$\ln D + P$	-121.50	14.10	4	<0.001
	$\ln D + \ln D^2 + P$	-114.00	21.50	5	<0.001
	$\ln D + T_a + P$	-109.90	25.60	5	<0.001
	$\ln D + \ln D^2 + T_a + P$	-102.30	33.20	6	<0.001

* symbols: $\ln D = \log_e$(density), P = "pond", T_a = air temperature, T_p = pond temperature

Table 3.3 Most informative models, according to Akaike's information criterion for small sample sizes (AICc), for Activity Level as a function of density, with "individual" nested within "pond" set as a random effect. Additional fixed variables include total body length, and mean pond temperature (for tadpoles), and mean air temperature (for toadlets). ω = AICc weight. Only the best six models for each stage are shown.

Stage	**No.**	**Model***	**AICc**	**ΔAICc**	**df**	**ω**
Tadpole	1	$\ln D + \ln D^2 + T_p + P_{Ind}$	45280.70	0.00	6	0.320
	2	$\ln D + \ln D^2 + L + T_p + P_{Ind}$	45280.80	0.20	7	0.300
	3	$\ln D + \ln D^2 + P_{Ind}$	45281.70	1.00	5	0.190
	4	$\ln D + \ln D^2 + L + P_{Ind}$	45281.70	1.10	6	0.190
	5	$L + P_{Ind}$	45302.30	21.70	4	<0.001
	6	$L + T_p + P_{Ind}$	45303.80	23.20	5	<0.001
Toadlet	1	$\ln D + L + T_a + P_{Ind}$	13349.90	0.00	6	0.300
	2	$L + P_{Ind}$	13350.40	0.50	4	0.230
	3	$\ln D + L + P_{Ind}$	13350.40	0.50	5	0.230
	4	$L + T_a + P_{Ind}$	13350.50	0.60	5	0.230
	5	$\ln D + T_a + P_{Ind}$	13364.80	14.90	5	<0.001
	6	$\ln D + P_{Ind}$	13367.00	17.10	4	<0.001

* abbreviations: $\ln D = \log_e(\text{density})$, P_{Ind} = individual nested within pond, T = air temperature, L = total body length

Table 3.4 Relationships between activity level vs. total body length for individuals from each rearing density group as tadpoles and then as toadlets. * significant at α = 0.05

Rearing Density (tadpoles/L)	**Tadpoles**					**Toadlets**				
	Log odds ratio	χ^2	*df*	*P*		Log odds ratio	χ^2	*df*	*P*	
0.08	-0.04	0.39	1	0.53		0.22	4.65	1	0.03	*
0.13	-0.11	3.77	1	0.05		0.30	2.91	1	0.09	
0.25	-0.23	5.34	1	0.02	*	0.33	46.87	1	<0.001	*
0.50	-0.17	3.15	1	0.08		-0.04	0.21	1	0.65	
1.00	0.16	4.68	1	0.03	*	-0.15	3.00	1	0.08	
1.67	0.18	4.52	1	0.03	*					

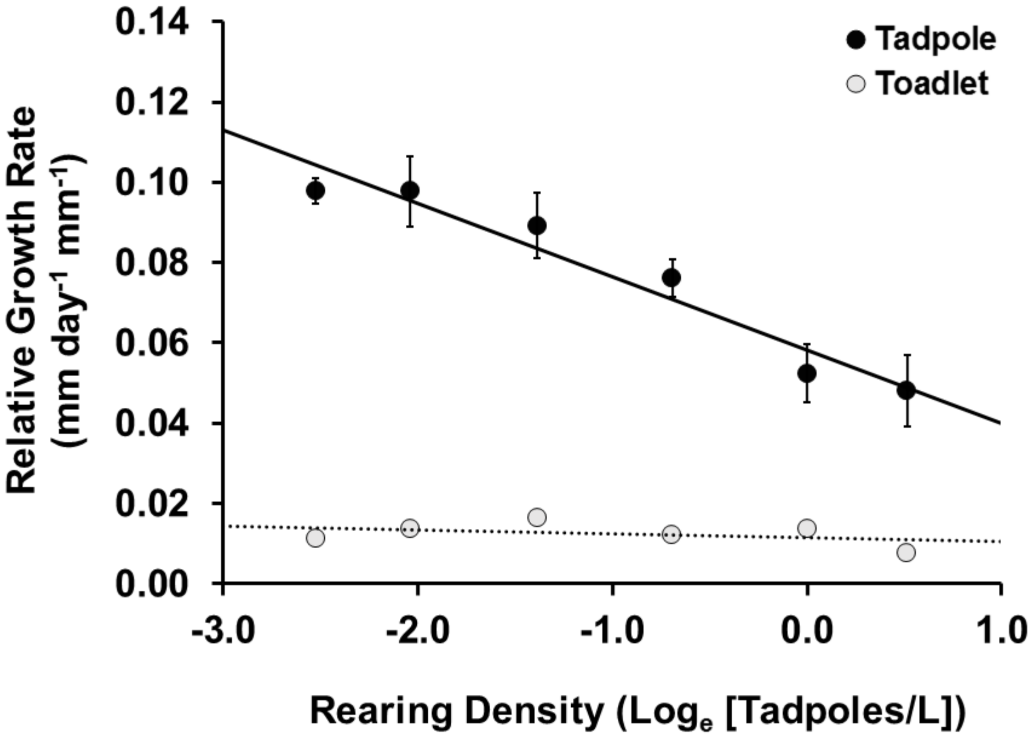

Fig. 3.1 Tadpole and toadlet relative growth rates as a function of density (log$_e$ transformed). Tadpole data fit a negative linear relationship, whereas the toadlet data did not show any trend. Error bars indicate standard error.

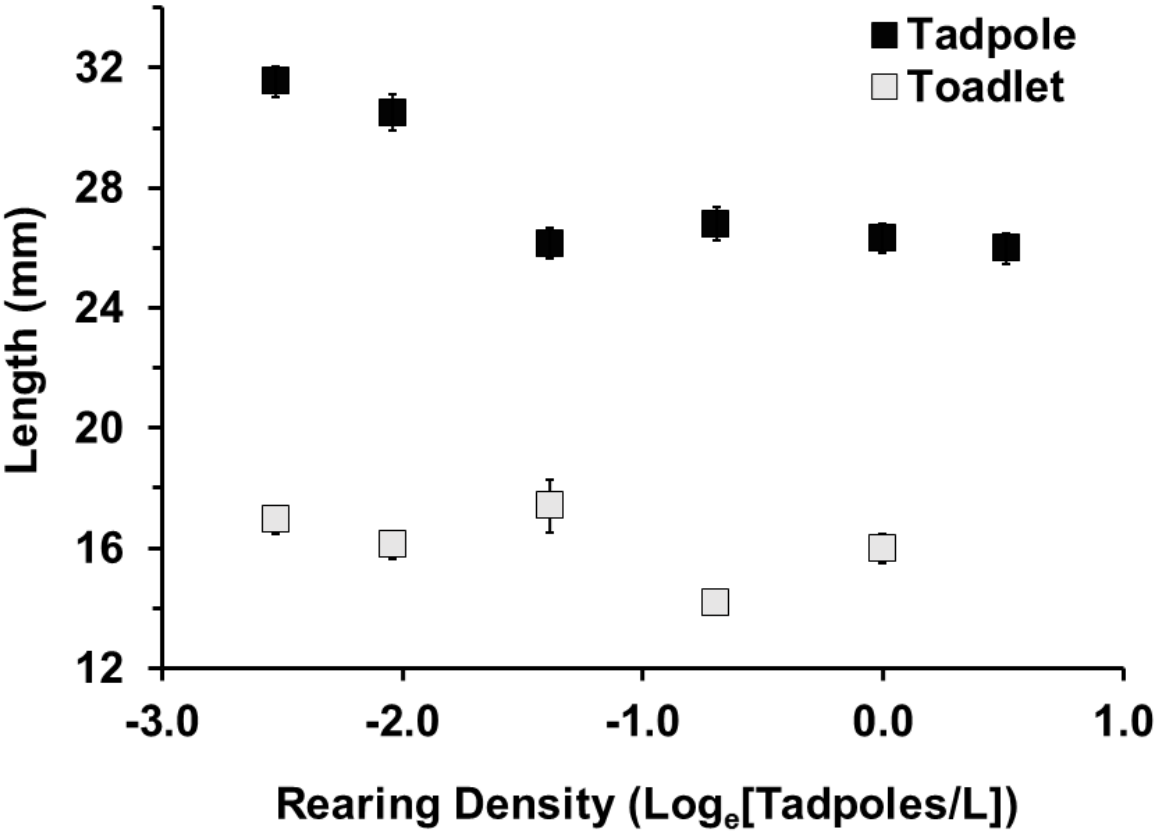

Fig. 3.2 Mean body sizes for tadpoles (total length) and toadlets (snout-vent length) that were subjected to activity level trials, from each density treatment. Although there are strong negative correlations with density at both stages, there is also a high degree of size range overlap among some density groups. Error bars indicate standard error.

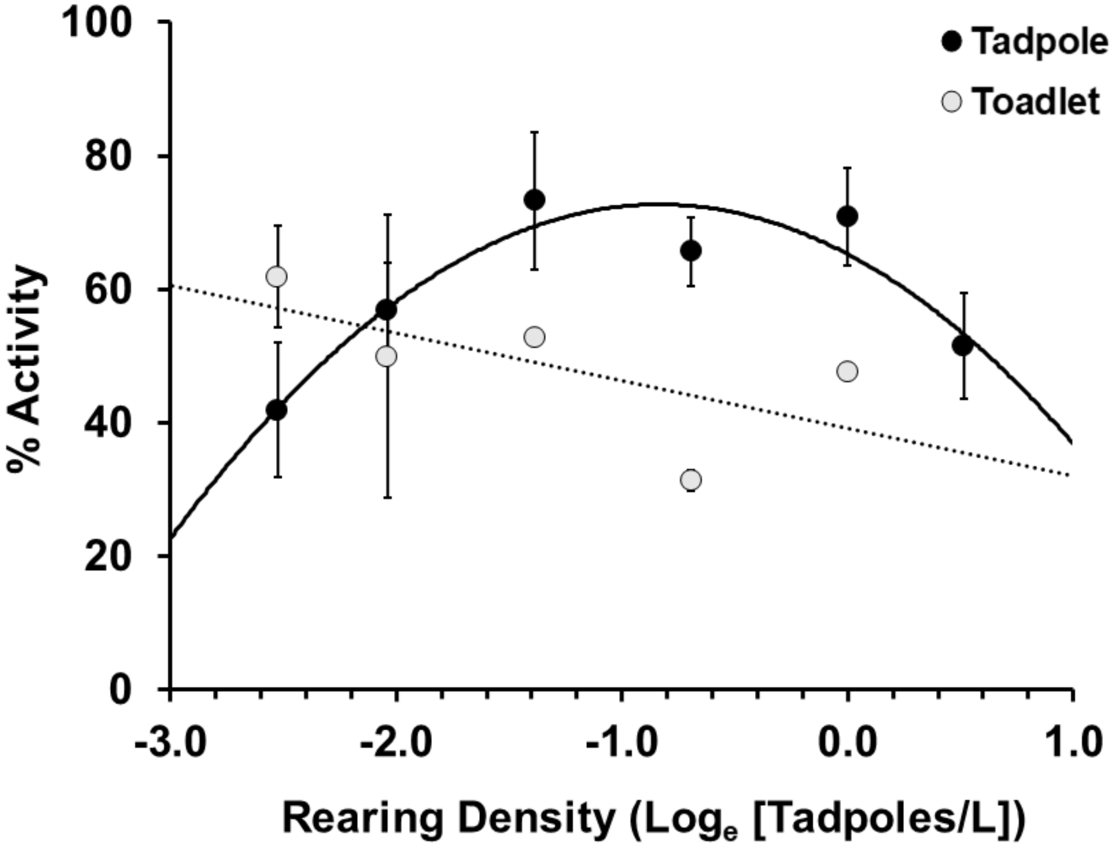

Fig. 3.3 Tadpole and toadlet activity level, portrayed as a percentage of time active, shown as a function of density (log$_e$ transformed). The tadpole fit line conforms to a quadratic relationship whereby activity peaks at an intermediate density level. The toadlet fit line is linear and negative. Error bars show standard error.

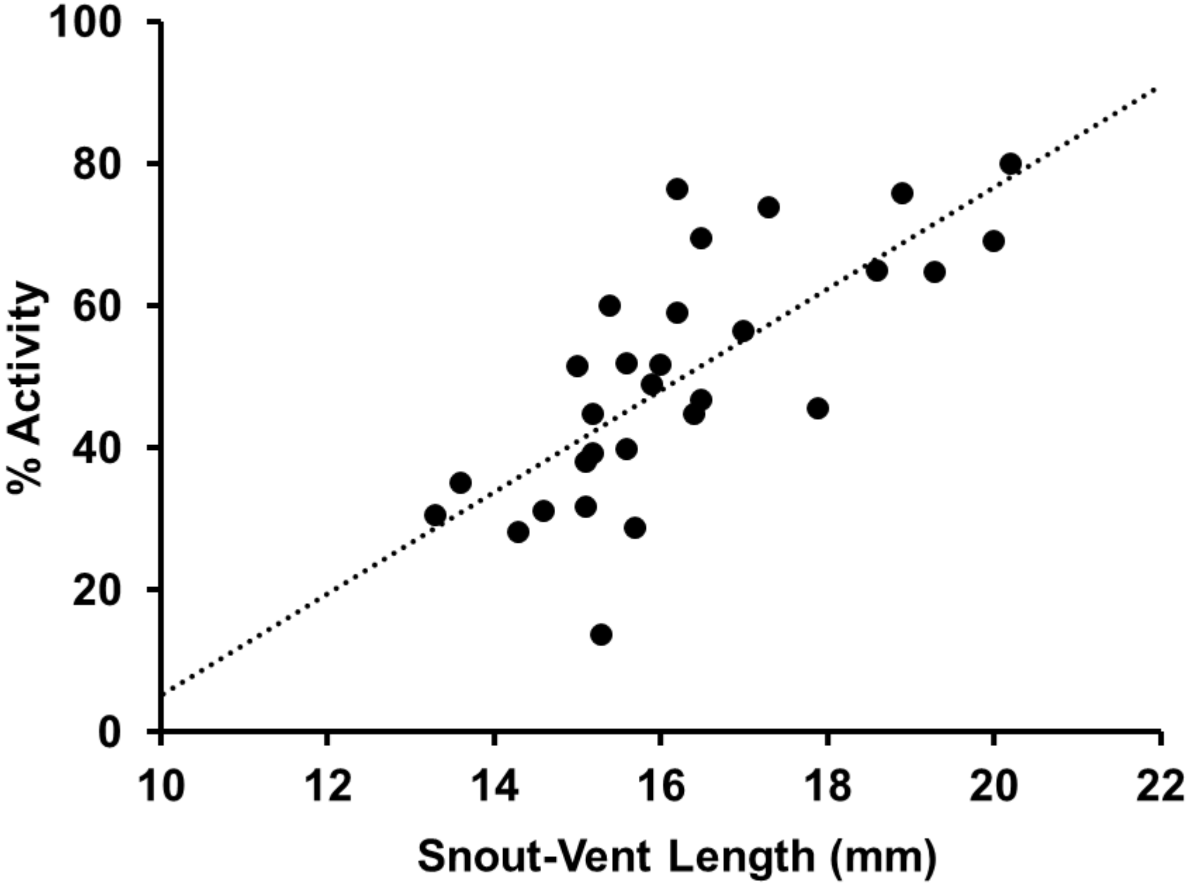

Fig. 3.4 Toadlet activity, shown as a percentage of time active, displayed as a function of individual body length (snout-vent length).

3.9 Appendix

3.9.1 Summary of mean activity level, relative growth rates, body mass and initial body length per density treatment group for tadpoles and toadlets, ± standard error. N_1 is the sample size associated with the activity tests and their mean group body mass, and N_2 is the sample size associated with the relative growth rate and initial body size calculations.

Rearing	Tadpoles					Toadlets					
Density (tadpoles L^{-1})	N_1	Activity Level	N_2	Relative Growth rate (day^{-1})	Initial Body Length (mm)	N_1	Activity Level	Body Mass (g)	N_2	Relative Growth rate (day^{-1})	Initial Body Length (mm)
0.08	18	0.42 ± 0.10	20	0.098 ± 0.005	6.95 ± 0.11	7	0.62 ± 0.04	0.49 ± 0.03	10	0.011 ± 0.002	12.52 ± 0.52
0.13	18	0.57 ± 0.07	20	0.098 ± 0.009	6.61 ± 0.23	7	0.50 ± 0.08	0.41 ± 0.04	10	0.014 ± 0.000	10.84 ± 0.36
0.25	18	0.73 ± 0.10	20	0.089 ± 0.011	6.55 ± 0.19	7	0.53 ± 0.07	0.51 ± 0.08	10	0.016 ± 0.001	10.46 ± 0.22
0.50	18	0.66 ± 0.05	20	0.076 ± 0.005	6.70 ± 0.17	5	0.31 ± 0.01	0.27 ± 0.02	10	0.012 ± 0.001	9.19 ± 0.40
1.00	18	0.71 ± 0.07	20	0.052 ± 0.007	6.86 ± 0.07	3	0.48 ± 0.02	0.39 ± 0.03	10	0.014 ± 0.002	9.08 ± 0.46
1.67	18	0.51 ± 0.08	20	0.048 ± 0.009	6.74 ± 0.02	0	-	-	10	0.008 ± 0.002	8.81 ± 0.43

Preface to Chapter 4

In Chapter 3, I was able to verify the presence of carry-over effects in these toads by comparing measures of activity behaviour between the tadpole and toadlet life stages. However, the complexity of the tadpole results suggested that there was an intricate interaction of various factors that were difficult to tease apart under natural field conditions. Therefore, Chapter 4 focuses on examining the interacting effects of density, temperature and even individual body size in tadpoles using a controlled multifactorial experimental design.

Chapter 4: Density and Temperature Effects on Tadpole Activity Level

Katharine T. Yagi and David M. Green

4.1 Abstract

Plastic responses to environmental conditions are mechanisms that allow animals to adapt and survive to changes in their environment. Larval amphibians tend to experience a wide range of conditions, including high densities and temperatures, because of the stochastic nature of their ephemeral habitat. Therefore, larval amphibians usually exhibit plastic behavioural responses to their environment, such as altering activity level, to optimize their growth and survival. As a follow-up to a previous study where we examined individual tadpole activity as a response to various levels of density, and found a complex relationship among density, temperature and body size, we sought to examine more precisely how these three variables interact to influence this plastic behavioural response. We hypothesized that if intermediate densities yield the highest activity levels, and increasing temperature causes an increase in activity, then we should find the same pattern of activity *versus* density that is amplified under increasingly warmer temperatures. We used tadpoles that had been raised under three density levels (0.08, 0.25 and 1.67 tadpoles/L) and exposed each group to three short-term temperature baths (16˚C, 23˚C, 30˚C), recorded body sizes, and monitored individual tadpole activity for eight individuals per density-temperature trial. Intermediate densities did not give rise to the highest activity levels as predicted, however increased temperature did yield higher mean activity. Activity level was positively correlated with tadpole size only under conditions of the highest density (1.67 tadpoles/L) and temperature (30˚C), suggesting that the size of the animal only becomes behaviourally relevant under more stressful conditions, and that behavioural plasticity may be governed by the combined effects of multiple factors like density, temperature, and body size.

4.2 Introduction

Behavioural responses to certain variables, such as temperature (Warkentin 1992; Arendt and Hoang, 2005; Niehaus et al. 2006; Niehaus et al., 2012) and intra-specific competition (Werner, 1991; Griffiths and Foster, 1998; Relyea, 2002; Gómez and Kehr, 2013) are typically adaptive and plastic responses to environmental variation (Lima and Dill, 1990; Relyea, 2001; Relyea, 2002; Van Buskirk, 2002). Phenotype, such as body size, is also known to have plastic responses to environmental change (Dewitt et al., 1998; Agrawal, 2001), but is also linked to behaviour such that a behavioural response to an environmental condition can be dependent on individual internal state (Dingemanse et al., 2010).

Larval amphibians are prone to experiencing a wide range of densities and temperatures due to the stochastic nature of their ephemeral habitat (Heilmeter et al., 2005; Kern et al., 2014; Stein and Blaustein, 2015). Thus, they tend to exhibit behaviourally plastic responses to such conditions, like altering their level of activity (Skelly and Werner, 1990; Smith and Van Buskirk, 1995; Hatch and Blaustein, 2000), to optimize factors like growth rate, timing to metamorphosis and survival. In tadpoles, the benefits of a high activity level include an increased foraging rate which in turn facilitates an increased rate of somatic growth (Skelly and Werner, 1990; Werner and Anholt, 1993; Relyea and Mills, 2001), and an increased competitive ability (Woodward, 1982; Morin, 1983; Griffiths and Foster, 1998). However, with a greater amount of energy expended on movement, comes an increased risk of predation (Werner and Anholt, 1993; Skelly, 1994; Anholt et al., 2000) and a shift in energy allocation, away from lipid storage, which is important for survival through metamorphosis (Marshall et al., 2003; Scott et al., 2007).

The effect of rearing density on tadpole activity level was examined in a previous study (Yagi and Green, in review), where the results revealed a quadratic relationship between activity and density, with intermediate densities gave rise to the highest mean tadpole activity levels. More specifically, the resulting explanatory model included density, temperature and tadpole body size as fixed predictors. The study also found that the relationship between activity level and body size changed from negative to positive at the two highest tested density levels (1.00 and 1.67 tadpoles/L). Therefore, as a follow-up to this study, we conducted a multifactorial experiment aimed to examine more closely, this complex relationship between tadpole activity, density, temperature and body size.

We hypothesized that if increasing density reveals a relationship where intermediate densities yield the highest activity levels, and increasing temperature causes an increase in activity then we should find an analogous pattern of activity among density treatments that becomes amplified under warmer temperature regimes. In addition, we predicted that body size would reveal a significant interaction with density and temperature.

4.3 Materials and Methods

4.3.1 Study Area and Tadpole Density.—This study was conducted at Long Point, Ontario, Canada (between 42˚34′33″42˚35′3″N and 80˚22′15″–80˚28′24″W; datum = NAD83), in small constructed ponds in the Thoroughfare Point Unit of the Long Point National Wildlife Area (NWA) left to naturalize (Yagi and Green, 2016).

We raised Fowler’s toad tadpoles in three density levels, that reflect the range of densities tested in Yagi and Green (in Review): Low (0.08 tadpoles/L), Medium (0.25 tadpoles/L) and High (1.67 tadpoles/L). Six custom-built floating mesh pens (John Radford, Ajax, ON, Canada) of three sizes (i.e. two of each size); 1200 L, 400 L, and 60 L, were used. Each contained 100 tadpoles, creating the respective Low, Medium, and High density levels. All six mesh pens were housed in one pond located in the NWA in order to subject all individuals to the same water conditions, and eliminate potential between-pond variation (Yagi and Green, 2016).

All tadpoles were hatched from one egg mass that was laid on 25 May 2015, and placed in a floating mesh enclosure in a pond until the eggs hatched and larvae grew to developmental stage 25 (Gosner, 1960). Upon reaching this stage on 6 June 2015, the tadpoles were counted into six groups of 100, and each group was randomly assigned to one of the six density treatments. Once tadpoles reached approximately stage 40 (Gosner, 1960), a sample of 12 tadpoles were collected from one density group and brought indoors to be part of a multifactorial experiment. Stage 40 was chosen because it was an easily identifiable stage prior to metamorphosis that allowed tadpoles time to grow under their assigned density treatment. Not all density groups were ready to be tested at the same time, therefore the lowest density group was tested on 5 and 6 July 2015, the medium density group was tested on 9 and 10 July 2015, and the highest density group was tested on 13 and 14 July 2015. Tadpoles were released back to their density treatments to complete their development, once the experiment was completed.

4.3.2 Multifactorial Experiment set-up.—Twelve glass dishes (20 cm^2) were used in this experiment. Four dishes, each with ca. 2 cm of pond water, were placed in each of three large plastic pools (Diameter of 90 cm) filled with approximately 7 cm of tap water. Each pool functioned as a water bath for the dishes. Water temperature was maintained at regimes averaging to 15.6°C ± 0.1 SE, 22.5°C ± 0.1 SE, and 30.0°C ± 0.1 SE, which we henceforth refer to as groups 16˚C, 23˚C and 30˚C, respectively.

The cold regime was maintained using multiple ice packs and ice cubes, and the hot regime was maintained by using three aquarium heating coils (Fluval, model B-38150, Rolf C. Hagen Inc., Montreal, QC, Canada) set at 27˚C. To ensure the heaters did not melt the plastic pool they were each secured on top of one of three terracotta plates underwater. A 2-inch-thick sheet of Styrofoam was placed underneath each large pool for insulation from the concrete floor. Finally, a submersible water pump (Repti Flo 200, model PT-2090, Rolf C. Hagen Inc., Montreal, QC, Canada) was secured to the bottom of each pool and oriented to face the heaters, using the water flow to maintain a homogenous temperature for the duration of the experiment.

4.3.3 Measuring tadpole activity.—For every test session, the sampled tadpoles were randomly placed into a dish and given 30 minutes to acclimate to the test conditions. Digital photographs were taken of each tadpole, and total body length, from tip of the rostrum to tip of the tail, was measured using the software ImageJ (v1.51g, Schindelin et al., 2012). A total of eight tadpoles were tested for each of the nine density-by-temperature treatments ($N_{total} = 72$).

Tadpole movement was monitored using an infrared closed circuit television camera (3xLOGIC, model VX-3S-OD-I-VF, 3xLOGIC Inc., CO, USA; maximum image resolution 2048 x 1536 px), digital video recorder (VIGIL, model MVR-4IP-2TB, 3xLOGIC Inc., CO, USA), and processed with VIGIL video recording software (v8.0, 3xLOGIC Inc). The camera was set to capture an image every four seconds for a total of 60 min. Tadpole activity was scored by recording “active” if the animal had moved during a 4 second interval, or “inactive” if it had not. For each tadpole, we counted the number “active” periods and calculated a percentage of activity out of the total number of time intervals recorded. For comparison, we calculated means of the % activity per density and temperature group, and called this “Activity Level” (Yagi and Green, in review).

4.3.4 Analysis. — All statistical analyses were carried out using appropriate packages in R v3.3.2 (R Core Team, 2015). The body length data was tested for normality using Shapiro-Wilk's test. Due to the known correlation between density and tadpole body size in our system (Yagi and Green, 2016), we verified this relationship by comparing tadpole length between the three density groups using ANOVA, via the aov function in R. We used Tukey's post hoc test to assess differences between each of the nine subgroups.

Since our data are bound between 0 and 1, we used separate beta regressions to examine the effect of density on Activity Level, and the effect of temperature on Activity Level, using the 'betareg' function in R. Each model was analyzed using Chi-square tests with the 'Anova' function from R's 'car' package. We also used a beta regression to test for differences in Activity Level among the nine density and temperature subgroups. A *post hoc* test of pairwise comparisons was used to determine specific differences between subgroups, using the lsmeans function from the 'lsmeans' package in R.

We examined the full dataset using a multiple logistic regression, using the binary 'activity' response variable, and the fixed categorical predictors Density and Temperature and the continuous variable, tadpole length. In addition, we included treatment day (i.e. the number of days the tadpoles were exposed to their density treatment), as a random effect variable to account for the potential temporal bias, and ran the mixed logistic regression under a binomial family using R's 'glmer' function. We analyzed the model using the 'Anova' function, or a Wald Chi square test, from the 'car' package in R.

We assessed the significance each of the nine activity *versus* body length relationships using separate logistic regressions for each density group with temperature and length set as interacting terms, using the glm function in R. All *P* values were assessed using $\alpha = 0.05$.

4.4 Results

4.4.1 Tadpole sizes.—Tadpole body length overlapped between all three density levels, where the mean length ranged from 26.6 to 32.6 mm, with a mean of 28.8 mm ± 0.33 (SE), for the Low density group, 24.6 to 30.0 mm, with a mean of 26.8 mm ± 0.27 (SE), for the Medium density group, and 21.5 to 31.0 mm, with a mean of 26.9 mm ± 0.54 (SE) for the High density group. The variable 'Length' was also found to be normally distributed ($W = 0.99$, $P = 0.65$). The results of the ANOVA revealed, as expected, that mean tadpole length does differ between density groups ($F_{2,69} = 8.13$, $P < 0.001$), more specifically between low and medium density ($P = 0.002$), and between low and high density ($P = 0.003$). Tadpole length did not differ between medium and high density groups ($P = 0.979$).

4.4.2 Patterns of Activity level.—Activity Level increased with temperature as predicted, with mean Activity Level at 42.4% ± 1.4 (SE) for the 16˚C group, 53.6% ± 3.3 (SE) for the 23˚C group, and 55.3% ± 2.8 (SE) for the 30˚C group. Results of the chi-square test revealed that temperature had a significant effect on Activity Level ($\chi^2 = 22.12$, df = 2, $P < 0.001$), and more precisely that the 16˚C group differed significantly from the 23˚C group ($P = 0.004$), and the 30˚C group ($P < 0.001$).

Activity Level also increased with density, although this differs from our original prediction that the intermediate density group would have the highest mean activity. For the Low density group, mean Activity Level was 45.0% ± 1.8 (SE), for the Medium density group it was 48.4% ± 2.5 (SE), and for the High density group it was 57.9% ± 3.4 (SE). The chi-square tests revealed that density also had a significant effect on Activity Level ($\chi^2 = 11.15$, df = 2, $P =$

0.004), and specifically that only the Low and High density groups significantly differed (*P* = 0.003).

Out of the nine subgroups, the lowest mean Activity Level was 40.2% ± 3.3 (SE), which came from the Low density-16˚C subgroup, and the highest mean Activity Level was 66.0% ± 6.1 (SE), which came from the High density-30˚C subgroup (Fig. 4.1). Results from the beta regression showed that both predictor terms were significant but not their interaction (Density: $\chi^2 = 9.63$, df = 2, $P = 0.01$, Temperature: $\chi^2 = 22.09$, df = 2, $P < 0.001$, Density × Temperature: $\chi^2 = 7.83$, df = 4, $P = 0.10$). The post hoc analysis showed four significant differences between the nine subgroups; within the 30˚C temperature group, High density differed from both Medium ($P = 0.005$) and Low ($P = 0.004$), within the Low density group, 30˚C differed from 16˚C ($P = 0.049$), and within the High density group, 16˚C differed from 30˚C ($P < 0.001$; Fig. 4.1).

For the full mixed model, with Density, Temperature, and Length set as interaction terms, the analysis revealed that the only term that did not show significance was Length ($\chi^2 = 0.46$, df = 2, $P = 0.50$). However, all interactions that included Length were significant (Density × Length: $\chi^2 = 183.85$, df = 2, P < 0.001, Temperature × Length: $\chi^2 = 186.25$, df = 2, P < 0.001, Density × Temperature × Length: $\chi^2 = 140.44$, df = 4, P < 0.001).

There were multiple significant interactions between Length and Temperature among the three density groups (Table 4.1). For the Low density group, all three temperature levels showed a negative log odds ratio, and only the 16˚C and 23˚C temperature groups showed significant relationships with Length (16˚C: Z = -2.07, $P = 0.04$, 23˚C: Z = -3.45, $P = 0.001$). More specifically, since the 16˚C group had a log odds ratio of -0.097, this means that for every one-unit increase in Length, there is a 9.7% decrease in tadpole activity from this group (Table 4.1; Fig. 4.2). For the Medium density group, the 16˚C and 23˚C temperature groups showed negative log odds ratios, while the 30˚C temperature group showed a positive log odds ratio, and only the 16˚C and 30˚C groups showed significant results (16˚C: Z = -2.04, *P* = 0.04, 30˚C: Z = 2.12, *P* = 0.03; Table 4.1). For the High density group, all three temperature groups showed positive log odds ratios, and all were significant (Table 4.1; Fig. 4.2).

4.5 Discussion

Since activity level increased with density and temperature, rather than showing intermediate densities yielding the highest mean tadpole activity, our results do not completely coincide with our hypothesis. Our results showed that both density and temperature are generally positively correlated with activity level (Fig. 4.1). In addition, it was under the highest density and two warmest temperature conditions where we observed the largest increase in Activity Level per unit increase in body size (Fig. 4.2). Meanwhile, the lowest density group showed significant decrease in Activity Level with tadpole body size under all three temperature regimes, and the intermediate density group showed a mixed response. The results seem to be complicated, it seems that tadpoles were not prompted to put energy towards movement until they were exposed to two external cues at their relative extremes, together. In addition, it was the larger tadpoles from the high-density and 23˚C/30˚C treatment groups that responded behaviourally to these conditions, compared to the smaller, seemingly less-competitive individuals. All three density groups exhibited a relatively complete range of body size, although the group means were not all the same, allowing us to only visually compare across groups. Therefore, our results suggest that, in terms of expending energy on movement, the size of the animal only becomes relevant under more stressful conditions, and that behavioural plasticity may be governed by the combined effects of density, temperature, and body size.

Living at high densities, or in a resource-limited environment, induces stress in tadpoles (Glennemeier and Denver, 2002; Crespi and Warne, 2013) and can alter the behaviour of individuals, and eventually allocation of resources and pathways of energy expenditure (Takahashi and Pauley, 2010). Large individuals generally have greater energy stores, which can be a benefit when living under stressful conditions (Scott et al., 2007). Also, it is relevant to consider individual personality when examining behaviour across various environmental gradients (Dingemanse et al., 2010). With density and temperature being two common challenges tadpoles would experience in nature, it is possible that the variation in trends we observed are caused by differences in individual preferences, yet monitoring individuals across all gradients would be required to confirm this (Dingemanse et al., 2010).

We observed a slight negative relationship between tadpole length as a function of activity level for the low-density treatment group. An explanation for this might be that the largest individuals under low stress conditions have no provocation for an increased movement behaviour, and the smaller individuals of the same group need to maximize their foraging efforts to counteract the presence of larger and more competitive siblings, thus showing a more active behaviour (Relyea, 2001). Large animals also have greater thermal inertia than relatively smaller ones, so their bodies approach the temperature of their surroundings at a slower rate (Seebacher and Shine, 2004). It is possible that the larger tadpoles in our experiment, which generally correlate with the lowest density group, were at a slower rate by the given thermal regimes due to their larger surface area to volume ratio.

Since temperature is usually species-specific, Bufonid tadpoles have been documented preferring relatively warmer thermal regimes (i.e. 30˚C; Sherman, 1980; Noland and Ultsch, 1981; Espinoza and Quinteros, 2008). It is also understood that Bufonid tadpoles naturally exhibit aggregation behaviour (Wilbur, 1980, Griffiths and Foster, 1998; Yagi and Green, 2016). It is likely that our observation of the high-density tadpole group showing the strongest activity response when put under the 23˚C and 30˚C regimes was caused by their treatment conditions closely resembling their natural densities and thermal optimum.

Thermal conditions outside the optimal range may induce maladaptive behaviour in tadpoles, such as decreased movement rates and reduced foraging efficiency at below-optimal temperatures (Niehaus et al., 2012), or over-active behaviour at extremely high temperatures leading to excess expenditure of energy and sub-optimal growth (Niehaus et al., 2012). So, it is likely that animals will not only attempt to cope with their environmental conditions, but take maximum advantage them. Therefore, it is possible that the range of thermal tolerance in Fowler’s toad tadpoles differs based on body size, where the optimal behavioural response in smaller, crowded individuals is to reduce movement when exposed to temperatures outside their thermal optimum (i.e. 30˚C in this case).

Ephemeral pools, in which most larval amphibians inhabit, are known for their large thermal fluctuations (Heilmeter et al., 2005) and can allow for substantial behavioural changes over a short period (Kern et al., 2014). While fluctuating temperatures during development can lead to sub-optimal growth rates (Dong et al., 2006; Neihaus et al., 2006; Du and Shine, 2010), organisms that are typically exposed to these conditions may exhibit increased thermal tolerances (Sinclair et al. 2006) and increased range of performance (Huey and Kingsolver, 1989; Yagi and Litzgus, 2013). The tadpoles in our study were exposed to a variety of thermal and crowding conditions which enlisted a variety of responses. Although we were able to see some trends in our data, there was still much variation. A major limitation to this study was the small sample size per group, and coupled with the fact that not all tadpole groups were tested on the same day, this may have contributed to the unexplained variation in the resulting data.

Our study gives some empirical evidence of behavioural plasticity in tadpoles, caused by three interacting variables; density, temperature and body size. We postulate that understanding this complex relationship requires the consideration of the optimal environmental conditions of the study species. Further examination of the interacting effects of such variables on amphibian behaviour, over multiple life stages, is required to fully understand the impacts they have on individual fecundity, fitness, and long-term population dynamics.

4.6 Acknowledgments

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4.8 Tables and Figures

Table 4.1 Results from three logistic regressions, one for each Density category, examining the Activity *vs.* Length relationship, using Temperature as an interacting term. For each subgroup, the log odds ratio, % change per unit of Length, Z value, standard error and P values are reported. Significant log odds ratios are indicated with an *.

Density Level	**Temperature**	***n***	**Log Odds Ratio**	**%**	**Z**	**SE**	***P***
Low	Cold	8	-0.097	-9.23	-2.07	0.02	0.039*
	Warm	8	-0.052	-5.04	-3.45	0.01	0.001*
	Hot	8	-0.080	-7.64	-1.22	0.02	0.223
Medium	Cold	8	-0.072	-6.93	-2.04	0.04	0.042*
	Warm	8	-0.080	-7.71	-0.18	0.05	0.856
	Hot	8	0.009	0.91	2.12	0.04	0.034*
High	Cold	8	0.064	6.59	7.73	0.01	<0.001*
	Warm	8	0.301	35.14	15.71	0.02	<0.001*
	Hot	8	0.175	19.14	7.66	0.01	<0.001*

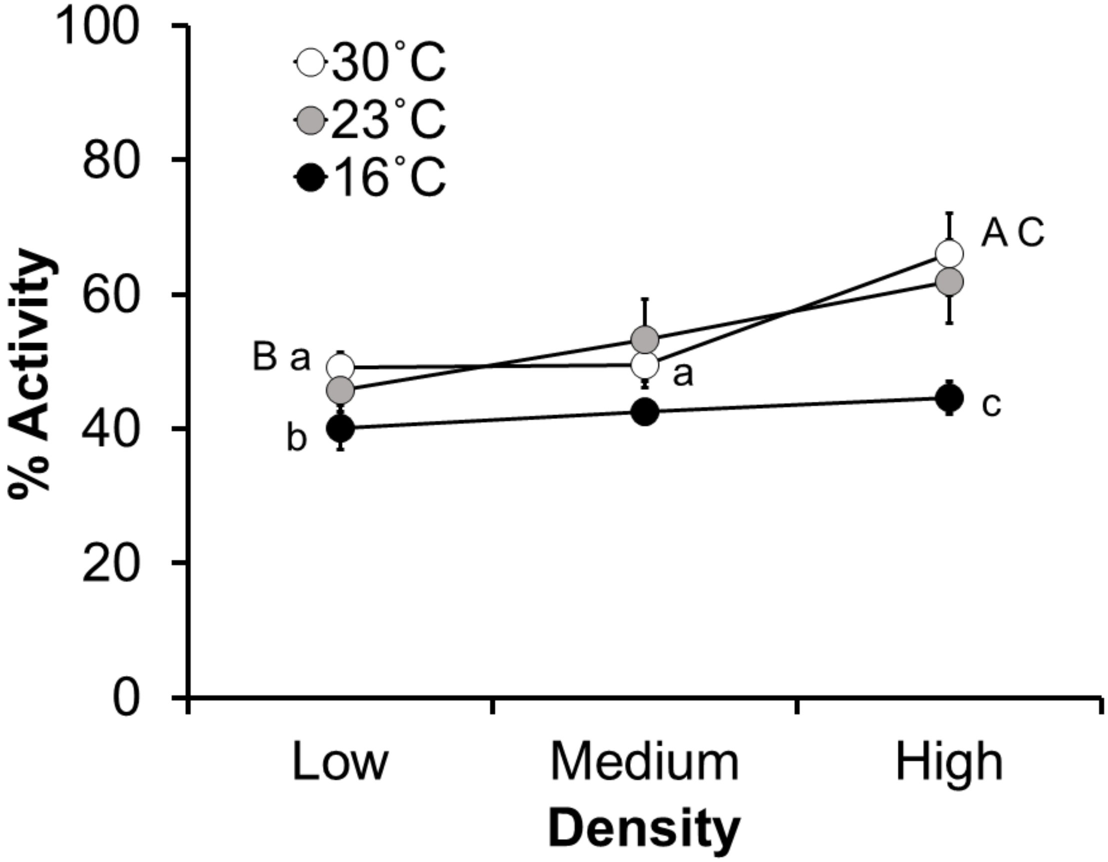

Fig. 4.1 Interaction plot from the multifactorial experiment on tadpole % activity. Activity Level increased with density and temperature under both ‘Low’ and ‘High’ density conditions, but when under ‘Medium’ density conditions, Activity Level is highest when under ‘Warm’ temperatures. All error bars represent standard error. Only comparisons of significantly different subgroups are distinguished with a letter (A/a, B/b, or C/c), where the lower-case letter distinguishes which subgroup is statistically different from the uppercase counterparts.

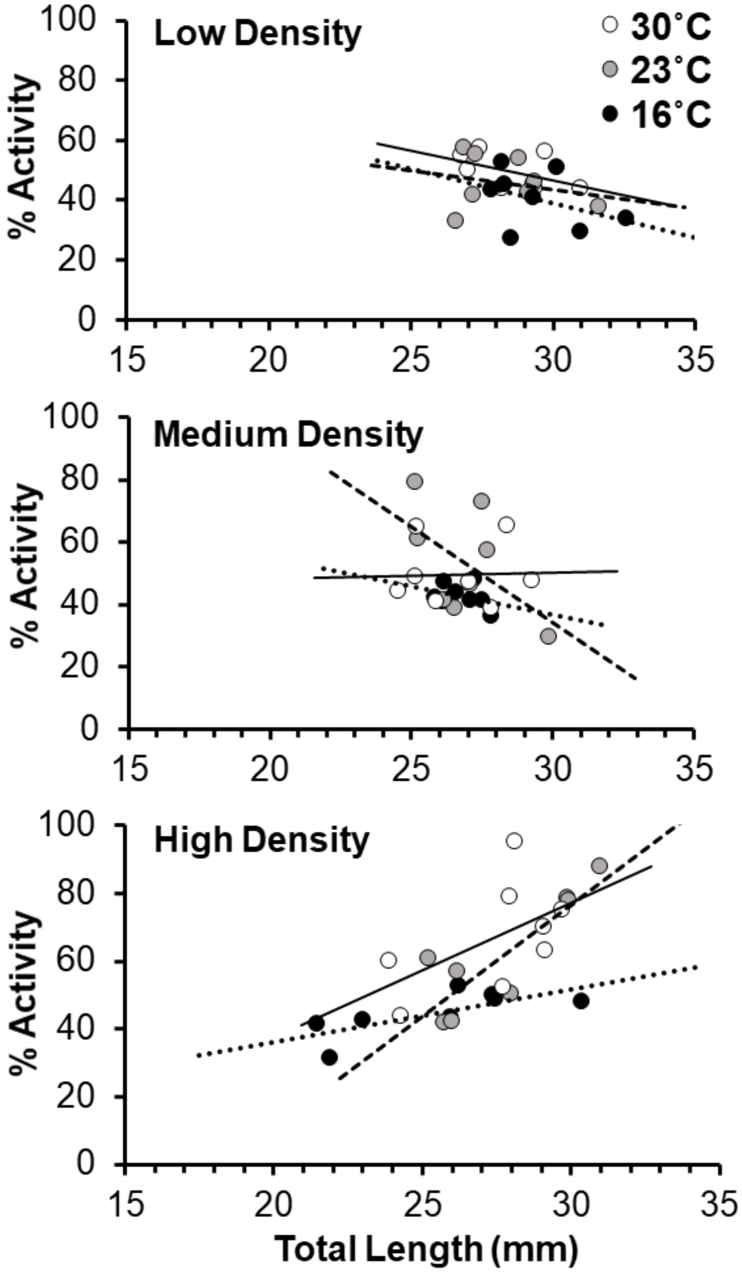

Fig. 4.2 Results from the multifactorial experiment displaying activity level as a function of tadpole body length. Each panel represents a density group while each colour represents a temperature group. Activity level was transformed with the natural logarithm. Regression lines are plotted on each panel for each temperature group.

Preface to Chapter 5

In Chapters 3 and 4, I developed evidence of carry-over effects being present in this toad population, and even went further to tease apart the major factors driving such behaviour at the tadpole stage. In Chapter 5, I shift my attention to the toads' terrestrial life stages, and using individuals exposed to density-treatments I examine the impact of their early life experience on their movement endurance. Since all toadlets from the density-treatments were released back into the field, I was able to expand the focus of this study to natural toad movements by applying the knowledge of their condition (or body size) to their actual movement distances in the field.

Chapter 5: Performance and Movement in Relation to Post-Metamorphic Body size in a Pond-breeding Amphibian

Katharine T. Yagi and David M. Green. In Review at *Journal of Herpetology*.

5.1 Abstract

We examined the effect of body size on locomotor performance and movement behavior in early post-metamorphic toadlets of Fowler's Toad (*Anaxyrus fowleri*). Dispersal, if strictly density-dependent, should be favored among the relatively small toadlets that emerge from crowded growth conditions but not among the relatively large toadlets that result from low density conditions. However, smaller animals may have less physical capability to disperse than larger ones. The net result may thus be that actual dispersal probability is greatest among intermediate-sized toadlets. Using toadlets of various sizes purposefully raised by manipulating their densities as tadpoles, we tested toadlet locomotor jumping endurance in relation to body size. We also compared the animals' initial body size against their overall movements in the wild, determined using capture-recapture methods, over two years as they grew from toadlets to adults. We calculated movement rate and dispersal probability for each individual recaptured more than twice, and determined if successful movements were strategy correlated or uncorrelated. Our results show that toadlets of intermediate size are most likely to disperse farthest, even though they do not necessarily exhibit the highest levels of endurance. Therefore, knowledge of individual life experience across multiple life stages may be necessary to understand dispersal tendencies in amphibians, and may be required in future studies aiming to predict dispersal and population dynamics.

5.2 Introduction

Dispersal, a key process that profoundly affects the structure and dynamics of populations (Bowler and Benton, 2005; Barton et al., 2009), can be defined as the displacement of an individual during its lifetime from its birthplace to a breeding site somewhere else (Clobert et al., 2009). Decreasing habitat quality and increasing population density have both been shown to increase the probability for active dispersal in animals (Travis et al., 2009; Mathieu et al., 2010; Innocent et al., 2010). In contrast to their potential for dispersal, realized dispersal (i.e. actual dispersal) may decrease with density (Ims and Andreassen, 2005; Meylan et al., 2007) and be influenced by habitat quality (Travis and Dytham, 1999; Matthysen, 2005).

The probability of dispersal may have a genetic component stemming from the inheritance of certain physical traits (Clobert et al., 2001; Bowler and Benton, 2005; Ronce, 2007) or behaviors (Clobert et al., 2009; Cote et al., 2010). It can also be dependent on an individual’s physical condition (Clobert et al., 2009; Bonte and de la Peña, 2009; Gyllenberg et al., 2011) since dispersal itself is a costly process (Bonte et al. 2012), which may be influenced by maternal effects (Williams, 1994; Sakai and Harada, 2001; Sinervo et al., 2006), habitat quality (Maceda-Veiga et al., 2014; Meillère et al., 2015), competition (Lorenzen and Enberg, 2002; Browne et al. 2003; Yagi and Green, 2016) and carry-over effects (Pechenick et al., 1998; Benard and McCauley, 2008; O’Connor et al., 2014). Realized, and probability of dispersal are related terms, in that they both can refer to a measure of dispersal for individuals or populations, but the probability to disperse is mediated by individual traits, which in turn ultimately influence the success of their realized dispersal.

Animal movement falls into two basic patterns; correlated and uncorrelated movement. Correlated movements are those where the animal moves in a directed manner and the direction of a movement step is dependent on the immediately previous one (Byers, 2001; Bartumeus and Levin, 2008; Barton et al., 2009). The initiation of correlated movement may often be caused by external triggers, like predation, competition, or change in habitat quality (Bowler and Benton, 2005). There is also some evidence of correlated movement based on the individual's internal condition (Bonte and De La Peña, 2009), and their genetic predisposition for dispersal (Clobert et al., 2001). Uncorrelated movements, however, follow a random-walk pattern, as may be typical of searching behavior in a homogenous landscape (Turchin, 1998; Mårell et al., 2002; Codling et al., 2008). Both movement patterns can occur throughout an individual's lifetime and at various spatial scales (Nathan et al., 2008). Such common animal movement patterns can result in dispersal as an end-product, even though it may not be the initial intention of their movements (Van Dyck and Baguette, 2005; Conradt and Roper, 2006). Movement behavior with both correlated and uncorrelated patterns has been associated with "fat-tailed" probability distance curves similar to a power distribution (Nathan et al., 2008), also called a Lévy walk (Viswanathan et al., 2000). This pattern includes random walks, with occasional long-distance directed movements, and has been used to classify searching behavior in animals living within a patchy resource landscape (Bartumeus et al., 2005; Benhamou, 2007).

Amphibians, on the whole, are not typically long-distance dispersers compared to many other vertebrates (Cushman, 2006, but see Smith and Green, 2006) in that they typically show high fidelity to breeding sites (Gamble et al., 2007; Pittman et al., 2014), have specific habitat tolerances (Gibbs, 1998), and can be susceptible to desiccation when moving across an open landscape (Rittenhouse et al., 2008; Mitchell and Bergmann, 2016). In addition, amphibians tend to experience high mortality rates when crossing roads (Beebee, 2013), a circumstance that has become inevitable in many urbanizing countries. Consequently, the ability of amphibians to move across the landscape may depend on body condition (Pittman et al., 2014), or body size (Alvarez and Nicieza, 2002; Tejeo et al., 2000; Cabrera-Guzmán et al., 2013) to a considerable degree. Body condition, or the relative level of fat stores, is strongly correlated with body size, particularly in amphibian metamorphs (Scott et al., 2007). Large body size has been linked to good locomotor performance in newly metamorphosed frogs (Álvarez and Nicieza, 2002), and to large dispersal distances across various taxa (Jenkins et al., 2007; Shurin et al., 2009), though there are exceptions (Gordon and Hellman, 2015).

If the body size of a young-of-the-year toad (i.e. a “toadlet”) is related to its dispersal ability, then there is likely to be a significant relationship between the individual’s snout-vent length (SVL) and its relative locomotor performance. This may be a linear relationship. However, dispersal also should depend on movement directionality, not just movement ability, and the favorability of dispersal as a survival strategy. It is plausible that smaller individuals, especially if they are the products of crowded, low quality growing conditions as tadpoles, may be incapable of dispersing effectively because of their small size and poor endurance, even though dispersal may be favored. Conversely, larger individuals, which may be the products of uncrowded, high quality conditions as tadpoles, might be able to disperse with ease but do not because dispersal away from optimal conditions would not be favored. As such, and if dispersal probabilities are thus reduced among both the largest and the smallest individuals, then the largest overall movements should be characteristic of intermediate-sized individuals, resulting in a hump-shaped curve when comparing movement distance against body size for animals of equal age. To test these hypotheses, we used both wild-caught toadlets and toadlets that had been purposely grown to be of varying sizes by manipulating larval density (Yagi and Green, 2016) and compared body size against 1) relative locomotor performance, assessed as endurance when jumping, and 2) movement patterns in the wild, assessed using mark/recapture over time until, in some cases, adulthood.

5.3 Materials and Methods

5.3.1 Study site and species.— This study was conducted in the Thoroughfare Point Unit of the Long Point National Wildlife Area (NWA; between 42˚34′33″N–42˚35′3″N and 80˚ 22′15″–80˚28′24″W). Fowler's toads (*Anaxyrus fowleri*) are a relatively small and widespread toad in eastern North America, and in Canada are adapted for living in sand dune-shoreline ecosystems (Greenberg and Green, 2013). In Canada, this species is classified as endangered due to their genetic isolation from the main populations in the United States, and the continuing habitat loss from invasive species and shoreline development in Canada (Greenberg and Green, 2013). Therefore, adult Fowler's toads in Long Point, Ontario, primarily use the natural beaches as their main foraging habitat and dispersal corridor (Greenberg and Green, 2013), most of which are assumed to be continuous, unaltered habitat. Natural adult toad densities have been shown to fluctuate annually, ranging from 4 to 93 toads per km, and natural tadpole densities have not been estimated in Long Point due to the nature of their ephemeral and dynamic beach habitat (Yagi and Green, 2016). However, mean female clutch size in Long Point Fowler's toads was measured to be 4443 eggs per clutch, from a sample of 34 individuals (Green, 2015), and tadpole survival to metamorphosis ranged from 5% to 43% as experimental density decreased from 5 to 0.1 tadpoles/L (Yagi and Green, 2016).

We produced young-of-the-year toads, or toadlets, of various sizes by manipulating tadpole density at six levels; 0.08, 0.13, 0.25, 0.5, 1.0 and 1.67 tadpoles/L, replicated in three ponds in 2014 (Yagi and Green, 2016). Three measurements were taken from all toadlets, whether performance-tested or not: snout-vent length (SVL) and leg length (in millimeters), and mass (in grams). Leg length was measured from the urostyle to the tip of the longest toe. Relative leg length was calculated by dividing leg length by SVL. Body condition was calculated using residuals from the regression of SVL versus mass; positive values indicated a good condition (i.e. heavier than average given their length), and negative values indicated a poor condition (i.e. lighter than average given their length). All length measures were collected using 15 mm dial calipers. Mass was measured using a portable weight scale (0.01 g, Ohaus, SP202). All toadlets were digitally photographed for identification (Schoen et al., 2015) and released into the adult habitat from the same point along the beach, at night.

5.3.2 Locomotor performance. — Jumping endurance was estimated for 29 toadlets by quantifying their jumping fatigue, or measuring the change in length of 60 consecutive series of hops. To do this, we placed toadlets individually into a plastic circular arena (diameter of 91 cm), with the bottom filled with 2 cm of moist sand. The test was initiated after a 60-minute acclimation period. We instigated movement by gently prodding each toadlet on the urostyle, up to a maximum of 60 times. We called this variable “Jump No.”, or the number identifying the jump in order from the first to the sixtieth. All jumping endurance tests were recorded using an infrared camera (model CMC-3MP-OD-I), and DVR unit (VIGIL, DRX-50-16-500; CAMACC systems Inc.). Because toads tend to not move in singular jumps, like frogs, but in a series of short hops (Reilly et al., 2015), we called the length of a series of hops a ‘movement segment’. All jumping tests were recorded at a rate of 7.5 frames per second, and the footage was analyzed afterward by taking a freeze-frame image after each ‘movement segment’ and measuring its length using the image processing software, ImageJ (Fiji v1.51g; Schindelin et al., 2012).

To calculate toadlet endurance levels ($n = 29$), we standardized the length of each measured ‘movement segment’ against the toadlets’ SVL, by taking the residuals from a regression of ‘movement segment’ versus SVL. Using these new values, we used the slope of the regression of standardized ‘movement segment’ versus Jump No., or acceleration (mm/jump2), to calculate endurance level per toadlet, EN, according to the formula: $EN = \text{Log}_e(\text{slope} + 1)$. EN values that were neutral or positive indicated good endurance; values of EN that were negative indicated poor endurance. A natural log transformation was used to attain normality.

5.3.3 Movement strategies.— We monitored the post-release movement of both density-treated, and any wild-caught toadlets for the remainder of the toads’ 2014 active season, and their movements as juveniles for the 2015 and 2016 seasons from 1 May to 20 August. We employed capture-recapture surveys, and conducted them along known dispersal corridors (i.e. beaches and sand dunes) of the study area. We noted the sex if secondary sex traits were visible, recorded location coordinates using a hand-held GPS unit for all individuals encountered.

To quantify an individual’s movement strategy (i.e. correlated vs. uncorrelated movement), we used the toadlet encounter data to determine the distance between each consecutive recapture event, and the Euclidean distance, i.e. the distance between their initial point of release and last capture location, per individual using Excel (Microsoft, Office 365). We calculated a dispersal ratio, DR, by dividing the straight-line (Euclidean) displacement between the very first and very last capture points ($\text{Dist}_{\text{AtoB}}$), by the sum of the distances between consecutive recaptures ($\text{Dist}_{\text{Total}}$). For each toadlet DR, values close to 1 indicated correlated movement, whereas values close to 0 indicated uncorrelated movement. DRs were calculated for any toadlet with ≥ 2 recapture events. Mean distance moved per day ($\mathrm{Dist}_{\mathrm{day}}$), or movement rate, was calculated per individual using $\mathrm{Dist}_{\mathrm{Total}}$ divided by number of active days between the first and last capture, removing the number of days between 15 October and 15 April each year to account for winter dormancy. Adult, or Realized, dispersal events, were determined only for toads that had reached sexual maturity (in 2016). We considered those with $\mathrm{Dist}_{\mathrm{AtoB}} \geq 1000$ m to have a positive dispersal trajectory and were given a binary response of “yes”, and a “no” was given for anything otherwise. Movement data from wild-caught and density-treated toadlets were examined separately, and again when combined, to observe a complete range of body sizes and its effect on movement and dispersal probability.

5.3.4 Analysis. — All statistical analyses were conducted using R (v3.3.2; R Core Team, 2015). The normality assumptions of linear regression were tested using the Shapiro-Wilk normality test. To achieve normal distributions for the analysis, we used a $\log_e$ transformation on $\mathrm{Dist}_{\mathrm{AtoB}}$ and $\mathrm{Dist}_{\mathrm{day}}$. Using only the density-treated toadlet data, we used model selection (Akaike, 1987) to examine the explanatory power of the predictors Density, SVL, relative leg length, and body condition, on *EN*, using the ‘lmer’ function from the ‘lme4’ package in R. Two additional variables were included in these models: mean air temperature as a fixed predictor and pond origin as a random effect term. The model with the highest AICc weight was analyzed using R’s summary function. We also examined linear regressions using *EN* as the predictor variable and tested $\mathrm{Dist}_{\mathrm{AtoB}}$ and $\mathrm{Dist}_{\mathrm{day}}$ as responses.

Using the movement data of both density-treated and wild-caught toadlets, we employed separate linear regressions to assess the movement response variables, $\mathrm{Dist}_{\mathrm{AtoB}}$, and $\mathrm{Dist}_{\mathrm{day}}$, as a function of toadlet SVL. We also employed the same analysis on the combined data of density-treated and wild-caught toadlets. To determine if the relationships between toadlet SVL and

$\text{Dist}_{\text{AtoB}}$, and between SVL and Dist_{day} differed among wild-caught and density-treated toads we used two separate analyses of covariance (ANCOVA), with the two movement variables as separate responses, with toadlet SVL and data 'Type' (i.e. wild-caught or density-treated) as interacting predictors. In addition, we used a Kruskal-Wallis non-parametric comparison of means to assess differences in these movement variables between males, females and juveniles. Toads were categorized as juveniles if they were ≥ 45 and ≤ 55 mm in SVL and did not exhibit secondary sex characteristics.

Since the dispersal ratio data was bounded between 0 and 1, we conducted beta regressions, to determine if dispersal ratio can be predicted by SVL, including SVL as a polynomial term. We examined this relationship for density-treated and wild-caught toadlets separately, and again using the combined dataset. Using only the density-treated toadlets, we also looked at a beta-regression with *EN* as the predictor and dispersal ratio as the response. We performed all beta regressions using the betareg command, with a 'logit' link function in the R package 'betareg', and analyzed using the 'Anova' function from the 'car' package in R.

Dispersal probability (D_p), was calculated by using a logistic regression on the binary dispersal data, using R's 'glm' function under the binomial family and logit link function. We used toadlet SVL as the primary predictor, and included it as a polynomial term to assess if the relationship is quadratic, as predicted. Finally, realized dispersal (D_r) was calculated for toads identified as adults in 2016, and analyzed using a logistic regression on their binary dispersal data with adult SVL as the primary predictor, again using R's the glm function. The 'Anova' function from R's 'car' package was used to assess the significance of both models.

5.4 Results

5.4.1 Toadlet locomotor performance.— Toadlets used in the performance trials ranged from 13.3 mm SVL to 20.2 mm SVL. Relative leg length ranged from 0.93 to 1.10 ± 0.02 (SE). The highest mean *EN* value, -0.10 ± 0.09 (SE), came from density level 0.25 tadpoles/L, while the lowest mean *EN* value, -0.27 ± 0.04 (SE), came from density level 0.50 tadpoles/L (Table 5.1). The results indicated that *EN* is most strongly predicted by SVL ($R^2 = 0.58$, $P < 0.001$) rather than Density ($R^2 = 0.02$, $P = 0.46$; Table 5.2), per the linear equation: $EN = 0.06*\text{SVL} – 1.19$ (Fig. 5.1).

5.4.2 Displacement and movement rate.— Toad movement data included recaptures of 74 individuals between 2014 and 2016, 13 of which were released as density-treated toadlets, and 61 were originally wild-caught toadlets in 2014. Our monitoring showed secondary sex characteristics for most individuals by 2016, revealing 34 toads to be female, 35 to be male. Five individuals not recaptured in 2016 were categorized as juveniles. Mean juvenile $\text{Dist}_{\text{AtoB}}$ was 232.67 m ± 115.4 SE, and Dist_{day} was 2.24 m/day ± 0.92 SE. For females, mean $\text{Dist}_{\text{AtoB}}$ was1039.77 m ± 134.43 SE, and mean Dist_{day} was 15.77 m/day ± 3.55 SE. For males, mean $\text{Dist}_{\text{AtoB}}$ was 1473.26 m ± 364.0 SE, and mean Dist_{day} was 15.03 m/day ± 2.78 SE (Table 5.3).

With *EN* as the predictor, we did not find a significant relationship in density-treated toadlets with Dist_{day} ($R^2 = 0.01$, $F_{1,6} = 0.04$, $P = 0.85$), nor with $\text{Dist}_{\text{AtoB}}$ ($R^2 = 0.17$, $F_{1,6} = 2.40$, $P = 0.17$) although the trend was negative. Toadlet SVL was not a significant predictor for Dist_{day} in density-treated toadlets ($F_{2,10} = 0.55$, $P = 0.59$), nor in wild-caught toadlets ($F_{2,58} = 0.26$, $P = 0.77$). Similarly, SVL was not a significant predictor for $\text{Dist}_{\text{AtoB}}$ in density-treated toadlets ($F_{2,10} = 1.59$, $P = 0.25$) or for wild-caught toadlets ($F_{2,58} = 0.62$, $P = 0.54$). However, when using the combined movement data (i.e. both density-treated and wild-caught toadlets), toadlet SVL was a significant quadratic predictor for $\mathrm{Dist}_{\mathrm{day}}$ ($R^2 = 0.12$, $F_{2,71} = 4.94$, $P < 0.01$; Fig. 5.2), and for $\mathrm{Dist}_{\mathrm{AtoB}}$ ($R^2 = 0.10$, $F_{2,71} = 3.49$, $P = 0.04$).

There was no interaction between wild-caught and density-treated toadlets in the relationship between $\mathrm{Dist}_{\mathrm{day}}$ and toadlet SVL ($F_{1,69} = 0.09$, $P = 0.77$). Similarly, there was no interaction between these two groups in the relationship between $\mathrm{Dist}_{\mathrm{AtoB}}$ and toadlet SVL ($F_{1,69} = 0.19$, $P = 0.67$). For the density-treated toadlets, there was no significant difference between males, females and juveniles for $\mathrm{Dist}_{\mathrm{day}}$ ($\chi^2 = 0.56$, df = 2, $P = 0.75$), or for $\mathrm{Dist}_{\mathrm{AtoB}}$ ($\chi^2 = 1.42$, df = 2, $P = 0.49$). For the wild-caught toadlets, there was a significant difference in $\mathrm{Dist}_{\mathrm{day}}$ among males, females and juveniles, where juveniles had a much lower movement rate at 1.59 m/day compared to the adults ($\chi^2 = 6.97$, df = 2, $P = 0.03$; Table 5.3), but there was no difference in $\mathrm{Dist}_{\mathrm{AtoB}}$ ($\chi^2 = 4.27$, df = 2, $P = 0.12$).

5.4.3 Movement strategies.— Mean dispersal ratio was highest in juveniles for both density-treated (0.44 ± 0.20 SE) and wild-caught toadlets (0.48 ± 0.21 SE), and lowest for density-treated males (0.18 ± 0.07 SE) and wild-caught females (0.38 ± 0.05 SE). For the density-treated toadlets, there was no relationship between dispersal ratio and toadlet SVL using the mean model (pseudo $R^2 = 0.002$, SVL: Z = 0.92, SE = 0.11, $P = 0.36$; SVL^2: Z = -0.71, SE = 0.002, $P = 0.48$). For the wild-caught toadlets, the mean model did show a significant quadratic relationship between dispersal ratio and toadlet SVL (pseudo $R^2 = 0.09$, SVL: Z = -2.23, SE = 0.32, $P = 0.03$; SVL^2: Z = 2.21, SE = 0.01, $P = 0.03$), however the precision model was not significant (phi SVL: Z = -0.08, SE = 0.33, $P = 0.93$; phi SVL^2: Z = 0.18, SE = 0.01, $P = 0.86$).

For the combined dataset, the mean model resulting from the beta regression analysis did not reveal a significant quadratic relationship between dispersal ratio and toadlet SVL (pseudo

$R^2 = 0.04$, SVL: Z = 0.84, SE = 0.12, $P = 0.40$; SVL^2: Z = -0.60, SE = 0.002, $P = 0.55$), However, the precision model did reveal significant coefficients (phi SVL: Z = -3.09, SE = 0.15, $P < 0.01$; phi SVL^2: Z = 2.98, SE = 0.003, $P < 0.01$). With *EN* as the predictor, there was a significant quadratic relationship with dispersal ratio in both the mean model (*EN*: Z = -2.15, SE = 7.28, $P = 0.03$; EN^2: Z = -2.06, SE = 22.32, $P = 0.04$) and the precision model (phi *EN*: Z = 2.69, SE = 16.55, $P = 0.007$; phi EN^2: Z = 3.95, SE = 60.54, $P < 0.001$). There was no difference between sexes ($\chi^2 = 0.80$, df = 2, $P = 0.67$)

5.4.4 Probability and realized dispersal.— Out of the 13 density-treated toadlets recaptured over the 2014 through 2016 active seasons, only seven were recaptured as adults in 2016. All seven individuals were males, and did not move farther than 500 m in $Dist_{AtoB}$. Out of the 61 wild-caught toadlets that were recaptured over the 2014 through 2016 seasons, 55 individuals were recaptured as adults in 2016, 27 of which were females and 28 were males.

Looking at only wild-caught toadlets, there was no relationship between dispersal probability and their SVL. There was also no difference in dispersal probability between wild-caught males and females (Z = -1.08, SE = 0.56, $P = 0.28$). However, when we combine both density-treated and wild-caught dispersal data, there was a significant quadratic, relationship between toadlet SVL and dispersal probability ($\chi^2 = 4.60$, df = 1, $P = 0.03$; Fig. 5.3), resulting in the fitted model: $D_p = 1 / (1 + \exp(-(0.697*SVL – 0.0113*SVL^2 – 10.354)))$. Additionally, dispersal probability revealed a significant positive relationship with adult SVL ($\chi^2 = 14.04$, df = 1, $P < 0.01$; Fig. 5.3), with a fitted model: $D_p = 1 / (1 + \exp(-(0.185*SVL – 12.18)))$.

5.5 Discussion

Our results are consistent with the hypothesis that toadlet endurance is best predicted by body size, as seen in laboratory studies (Beck and Congdon, 2000; Cabrera-Guzmán et al., 2013). However, because the size range for this analysis was relatively narrow compared to the range of sizes observed in the combined movement dataset, we might only be able to see one part of a potentially complex relationship. Also, because toadlet body size is, in turn, strongly dependent on tadpole density (Yagi and Green, 2016), these results are also consistent with other studies showing that amphibian larval density conditions have an impact on post-metamorphic performance (John-Alder and Morin, 1990; Alvarez and Nicieza, 2002; Tejedo et al., 2010).

We did not observe a significant relationship between toadlet size and movement rate when the density-treated and wild-caught datasets were kept separate. We did observe a significant relationship between SVL and movement strategy (i.e. dispersal ratio) for the wild-caught toadlets under the mean model from the beta regression, but not for the density-treated group. When looking at the data separately, it appears that the number of data points and the breadth of sizes likely made a difference statistically when trying to see such a trend, where there were only 13 points for the density-treated group and 61 points for the wild-caught. We did however, find a significant quadratic relationship between toadlet size and movement rate (Fig. 5.2), toadlet size and Euclidean displacement, and between toadlet size and dispersal probability (Fig. 5.3) when looking at the combined dataset with toadlets from a broad size range. These results are consistent with the hypothesis that toadlets of intermediate size will have the fastest terrestrial movement and probability of dispersal in the wild.

Endurance was found to be a significant quadratic predictor for movement strategy, where intermediately-abled individuals showed a more correlated movement behavior. Since endurance is closely linked to body size (Fig. 5.1), our results further support the idea that those individuals with the highest endurance level or largest body size do not necessarily choose a movement strategy that will reflect dispersal behavior. This part of the study was similar to that of Llewelyn et al. (2010), which compared laboratory-tested endurance levels of invasive Cane toads (*Rhinella marina*) against dispersal behavior observed from other individuals from the same populations in Australia. However, our results were different, since the individuals that showed highest endurances in the lab did not reveal the same, dispersal-like behavior in the field. The difference in our results is likely a tribute to the clear differences in the ecology of the two species, and the fact that Cane toads are invasive and resilient in the Australian populations, whereas Fowler's toads exist on the periphery of their native range in Long Point (Greenberg and Green, 2013).

Positive correlation between successive movements, or directional movement, is understood to reflect dispersal behavior (Byers, 2001; Codling and Hill, 2005). However, juvenile amphibian dispersal is considered a more diffusive process (Codling et al., 2008), indicative of uncorrelated movement, which might explain our lack of correlated movement results in our study population. An uncorrelated movement strategy has been associated with searching behavior (Smouse et al., 2010), a potentially costly process if resources are limiting, and may influence the animal's decision to disperse (Morris, 1992; Stamps et al., 2005). Therefore, it is possible that our measures of uncorrelated movement in many individuals does not necessarily mean they will remain local and breed in their natal pond. Rather, their movement pattern might gradually lead them away from their natal site until they reach adulthood, and breed at a new, distant pond. Consequently, there is evidence of poor agreement between field assessments of amphibian dispersal, and estimates of gene flow using molecular techniques (Sinsch, 2014). In addition, we did not find any differences between sexes in our measures of movement, which is somewhat surprising since many studies have shown a strong sex-biased dispersal trait in various taxa (Perrin and Mazlov, 2000; Lawson Handley and Perrin, 2007; Trochet et al., 2016).

Unlike the toadlets, adult-sized toads exhibited a positive, logistic the relationship between SVL and realized dispersal probability (Fig. 5.3). This positive trend between body size and dispersal agrees with some past studies (Jenkins et al., 2007; Shurin et al., 2009), although both trends have been detected under different circumstances (Bonte and De La Pena, 2009; Cote and Clobert, 2010). Since our measure for dispersal is the displacement of the animal between 2014 and 2016, and we are using the same individuals in both regressions, we may interpret these results as the toads having different growth rates once released into the terrestrial habitat. Based on Fig. 5.3, the intermediate sized toadlets were more likely to disperse, and as adults these toads became the largest in their cohort. Based on this observation, one would have to consider that adult size might be the response to their dispersal behavior, rather than the cause. This idea agrees with our finding that most toads exhibited uncorrelated movement (dispersal ratio < 0.5), analogous to foraging behavior (Mårell et al., 2002), and amphibian diffusive dispersal (Codling et al., 2008; Semlitsch, 2008). If those intermediate-sized individuals were acquiring food resources efficiently over their adolescence, then they would reach larger adult sizes by the end of the study, and giving us our positive size *vs*. dispersal probability relationship.

Our results show that small size, which can result from crowded larval growth conditions, is associated with lower movement and performance levels in toads, which may result in lower dispersal probability. We were also able to demonstrate that an intermediate size might be favored for dispersal in toadlets due to a combination of early life density-dependent conditions, and their subsequent vagility. Although we see no clear trend concerning correlated movement strategy in relation to body size, final displacement distances were quite large in some individuals. Therefore, an uncorrelated movement strategy may eventually lead to actual dispersal. These factors all add a level of complexity to dispersal models wherein high population density typically are thought to yield higher dispersal rates. Since dispersal is a key component in sustaining populations, especially those that are small and peripheral, a clear understanding of density-dependent vs. size-dependent dispersal propensities is critical in the management of such vulnerable populations.

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5.8 Tables and Figures

Table 5.1 Summary of endurance-tested Fowler's toad toadlets (*Anaxyrus fowleri*); mean toadlet SVL, body condition, relative leg length and endurance (*EN*) calculated per density treatment, with variance given as standard error. Endurance values are shown as Log$_e$ transformed values.

Rearing Density (tadpoles/L)	***N***	**Toadlet SVL (mm)**	**Body Condition**	**Relative Leg Length (mm)**	***EN***
0.08	7	16.94 ± 0.47	-0.04 ± 0.29	1.07 ± 0.02	-0.12 ± 0.03
0.13	7	16.10 ± 0.45	0.13 ± 0.19	1.03 ± 0.02	-0.13 ± 0.05
0.25	7	17.40 ± 0.87	0.52 ± 0.25	0.99 ± 0.01	-0.10 ± 0.09
0.50	5	14.18 ± 0.33	-0.18 ± 0.26	1.08 ± 0.02	-0.27 ± 0.04
1.00	3	15.97 ± 0.48	0.08 ± 0.24	1.10 ± 0.02	-0.13 ± 0.06

Table 5.2 Model selection output for the top six explanatory models, assessing how Fowler's toad (*Anaxyrus fowleri*) toadlet size (SVL), body condition, relative leg length, tadpole rearing density (Density) and air temperature (Temp) explain toadlet endurance (*EN*). Pond origin was set as a random effect term for all models (i.e. 1|Pond).

Model	**AICc**	**∆AICc**	**df**	**ω**
SVL + (1|Pond)	-30.60	0.00	4	0.97
SVL + Density + (1|Pond)	-22.20	8.40	5	0.02
SVL + Temp + (1|Pond)	-21.90	8.70	5	0.01
Body condition + (1|Pond)	-17.00	13.60	4	< 0.001
(1|Pond)	-16.50	14.10	3	< 0.001
Relative leg length + (1|Pond)	-16.4	14.2	4	< 0.001

Table 5.3 Summary of means for density-treated and wild-caught Fowler's toad toadlets (*Anaxyrus fowleri*); SVL at initial release in 2014 and at last capture, number of growth days in between initial and final SVL measures, total distance travelled, absolute linear displacement ($\text{Dist}_{\text{AtoB}}$), dispersal ratio ($\text{Dist}_{\text{AtoB}}$ /Total Distance) and distance moved per day (Dist_{day}), between males, females and juveniles. Only toadlets recaptured more than twice were used in these calculations. Variance is given as standard error.

	Density-treated			**Wild-caught**		
	Males	**Females**	**Juveniles**	**Males**	**Females**	**Juveniles**
Sample size (*N*)	6	5	2	29	29	3
Toadlet SVL (mm)	15.62 ± 0.60	16.04 ± 0.41	16.80 ± 0.70	31.54 ± 1.04	31.23 ± 1.05	29.53 ± 3.01
Final SVL (mm)	60.87 ± 1.81	59.34 ± 2.46	39.60 ± 0.00	63.09 ± 0.83	69.37 ± 0.95	46.23 ± 3.01
No. Growth Days	313.17 ± 13.18	209.20 ± 20.17	145.00 ± 13.50	276.48 ± 13.89	290.90 ± 9.49	308.67 ± 24.25
Total Distance (m)	1550.20 ± 448.24	933.44 ± 375.95	483.91 ± 293.94	5107.68 ± 968.01	5577.74 ± 1263.85	492.38 ± 228.82
$\text{Dist}_{\text{AtoB}}$ (m)	155.15 ± 45.50	321.22 ± 114.61	271.20 ± 225.20	1745.97 ± 384.65	1163.66 ± 133.57	206.99 ± 133.45
Dispersal Ratio	0.18 ± 0.07	0.36 ± 0.06	0.44 ± 0.20	0.45 ± 0.06	0.37 ± 0.05	0.48 ± 0.21
Dist_{day} (m)	4.87 ± 1.29	4.05 ± 1.15	3.22 ± 1.77	17.14 ± 2.93	17.79 ± 3.74	1.59 ± 0.79

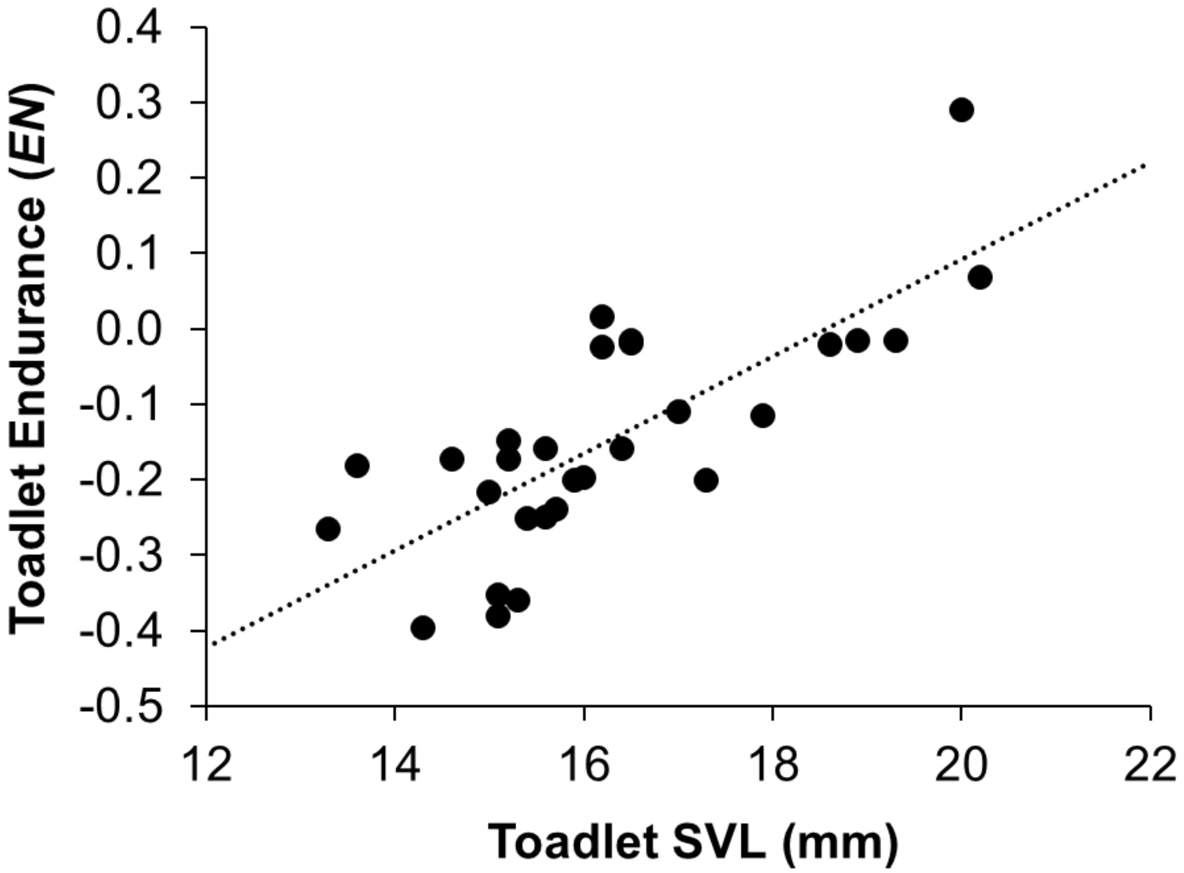

Fig. 5.1 Results of the locomotor performance tests in Fowler's toad (*Anaxyrus fowleri*) toadlets showing that endurance, *EN* (Log$_e$[Endurance+1]), is positively correlated with toadlet body snout-vent length, SVL (N=29).

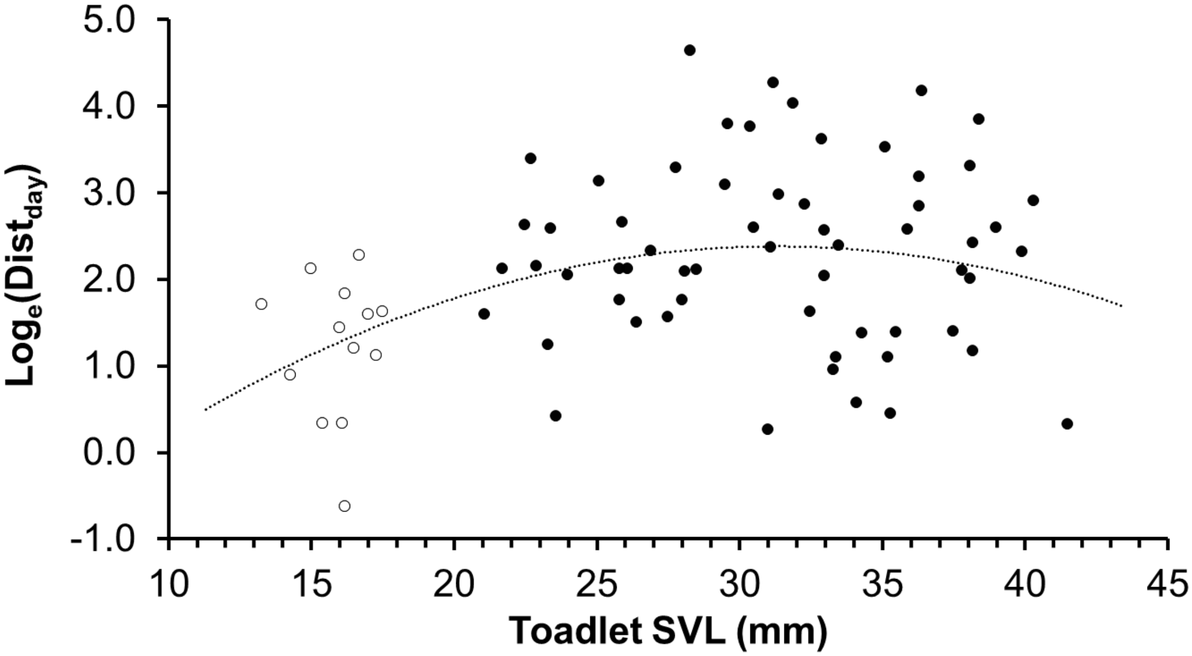

Fig. 5.2 Fowler's toad (*Anaxyrus fowleri*) movement rate, or Dist_{day}, from the combined dataset of density-treated [open points] and wild-caught toadlets [filled points], plotted against toadlet snout-vent length, SVL, (mm) showing a significant quadratic relationship for the full toadlet SVL range, where intermediate-sized toadlets displayed the greatest distance travelled per day. Density-treated and wild-caught toadlet data did not reveal significant relationships with SVL separately.

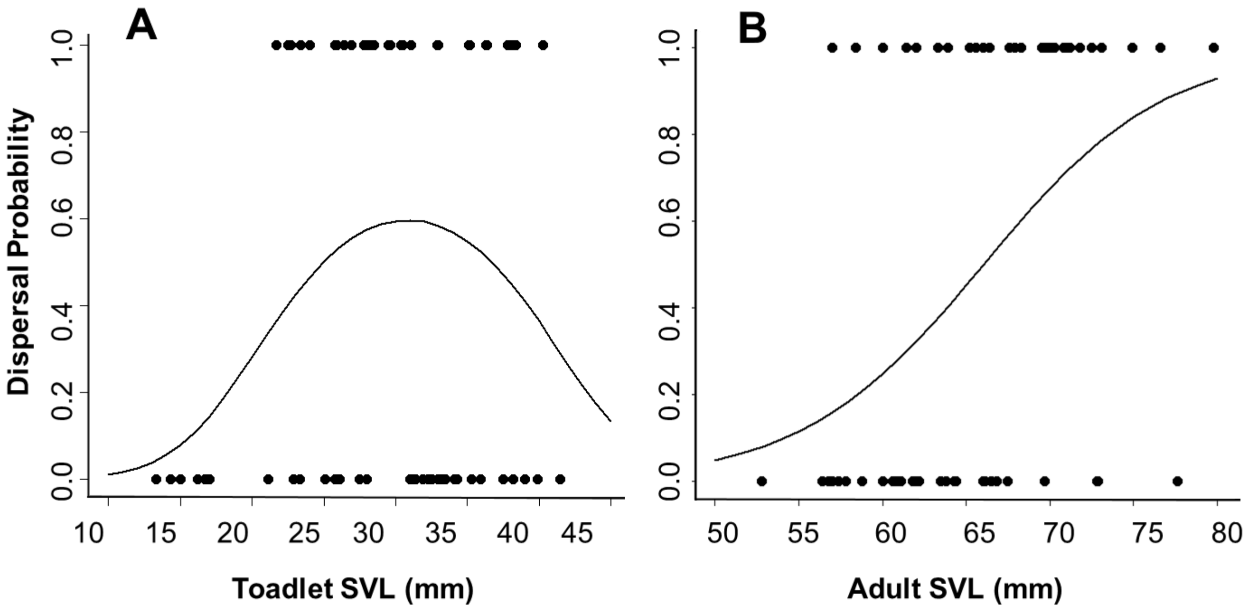

Fig. 5.3 Dispersal probability of Fowler's toads (*Anaxyrus fowleri*) from Long Point, Ontario, plotted against toadlet snout-vent length, SVL, (A) and probability of dispersal plotted against adult SVL (B), whereby a positive binary dispersal response (1) came from a $\text{Dist}_{\text{AtoB}} \geq 1000$ m, and a negative binary response (0) was given when $\text{Dist}_{\text{AtoB}} < 1000$ m, per individual. A significant quadratic relationship was observed between dispersal probability and toadlet SVL, where the intermediate sized toadlets are predicted to have the highest probability of dispersal (A). Using the same dataset, a positive logistic relationship was observed (B), where the largest adult toads showed the highest dispersal probability.

5.9 Appendix

5.9.1 Density-treated toadlets that were recaptured (*N*=13) between their release date in 2014 and their last recorded capture up to August 20 2016, where final SVL and the individuals sex were also recorded. Growth days refer to the number of days in between the release and final capture date, excluding the winter months (i.e. excluding the number of days from October 15 2014 to April 15 2015, and from October 15 2015 to April 15 2016). $\text{Dist}_{\text{AtoB}}$ refers to the distance between the release location and the location of the last recorded capture, whereas total distance refers to the cumulative sum of distances between each recapture location. Movement rate refers to the total distance moved per growth day, and Endurance (*EN*) measures were collected for *N* = 6 of these recaptures.

Rearing Density (tadpoles/L)	Sex	Toadlet SVL (mm)	Final SVL (mm)	Date of Release	Date of Last Capture	No. Recaps	No. Growth Days	*EN*	Total Distance (m)	AB Distance (m)	Movement Rate (m/day)	Dispersal Ratio
0.08	J	16.1	39.6	Jul 22 2014	Jun 5 2015	5	136	-	189.97	46.00	1.40	0.24
0.08	M	16.7	59.4	Aug 1 2014	Jul 21 2016	28	353	-0.11	3417.26	55.15	9.68	0.02
0.08	M	17	61.8	Jul 30 2014	May 21 2016	3	295	-0.11	1442.42	199.09	4.89	0.14
0.08	F	16.5	50.8	Aug 1 2014	Aug 13 2015	7	195	-	645.13	360.94	3.31	0.56
0.08	F	15.4	63.8	Aug 1 2014	Aug 6 2015	6	188	-0.25	262.08	56.64	1.39	0.22
0.08	F	17.3	62.4	Aug 1 2014	Jul 28 2015	9	179	-0.20	545.46	258.44	3.05	0.47

0.13	F	16	62.9	Aug 1 2014	Aug 13 2015	9	195	-0.20	821.67	195.01	4.21	0.24
0.13	M	16.2	57.8	Aug 1 2014	May 20 2016	5	291	0.02	1749.01	60.41	6.07	0.03
0.13	M	16.2	56.4	Aug 1 2014	May 20 2016	2	292	-0.02	155.48	72.01	0.53	0.46
0.25	J	17.5	48.2	Aug 4 2014	Jul 6 2015	4	154	-	777.85	496.40	5.05	0.64
0.25	F	15	56.8	Aug 15 2014	May 31 2016	3	289	-	2402.85	735.07	8.31	0.31
0.25	M	14.3	56.9	Aug 7 2014	Jul 27 2016	9	353	-	860.72	190.04	2.44	0.22
0.5	M	13.3	57.4	Aug 15 2014	May 24 2016	4	281	-0.27	1544.40	342.58	5.50	0.22

5.9.2 Summary of all 171 density-treated Fowler's toad (*Anaxyrus fowleri*) toadlets, showing the final number released from each density group in 2014 ($N_{released}$), their mean SVL at release, body condition, relative leg length, and the number of individuals recaptured more than once (N_{recap}). Variation is reported as standard error.

Rearing Density (tadpoles/L)	N_{released}	**SVL (mm)**	**Body Condition**	**Relative Leg Length**	N_{recap}
0.08	33	14.72 ± 0.34	0.02 ± 0.02	1.05 ± 0.01	6
0.13	24	14.02 ± 0.44	0.00 ± 0.03	1.01 ± 0.01	3
0.25	37	14.10 ± 0.47	-0.01 ± 0.02	1.03 ± 0.01	3
0.50	26	12.13 ± 0.30	-0.08 ± 0.02	1.04 ± 0.01	1
1.00	30	12.10 ± 0.30	0.02 ± 0.02	1.03 ± 0.04	-
1.67	21	10.72 ± 0.28	0.06 ± 0.02	1.07 ± 0.01	-

Preface to Chapter 6

In Chapter 6, I develop a simulation model of a metapopulation using empirical data from the Long Point Fowler's toad population, in order to predict its extinction risk. Therefore, the information provided by Chapter 5, as well as other publications focusing on this toad population were used to create equations and simulation parameters to predict the population's future under various conditions of habitat loss. This chapter attempts to fill a knowledge gap where empirical data informs dispersal models and provides important insights on outcomes of various habitat management scenarios.

Chapter 6: Dispersal rates and extinction risk in an endangered toad population: A Case Study on the Fowler's Toads (*Anaxyrus fowleri*) in Long Point, Ontario

Katharine T. Yagi and David M. Green. In preparation for submission to *Journal of Applied Ecology*.

6.1 Abstract

The risk of local extinction within a metapopulation is determined partially by the rate of dispersal between subpopulations. Rates of dispersal have been shown to be dependent on density and body condition, as mobile organisms may move in response to changes in habitat quality, density-dependent carry-over effects, and habitat disturbance by conservation practices. Amphibians, known for their susceptibility to human impacts, can have a complex dispersal process since they tend to show fidelity to breeding ponds and have slow movement rates. We used a well-studied, yet endangered population of Fowler's toads (*Anaxyrus fowleri*) in a case-study to predict its extinction risk via model simulations by integrating empirical information on its population dynamics, declining carrying capacity, and density- and condition-dependent dispersal under various scenarios of habitat loss. We predicted a low extinction probability when there are elevated dispersal rates of large bodied immigrants from adjacent subpopulations. The simulation model showed higher dispersal rates from populations with high abundance or fluctuating abundance, but emigrant body size was not different from the overall mean. We also found the highest extinction risk when carrying capacity fell to 8 toads, and when it fluctuated on a 7-year cycle between <17 and 100 toads. Our results give insight to how a population may

realistically respond to habitat management, and highlights the importance of long term population data. Further examination of the integration of density- and condition-dependent dispersal is needed to better inform predictive models.

6.2 Introduction

A metapopulation is a collection of subpopulations, whereby local extinctions are offset by dispersal-mediated rescue events (Hanski and Gilpin, 1991; Caley et al., 1996; Hastings and Botsford, 2006; Bell and Gonzalez, 2011). Dispersal itself is a multi-phase process often described by; 1) emigration, the departure of individuals from natal habitat, 2) transfer, which includes landscape exploration, and 3) immigration, the settlement of individuals into a new breeding habitat (Clobert et al., 2009; Bonte et al., 2012; Martin and Fahrig, 2016). However, measuring this process among natural populations is often challenging due to the difficulties in following individuals over large spatial- and time scales, as well as the high mortality risk associated with it (Bonte et al., 2012). Thus, many biologists tend to use models to estimate dispersal probabilities (i.e. dispersal rates), rather than use measures of realized, or actual dispersal, to predict the long-term persistence of populations (Travis et al., 1999; Poethke and Hovestadt, 2001; Ponchon et al., 2014).

It has been established that we should expect dispersal rates to increase when environmental conditions become unfavorable (Travis et al., 1999; Innocent et al., 2010) leading to the prediction that dispersal should increase with density (i.e. positive density-dependent dispersal; Matthysen, 2005; Clobert et al., 2009; De Meester and Bonte, 2010; Innocent et al., 2010; Altwegg et al., 2013). Dispersal can also decrease with density (i.e. negative density-dependent dispersal; Matthysen, 2005; Ims and Andreassen, 2005; Meylan et al., 2007), where dispersal is mediated by Allee effects or conspecific attraction (Roland et al., 2000; Fauvergue, 2013), the seasonality of resources (Matthysen, 2005; Baguette et al., 2011; Rodrigues and Johnstone 2014), or by density-dependent carry-over effects (Harrison et al., 2011; O'Connor et al., 2014; Betini et al., 2015). Seasonal resource availability can impact the condition and fecundity of females, which can translate into maternal effects on their offspring (Gill et al., 2001; Descamps et al., 2008; Warner et al., 2009), and can ultimately impact their dispersal behaviour (Ims and Hjermann 2001; Galliard and Clobert 2003). Therefore, another phenomenon, called condition-dependent dispersal, where the intrinsic capacity of an individual affects their survival and successful immigration, is another mechanism to consider when predicting long term metapopulation dynamics (Bowler and Benton, 2005; Bates et al., 2006; Clobert et al., 2009; Bonte and De La Peña, 2009; Harrison et al., 2011). Human impacts causing habitat loss, and habitat creation effect immediate, anthropogenic changes in habitat quality. However, it is not well understood how human intervention affects processes like dispersal and extinction risk when studying metapopulations.

Amphibian dispersal is complex due to common traits like pond fidelity (Reading et al., 1991; Smith and Green, 2006; Gamble et al., 2007), their dependency on habitat connectivity (Cushman, 2006; Semlitsch, 2008) and relatively slow terrestrial movement rates (Smith and Green, 2006). However, some amphibians have been documented to move large distances (Smith and Green, 2006; Semlitsch, 2008) allowing their distance-frequency curve to be leptokurtic, or "fat-tailed" (Nathan et al. 2008). Amphibians also show various degrees of density-dependence at different life stages (Altwegg, 2003), for example as larvae, density negatively correlates with survival, growth and metamorph size (Goater, 1994; Yagi and Green, 2016). Yet, it has been documented that juvenile frogs are attracted to areas inhabited by conspecifics (Pizzatto et al., 2016), and in many species, males aggregate to produce choruses, a cue to attract females during the breeding season (Bee, 2007; Swanson et al., 2007). These behaviours suggest there is a preference in adult amphibians, for living at higher densities (Courchamp et al., 2008). However, it has also been documented that adult body size can negatively correlate with abundance on an annual basis (Green and Middleton, 2010). Since body size is shown to be a strong indicator of performance and therefore dispersal ability (Cabrera-Guzman et al., 2013; Yagi and Green, Chapter 5), their dispersal probability may be dependent on population density from two perspectives; behavioural (i.e. social aggregations), and inherent (i.e. body condition or size).

We used a population of Fowler's toads (*Anaxyrus fowleri*) located in Long Point, Ontario, Canada, in a case-study to examine the impact of two dispersal-mediating mechanisms, density-dependent and condition-dependent dispersal, on the populations long-term viability under the pressure of gradual, yet extensive habitat loss (Greenberg and Green, 2013). This species, a beach-dune specialist classified as endangered in Canada (COSEWIC, 2010), exists in three remnant populations along the northern shore of Lake Erie in Ontario. The Long Point population has been monitored annually since 1989, where significant population decline became evident in 2002 due to the invasion of the Common reed (*Phragmites australis*) in prime amphibian breeding habitat (Greenberg and Green, 2013). A population viability analysis predicted >80% probability of extinction within 50 years under various scenarios of adult survival (COSEWIC, 2010). Thus, habitat management took place in 2012 where twelve small breeding ponds were created in a section of the invaded marshlands to aid in toad population recovery (Yagi and Green, 2016). Since invasive *Phragmites* is known for its adaptations to habitat change and overall resilience (Engloner, 2009), it is assumed that the maintenance of toad breeding habitat, by periodic removal of the *Phragmites*, will be required in the future.

Although the Long Point Fowler's toads are understood to be one population on a large-scale due to its isolation from the mainland (Fig. 6.1), we treated the site as a functional metapopulation for this study. From years of monitoring, movement of some individuals between three areas have been documented, which we considered to be the three-components of a metapopulation (Smith and Green, 2006; Fig. 6.1). Additionally, the mechanism of size-dependent (i.e. condition-dependent) dispersal was verified to be present in this population, where adult body size positively correlated with dispersal probability (Yagi and Green, Chapter 5).

First, we tested the hypothesis that if adult toads benefit from high density conditions during the breeding season, yet their body size decreases with abundance and is also positively correlated with dispersal, then we expect to find negative-density dependent dispersal in this toad population. Second, we hypothesized that if both density-dependent, and condition-dependent dispersal operate as mechanisms of the toads realized dispersal, then in a metapopulation where habitat loss is evident, declining populations will be sustained by elevated dispersal rates of large-bodied immigrants from adjacent subpopulations. Under conditions of habitat management, we expect to find elevated dispersal rates from the declining populations, but lower extinction probability as habitat availability will be systematically maintained and population size will be sustained by colonizers. Using a simulation model of a three-component metapopulation, where dispersal is implemented under two interrelated mechanisms, population density and body size, we recreated various scenarios of habitat loss based on empirical knowledge, and examined body size of emigrants, proportion of successful dispersers and the probability of local extinction.

6.3 Materials and Methods

***6.3.1 Population estimates*.**— Annual surveys consisted of nightly mark-recapture monitoring of the same study area, including the beaches and marsh habitat in Long Point, (between 42˚34′33″N–42˚35′3″N and 80˚ 22′15″–80˚28′24″W) for 41 nights encompassing the breeding season starting from 1 May to 10 June. From 1989 to 2011, adult toads were marked individually using toe-clipping techniques (Smith and Green, 2006), and from 2012 to 2016 toads were identified using digital photographs of their dorsal spots and the program *Foto Spottr* (Schoen et al., 2015). Upon each toad capture, UTM coordinates and snout-vent length (SVL) were recorded, along with the date and time.

All past population estimates were calculated using the program MARK (White and Burnham, 1999). Population estimates from 1989 to 2011 were reported in Greenberg and Green (2013), however only number of males were used in their calculations. We estimated total population size, N_{Total}, from 1989 to 1997 by assuming an even sex ratio (Green, 2013) and multiplying number of males by two (Table 6.1). N_{Total} (i.e. including males and females) was provided from 1998 to 2011 in Green (2013), and further analysis of the dataset from 2012 to 2016 allowed us to report the remaining estimates.

As reported in Greenberg and Green (2013), the carrying capacity (K) was initially 176 (± 59 SE) male toads in 1989, and declined at a rate of 8 toads per year from that point on, with the onset the *Phragmites* invasion being a major influence. Therefore, we estimated the initial carrying capacity for N_{Total} to be twice the size, at 352 toads, and to decrease by 16 toads per year from 1989 because Green (2013) showed the population sex ratio to be 1:1. To avoid carrying capacity falling below zero, we kept it at 16 toads in 2011 and to account for the addition of amphibian breeding ponds in 2012, we allowed carrying capacity to increase and remain constant at 32 toads from 2012 to 2016. This value was decided on as an assumption that the creation and annual maintenance of new breeding ponds would support a carrying capacity of 32 adult toads. Population density was calculated by dividing N_{Total} by K (i.e. N/K).

6.3.2 Calculating dispersal.— We assessed toad movement data from 2004 to 2016 by calculating the Euclidean distance between consecutive capture locations using UTM coordinates per individual toad via Excel (Microsoft, Office365). For each year, the total number of step distances greater than 1000 m was called 'dispersal frequency' (Smith and Green, 2006). We used the dispersal frequency data reported in Smith and Green (2006) to account for the years 1991 to 2003, therefore the full range of dispersal data included the years 1991 to 2016. Dispersal probability was calculated by using the 'dispersal frequency' value and dividing by the total number of recapture events (N_{recap}), and was called dispersal probability, or 'Pd'. This measure allowed us to estimate the level of long distance movement from the population in each year.

6.3.3 Analysis: dispersal probability.— All statistical analyses were carried out using R (v3.3.2, R Core Team, 2015). Due to the probability data being bound between 0 and 1, we used beta regressions to examine the effect of population density on dispersal probability. Pd was analyzed as the response variable to the predictor N_{Total}/K, using the betareg function from R's 'betareg' package.

6.3.4 Metapopulation model set-up.—To simulate the Long Point toad metapopulation system (Fig. 6.1), a three-component metapopulation was constructed mathematically in R (v3.3.2, R Core Team, 2015), and used in an overall analysis of extinction probability. Each subpopulation was designed to grow over a maximum of 100 years by use of three "for loops", nested within a "for loop" set to re-run the simulation over 1000 iterations. Population growth was defined by the equation for logistic growth:

[1] $N_{t+1}= N_t+ r_m*N_t(1-N_t/K_t))*e_t$

where N_t is the number of individuals in the population at time 't', with the stochastic variable e_t added. For all three subpopulations and all scenarios, N_1 was set at 100. 'r_m' is the mean annual population growth rate and was set at a constant. We calculated 'r_m' by first determining 'r_t', the annual growth rate, for each year in our dataset from 2012 to 2016, using the equation $r_t = \mathrm{Log}_e(N_{t+1}/N_t)$, and '$r_m$' was calculated by taking the mean these r_t values. We decided upon using the years after the amphibian ponds were installed, to better capture r_t values when population dynamics were relatively stable. K_t is the carrying capacity at time step 't'. Environmental stochasticity, or process error 'e', was included in the population growth model, using the following equation:

[2] $e = \exp*(x*sE-sE^2/2)$

Where x is a random value drawn from a normal distribution with a mean of zero and variance of 0.5, and sE is the standard deviation of the process error. For the purposes of this simulation, we kept sE constant at 0.02 (Table 6.1).

We included the density-dependent dispersal component in the model, by including an equation for probability of dispersal, 'Pd' as a function of population density 'N_{Total}/K'. We used the coefficients obtained from a linear model analysis, using R's 'lm' function, of the raw population data to form the equation. 'Pd', was set with an upper bound of 0.4 (i.e. if density were to fall below 0.1 or above 3) using a nested 'ifelse' command, to ensure realistic dispersal probabilities were maintained even if population densities were to fluctuate drastically in a simulation.

Mean adult body size, 'B' was included in the model as a variable dependent on population abundance. We used the results published in Green and Middleton (2013), of the relationship between population abundance and mean adult body size, and came up with the following equation:

[3] $B_t = -2.68*Log_e(N_t)+71.00$

We set initial body size, B_1, to be 58 mm to start the simulation off, which was the overall average adult toad size calculated from 1989 to 2016. Although mean size of females was larger than males (Green and Middleton, 2013), no difference in dispersal was found between sexes (Smith and Green, 2006) and therefore we did not include a sex-dependent dispersal function in the model.

Body size was also incorporated in this model as a mechanism of dispersal. We used 'B' to simulate the effect of individuals with poor body condition, or small size, being less likely to disperse due to their lower performance levels (Yagi and Green, Chapter 5). This was accomplished by using random samples of distance values obtained from two different Cauchy-distributions; one with a location of 0 and scale of 30 to represent a low probability of an individual moving long distances, and the other with a location of 0 and scale of 100 to represent relatively higher probability of an individual moving longer distances. These parameters were selected through trial and error and comparing various Cauchy distance curves to our empirical data. These dispersal distances, 'Dd,' were implemented in the for loop using an 'ifelse' command, where if the mean body size of the population fell below 58 mm, then the dispersal distance value is sampled from the short-distance Cauchy distribution, otherwise the dispersal distance value is sampled from the long-distance distribution.

The number of dispersers, 'Dn', was determined in two steps. First, the probability of dispersal of the current time step was multiplied by the total population size to acquire a tentative number of dispersers. Next, the number of successful dispersers was set to be dependent on the dispersal distance values sampled from either Cauchy distribution. We used a threshold distance of 1000 m, whereby values greater than this were considered a realized dispersal event and allowed the tentative number of dispersers to become actual dispersers. If the dispersal distance value was less than 1000 m, the number of dispersers was returned to zero.

At the end of each 'for loop', the total population size was adjusted by the number of emigrants leaving, and number of immigrants arriving from the two adjacent populations, using the acronym 'Em'. We set a constant dispersal mortality rate of 10% per dispersal group, making a total of 20% (Bonte et al., 2012). Therefore 40% of the dispersers leaving population A will enter population B, and the remaining 40% will enter population C, given their dispersal distances were large enough. All three populations were given the same set of parameters and equations, aside from the conditions set for carrying capacity for each simulation scenario.

6.3.5 Carrying capacity scenarios.—We ran a total of thirteen scenarios to assess the effect of gradual habitat loss on extinction probability, emigrant body size, and dispersal rates in all three sub-populations, which we called 'A', 'B' and 'C'. We considered population A to represent our case-study toad subpopulation, and the others being the two peripheral subpopulations as shown in Fig. 6.1. The first scenario was the control, which had carrying capacity remaining constant at 352 toads in all three subpopulations. Scenarios 2, 3 and 4 involved habitat loss in one (i.e. population A), two (i.e. populations A and B), and three subpopulations (i.e. A, B and C) respectively, with K decreasing at a rate of 16 toads per year, and reaching a minimum of 8 toads, thereafter remaining constant. Scenarios 5, 6 and 7 were like the previous three, where K was set to decrease at a rate of 16 toads per year, but here only attained a minimum of 16 toads and was kept constant thereafter. Scenarios 8, 9 and 10 had a fluctuating K in one, two and three sub populations, respectively. Here, K was set to decrease from 352 toads at a rate of 16 toads per year, and when a value less than 17 was reached, it was reset to 100. This pattern was repeated for the duration of the 100-year simulation for each iteration, to represent continuous habitat management on a 7-year cycle where the agent of habitat loss (i.e. invasive *Phragmites australis*) grows perpetually. Finally, scenario's 11, 12 and 13 were similar to the previous three in that K was set to fluctuate again, however when K reached a value less than 17 toads, it was reset to 200. This simulation was to reflect continuous habitat management on a longer 14-year cycle.

To assess the mean size of emigrants we repeated each simulation 10 times, and collected body size (B), and associated dispersal distance (Dd) data from each population from one iteration per replicate. Only body size values associated with distances larger than 1000 m were used to calculated the means per subpopulation, per scenario. The proportion of successful dispersers was calculated by dividing Em by N_{Total} for each year in a simulation, and taking the mean. This was repeated for 10 iterations to get an overall mean per population per scenario. The total number of extinction events was tallied over the 1000 iterations, using a final population size threshold where if $N_{100} < 5$ individuals, for any of the three subpopulations, it was counted as locally extinct. Probability of an extinction event to occur within the metapopulation was calculated by dividing the total number of extinction events by 3000. We took the mean of ten extinction values after running the simulation ten times.

6.4 Results

***6.4.1 Population estimates*.—** N_{Total} ranged from a maximum of 860 toads in 1991 to a minimum of 27 toads in 2011. From 2012 to 2016, N_{Total} fluctuated around a mean of 46 toads (± 7 SE), with an r_m of 0.11 (± 0.3 SE). Population density (N_{Total}/K) ranged from a maximum of 3.04 in 2002, to a minimum of 0.23 in 1989, and an overall mean of 1.61 (± 0.17 SE). Prior to 2002, mean population density was 1.26 (± 0.30 SE), and after 2002 was higher at 1.83 (± 0.17 SE).

***6.4.2 Dispersal probability*.—** The overall mean Pd was 18% (± 4 SE), where the lowest Pd was 0% in 2006 with a movement distances ranging from 1 to 976 m, and the highest Pd was in 2001 at 85%, with distances ranging from 1 – 5,075 m. Interestingly the second highest Pd was in 2002 at 74%, when population decline became evident, with distances ranging from 1 – 28,662 m. The mean Pd prior to 2001 was similar to the overall mean, 19% (± 3 SE), and after 2002 it was much lower at 8% (± 5 SE; Table 6.2). Results of the beta regression analysis revealed that population density (N_{Total}/K) was a significant quadratic predictor for dispersal probability (Pd) with the mean model (N_{Total}/K: Z = -2.95, $P = 0.003$; N_{Total}/K^2: Z = 2.98, $P = 0.003$; Fig. 6.3). The linear equation obtained via our empirical data, and used in the simulation to estimate Pd was; $Pd_t = 0.10*(N_t/K_t)^2 - 0.36*(N_t/K_t) + 0.42$.

***6.4.3 Simulations*.—** The smallest mean body size of emigrants was observed in scenario 1, where all three populations had mean toad size of ca. 55.5 mm ± 0.2 SE. However, the mean size of toads in the population was similar, at 55.8 mm ± 0.1 SE. The largest sized emigrants came from scenario 9, at 64.3 mm ± 0.4 SE in population B, which was only slightly larger than the overall mean body size from the population 63.1 mm ± 0.3 SE (Table 6.3).

The lowest percentage of successful dispersers came from scenario 1, where K was kept constant in all three populations, with an overall mean of 0.26% ± 0.1 SE. The highest percentage of dispersers came from scenario 9, defined by a fluctuating carrying capacity on a 7-year cycle in two populations, where population A had a mean of 2.1% ± 0.5 SE, followed by population B with a mean of 1.26% ± 0.3 SE. Interestingly, the percentage of successful dispersers proved to be high for all populations with a fluctuating carrying capacity, compared to those with a stable one (Table 6.3). A similar pattern was observed under scenarios 2 to 7, however the dispersal percentages were not as high (Table 6.3).

The probability of extinction was the lowest, at 0%, for scenarios 1, 5, 6 and 7, whereas the highest probability came from scenario 4, at 83.1% ± 1.1 SE, where all three sub-populations had decreasing K down to 8 toads. The second highest extinction probability came from scenario 10, at 78.2% ± 0.5 SE, where all three sub-populations had fluctuating K on a 7-year cycle (Table 6.3; Fig. 6.4). Scenarios 11, 12 and 13, where K fluctuated on a 14-year cycle showed a much lower extinction probability compared to the previous three scenarios, with K fluctuating on a 7-year cycle (Table 6.3).

6.5 Discussion

Dispersal probability was surprising in that the relationship with population density fit a quadratic, rather than our predicted negative relationship. We observed intermediate densities yielding the lowest dispersal probabilities, and observed higher probabilities when density was either very high or very low. The two highest recorded densities appeared to coincide with the onset of significant population decline in 2002 (Greenberg and Green 2013), which might explain the high degree of movement observed in these two years. However, this overall relationship might be explained by the competing effects of amphibian behaviour and density-dependence, where adults likely favor high densities during breeding seasons thus are prone to dispersal behaviour when density is too low (Roland et al., 2000; Fauvergue, 2013), and conversely when densities are too high dispersal is favored due to classic competition-driven behaviours (Lambin et al., 2001; Matthysen, 2005).

Our prediction that we should observe elevated dispersal rates of large bodied immigrants to maintain populations experiencing habitat loss was partially confirmed in our results. We did detect some rescue by colonizers in our scenarios where carrying capacity was set to decrease. When only one population had declining carrying capacity, the probability of extinction ranged from 0%, when carrying capacity was reduced to 16 toads, to 13.8%, when carrying capacity fluctuated between 100 and less than 17 toads. On the other end, when carrying capacity was set to decline in all populations, rescue by colonizers was less likely and therefore we observed the highest extinction probabilities. Extinction probability was always 0% when carrying capacity was kept high enough, which in our case was a minimum of 16 toads, and coincided with our scenarios 1, 5, 6 and 7. When carrying capacity was as low as 8 toads, we observed the largest extinction probabilities. Unlike our original prediction, the mean body size of emigrants was not necessarily larger than their population means. We observed dispersers of large sizes emerging from populations of low abundance, which coincides with the negative body size vs. abundance relationship used in the model.

Considering the perpetual growth of the invasive reeds and subsequent loss of breeding habitat, the implementation of continuous habitat management (e.g. *Phragmites* removal or pond creation) is a more realistic approach to take from a conservation perspective, on the future of the Long Point toad population. However, our simulation results suggest that creating such habitat disturbances may increase extinction risk if management occurs too frequently (i.e. every seven years or less). This may occur if habitat creation does not increase carrying capacity to a large enough value, thereby creating the need for frequent, continuous habitat management efforts. If habitat creation allows for a substantial increase in carrying capacity, the population would have time to rebound and respond positively to an increase in available habitat.

It is important to consider density and condition dependent dispersal as integrated processes of population dynamics (Harrison et al., 2011; Betini et al., 2015), and should be examined together when studying dispersal at the individual level (Travis et al., 2012; Aben et al., 2016). Our model simulations show how such factors may be integrated in real populations, however this may be a species-specific result and further research is needed to verify the strength of each process to determine whether the combination of density- and condition- dependence has an additive or opposing effect on dispersal.

Long-term population data, although difficult to acquire, is invaluable as it informs us of its past dynamics and allows us to formulate more accurate predictions of its future state. We were able to predict both positive and negative impacts of conservation efforts because of this, and how habitat management can be detrimental in cases where habitat disturbance plays large role in the population’s stability. Since amphibians are understood to be in decline on a global scale, our study also provides some insight on their metapopulation dynamics in a realistic situation of habitat loss and management. Further investigation of amphibian responses to habitat management, particularly in terms of dispersal, would be valuable to advance the accuracy of predictive models.

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6.8 Tables and Figures

Table 6.1 Definitions of parameters used in the simulation.

Parameter	Definition
$\mathbf{N_{Total}}$	Population size
r	Maximum population growth rate
K	Carrying capacity
$\mathbf{N_{Total}/K}$	Population density
e	Environmental stochasticity, or process error
sE	Standard deviation of the process error
Em	Number of emigrants entering or immigrants leaving the population
B	Mean body size (mm) of individuals in the population
Dd	Dispersal distance (m) of individuals, depends on **B**
Pd	Probability of dispersal, depends on $\mathbf{N_{Total}/K}$ and **B**
Dn	Number of potential dispersers, depends on **B**

Table 6.2 Population size estimates (N_{Total}), estimated carrying capacity (K), estimated population density (N_{Total}/K), mean population SVL (mm), and dispersal probabilities from 1989 to 2016. Carrying capacity (K) was estimated using values published in Greenberg and Green (2013), decreasing K by 16 toads per year until a minimum of 16 toads was reached in 2011. From 2012 to 2016, K was kept constant at 32 toads to coincide with the creation of amphibian breeding ponds.

Year	**N_{Total}**	**± SE**	**K**	**Density (N_{Total}/K)**	**Dispersal Probability (%)**
1989	82	4.2	352	0.23	-
1990	312	40.0	336	0.93	-
1991	860	29.0	320	2.69	18.4
1992	614	16.0	304	2.02	29.3
1993	788	22.7	288	2.74	11.1
1994	186	4.1	272	0.68	29.0
1995	86	2.6	256	0.34	2.5
1996	78	1.6	240	0.33	18.8
1997	62	0.6	224	0.28	33.3
1998	347	6.4	208	1.67	13.0
1999	146	8.5	192	0.76	9.1
2000	192	33.0	176	1.09	26.5
2001	93	8.0	160	0.58	85.4
2002	438	62.5	144	3.04	73.7
2003	261	34.5	128	2.04	22.2

2004	347	10.9	112	3.10	5.7
2005	170	8.7	96	1.77	11.3
2006	119	13.6	80	1.49	0.0
2007	96	3.7	64	1.50	4.3
2008	93	10.0	48	1.94	14.0
2009	65	3.3	32	2.03	5.6
2010	46	3.9	16	2.88	8.5
2011	27	0.0	16	1.69	6.0
2012	65	0.5	32	2.03	3.8
2013	30	1.0	32	0.94	18.0
2014	33	0.5	32	1.03	2.6
2015	57	0.5	32	1.78	4.3
2016	46	0.5	32	1.44	6.4

Table 6.3 Results from the simulations of ten different scenarios of habitat loss (i.e. decreasing K) in a metapopulation composed of three subpopulations (A, B and C), showing mean emigrant body size (mm), mean percentage of dispersers from the population and mean extinction probability.

No.	K Scenario	Emigrant Body size (mm) ± SE			% Dispersal ± SE			% Extinction ± SE
		A	**B**	**C**	**A**	**B**	**C**	
1	Constant in all populations	55.60 ± 0.2	55.51 ± 0.2	55.47 ± 0.2	0.26 ± 0.1	0.25 ± 0.1	0.28 ± 0.1	0.0 ± 0.0
2	Decreasing to 8 in A	64.10 ± 0.5	55.98 ± 0.3	55.95 ± 0.3	1.00 ± 0.3	0.61 ± 0.2	0.58 ± 0.2	5.77 ± 1.8
3	Decreasing to 8 in A & B	64.78 ± 0.4	64.39 ± 0.6	57.18 ± 0.5	0.75 ± 0.2	0.96 ± 0.2	0.65 ± 0.2	23.68 ± 3.3
4	Decreasing to 8 in A, B & C	63.06 ± 0.8	63.75 ± 0.8	63.84 ± 0.6	0.44 ± 0.2	0.83 ± 0.3	0.45 ± 0.2	83.14 ± 1.1
5	Decreasing to 16 in A	61.86 ± 0.5	56.54 ± 0.5	56.01 ± 0.4	0.79 ± 0.2	0.45 ± 0.2	0.31 ± 0.1	0.0 ± 0.0
6	Decreasing to 16 in A & B	62.53 ± 0.4	63.06 ± 0.3	56.29 ± 0.6	0.65 ± 0.2	0.90 ± 0.2	0.44 ± 0.2	0.0 ± 0.0
7	Decreasing to 16 in A, B & C	63.12 ± 0.4	62.77 ± 0.7	62.84 ± 0.6	1.29 ± 0.3	0.75 ± 0.2	0.97 ± 0.2	0.0 ± 0.0
8	Fluctuates in A, 7-year cycle	61.86 ± 1.0	56.23 ± 0.6	55.87 ± 0.3	1.40 ± 0.3	0.61 ± 0.2	0.20 ± 0.1	13.81 ± 1.0
9	Fluctuates in A & B, 7-year cycle	63.72 ± 0.5	64.31 ± 0.4	55.47 ± 0.1	2.09 ± 0.5	1.26 ± 0.3	0.50 ± 0.2	31.04 ± 2.2
10	Fluctuates in A, B & C, 7-year cycle	63.53 ± 0.5	63.56 ± 0.7	62.49 ± 0.9	1.35 ± 0.3	0.90 ± 0.3	1.15 ± 0.3	78.23 ± 0.5
11	Fluctuates in A, 14-year cycle	60.86 ± 0.5	55.78 ± 0.4	55.77 ± 0.4	1.11 ± 0.1	0.38 ± 0.1	0.39 ± 0.1	1.22 ± 0.1
12	Fluctuates in A & B, 14-year cycle	61.76 ± 0.5	61.40 ± 0.4	56.53 ± 0.6	1.04 ± 0.1	1.04 ± 0.1	0.42 ± 0.1	1.94 ± 0.2
13	Fluctuates in A, B & C, 14-year cycle	61.90 ± 0.3	61.68 ± 0.2	60.96 ± 0.4	1.12 ± 0.1	1.13 ± 0.1	1.12 ± 0.1	0.08 ± 0.0

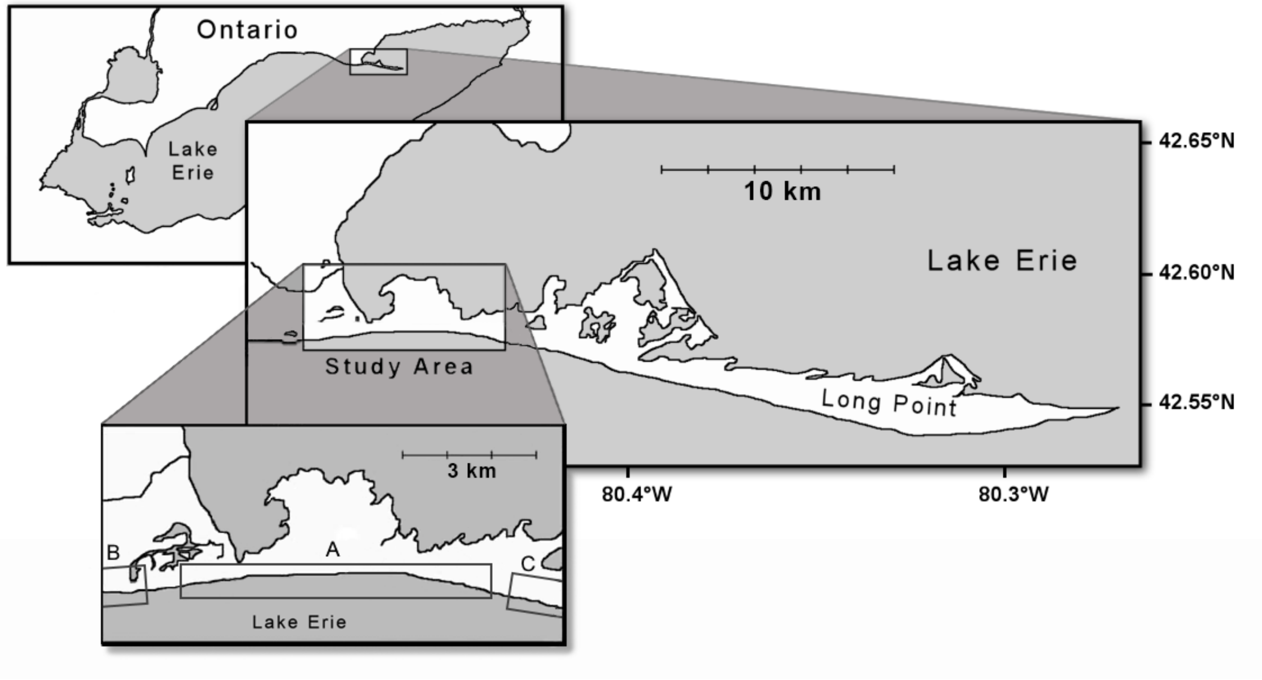

Fig. 6.1 A map of the study site in Long Point, Ontario Canada. Three sub-populations of Fowler’s toads (*Anaxyrus fowleri*) are outlined in gray rectangles.

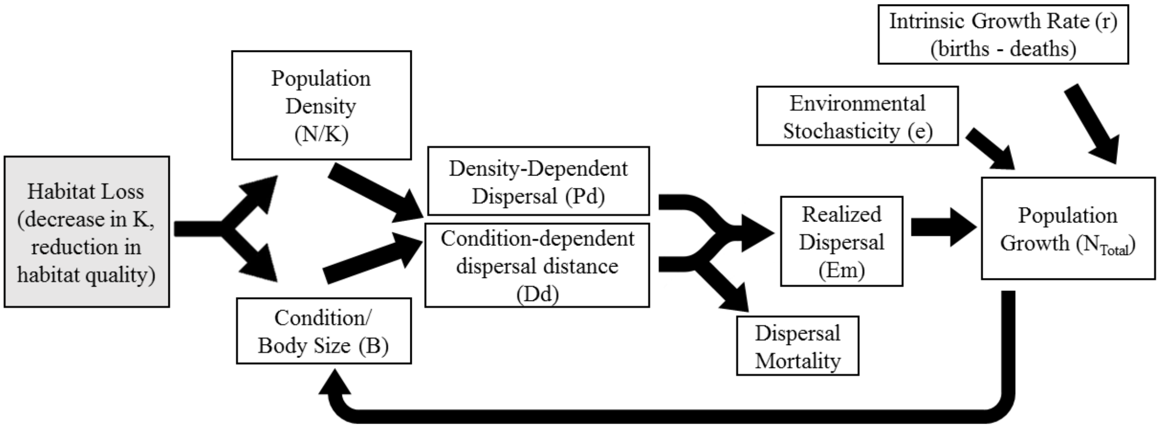

Fig. 6.2 A conceptual diagram depicting the function of the various components of the simulation model. Ultimately, habitat loss leads to changes in population density and individual body condition, both of which can impact realized dispersal.

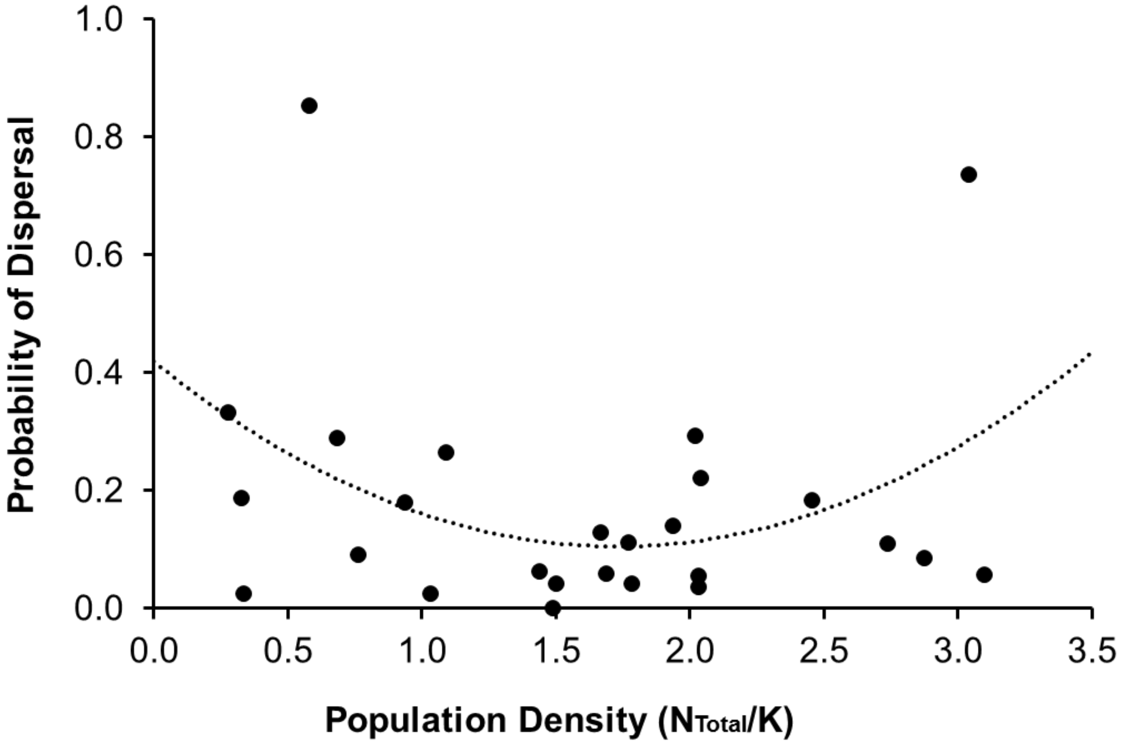

Fig. 6.3 Results from the empirical population data, the population density and dispersal probability relationship fits a quadratic curve, where high dispersal probability occurs under low and high population densities.

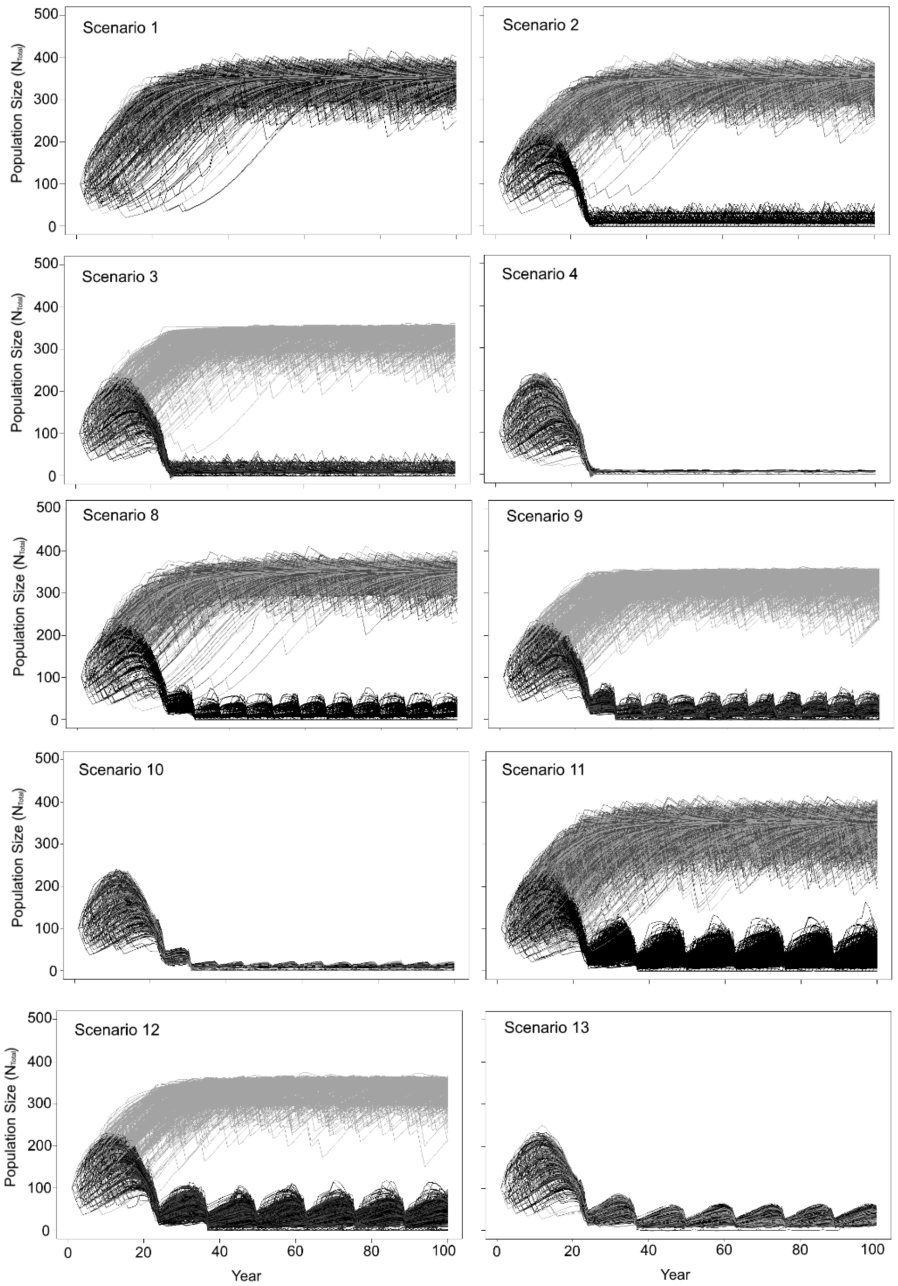

Fig. 6.4 Simulations of total population size (N_{Total}) over 100 years and 1000 iterations, from ten out of thirteen tested scenarios. Scenarios 5, 6 and 7 were visually similar to 2, 3 and 4, and were therefore not displayed. Population A is represented in black, population B is dark gray, and population C is in light gray.

6.9 Appendix

6.9.1 The R script used to run the population simulations. Each simulation required an adjustment of the carrying capacity parameter (K) for each sub population. K was manipulated under 13 scenarios of habitat loss.

```
#population extinction
yearmax<- 100
runmax<- 1000
year<- 1:yearmax
extinct<- 0 #extinction counter
Em1.count<- 0
Em2.count<- 0
Em3.count<- 0
N1<- numeric(100)
N2<- numeric(100)
N3<- numeric(100)
r1<- numeric(100) #pop growth rate
r2<- numeric(100)
r3<- numeric(100)
B1<- numeric(100)
B2<- numeric(100)
B3<- numeric(100)
Dn1<- numeric(100)
Dn2<- numeric(100)
Dn3<- numeric(100)
Dd1<- numeric(100)
Dd2<- numeric(100)
Dd3<- numeric(100)
Em1<- numeric(100)
Em2<- numeric(100)
Em3<- numeric(100)
curve1<- abs(rcauchy(200,0,30))#dispersal curve for low body condition toads
curve2<- abs(rcauchy(200,0,100))#dispersal curve for high body condition toads
Pd1<- numeric(100)
Pd2<- numeric(100)
Pd3<- numeric(100)
K1<- numeric(100)
K2<- numeric(100)
K3<- numeric(100)
N.K1<- numeric(100)
N.K2<- numeric(100)
N.K3<- numeric(100)
Em1.N1<- numeric(100)
```

```
Em2.N2<- numeric(100)
Em3.N3<- numeric(100)
for (i in 1:runmax){
  N1[1]<- 100
  N2[1]<- 100
  N3[1]<- 100
  sE<- 0.02 #standard deviation of process error
  e1<- exp(rnorm(100,0,0.5)*sE-sE^2/2) #process error (environmental stochasticity)
  e2<- exp(rnorm(100,0,0.5)*sE-sE^2/2)
  e3<- exp(rnorm(100,0,0.5)*sE-sE^2/2)
  K1<- 352 #carrying capacity starts at 350 toads
  K2<- 352
  K3<- 352
  N.K1[1]<- N1[1]/K1
  N.K2[1]<- N2[1]/K2
  N.K3[1]<- N3[1]/K3
  rmax<- 0.11
  #r1[1]<- 0.11
  #r2[1]<- 0.1066
  #r3[1]<- 0.1066
  B1[1]<- 58
  B2[1]<- 58
  B3[1]<- 58
  Pd1[1]<- 0.01 #initial dispersal proportion
  Pd2[1]<- 0.01
  Pd3[1]<- 0.01
  Dn1[1]<- 10
  Dn2[1]<- 10
  Dn3[1]<- 10
  for (t in 2:yearmax){
    K1[t]<- ifelse(K1[t-1]<17,8,K1[t-1]-16) #carrying capacity decreases
    N.K1[t]<- abs(N1[t-1]/K1[t-1])
    N1[t]<- N1[t-1]+rmax*N1[t-1]*(1-N.K1[t-1])*e1[t]
    N1[t]<- ifelse(N1[t]<5,0,N1[t])
    B1[t]<- ifelse(N1[t]<10,65,-2.68*log(N1[t])+71)
    Pd1[t]<- ifelse(N.K1[t]>3,0.4, ifelse(N.K1[t]<0.1,0.4,0.10*(N.K1[t]^2)-0.36*(N.K1[t])+0.42))
    Dn1[t]<- as.integer(Pd1[t]*N1[t]) #number of dispersers rounded to integer
    Dn1[t]<- ifelse(Dn1[t]<1,1,Dn1[t])#keeps number of dispersers above zero
    Dd1[t]<- ifelse(B1[t]<58, sample(curve1,1,replace=T), sample(curve2,1,replace=T))
    Em1[t]<- ifelse(Dd1[t]<1000,0,Dn1[t])
    Em1.N1[t]<- abs(Em1[t]/N1[t])
    N1[t]<- N1[t]-Em1[t]+0.4*Em2[t]+0.4*Em3[t]# mortality is 0.4 per Em instead of 0.5, 20% mortality
  }
  for (t in 2:yearmax){
    K2[t]<- ifelse(K2[t-1]<17,8,K2[t-1]-16) #carrying capacity decreases
    N.K2[t]<- abs(N2[t-1]/K2[t-1])
    N2[t]<- N2[t-1]+rmax*N2[t-1]*(1-N.K2[t-1])*e2[t]
```

```
    N2[t]<- ifelse(N2[t-1]<5,0,N2[t])
    B2[t]<- ifelse(N2[t]<10,65,-2.68*log(N2[t])+71
    Pd2[t]<- ifelse(N.K2[t]>3,0.4, ifelse(N.K2[t]<0.1,0.4,0.10*(N.K2[t]^2)-0.36*(N.K2[t])+0.42))
    Dn2[t]<- as.integer(Pd2[t]*N2[t]) #number of dispersers rounded to integer
    Dn2[t]<- ifelse(Dn2[t]<1,1,Dn2[t])
    Dd2[t]<- ifelse(B2[t]<58, sample(curve1,1, replace=T), sample(curve2,1,replace=T))
    Em2[t]<- ifelse(Dd2[t]<1000,0,Dn2[t])
    Em2.N2[t]<- abs(Em2[t]/N2[t])
    N2[t]<- N2[t]-Em2[t]+0.4*Em3[t]+0.4*Em1[t]
  }
  for (t in 2:yearmax){
    K3[t]<- ifelse(K3[t-1]<17,8,K3[t-1]-16) #carrying capacity decreases
    N.K3[t]<- abs(N3[t-1]/K3[t-1])
    N3[t]<- N3[t-1]+rmax*N3[t-1]*(1-N.K3[t-1])*e3[t]
    N3[t]<- ifelse(N3[t]<5,0,N3[t])
    B3[t]<- ifelse(N3[t]<10,65,-2.68*log(N3[t])+71)
    Pd3[t]<- ifelse(N.K3[t]>3,0.4, ifelse(N.K3[t]<0.1,0.4,0.10*(N.K3[t]^2)-0.36*(N.K3[t])+0.42))
    Dn3[t]<- as.integer(Pd3[t]*N3[t]) #number of dispersers rounded to integer
    Dn3[t]<- ifelse(Dn3[t]<1,1,Dn3[t])
    Dd3[t]<- ifelse (B3[t]<58, sample(curve1,1,replace=T), sample(curve2,1,replace=T))
    Em3[t]<- ifelse(Dd3[t]<1000,0,Dn3[t])
    Em3.N3[t]<- abs(Em3[t]/N3[t])
    N3[t]<- N3[t]-Em3[t]+0.4*Em1[t]+0.4*Em2[t]
  }
  #extinction counter
  extinct<- extinct + ifelse(N1[yearmax]<5,1,0)
  extinct<- extinct + ifelse(N2[yearmax]<5,1,0)
  extinct<- extinct + ifelse(N3[yearmax]<5,1,0)
  Em1.count<- Em1.count + sum(Em1[1:100])/sum(N1[1:100])
  Em2.count<- Em2.count + sum(Em2[1:100])/sum(N2[1:100])
  Em3.count<- Em3.count + sum(Em3[1:100])/sum(N3[1:100])
  lines(N1, typ="l", col="black")
  lines(N2, typ="l", col="gray27")
  lines(N3, typ="l", col="gray64")
}
extinct #will be inflated by ~3x
Exprob<- extinct/runmax
Exprob/3
#****---
Em1.count/1000
Em2.count/1000
Em3.count/1000
#base plot for drawing population size trajectories – run this code after a trial simulation, before the real simulation.
plot(N1, typ="l", col="white",xlab="Year",ylim=c(0,500),ylab="Population Size (N)",main = "Scenario 4")
```

Chapter 7: General Conclusions and Implications

Habitat loss continues to be the source of concern for many declining populations, especially for those that are small, isolated or otherwise have low dispersal rates. Therefore, ecologists must continue to compile information on their long-term dynamics. This type of data is not easy to amass due to the need for immediate action in many cases, but it provides invaluable information allowing us to make more accurate predictions and informed management decisions.

Although predictive dispersal models have been advancing in complexity to address the need for more realistic predictions of population response to habitat change (Hovestadt et al., 2010; Travis et al., 2012; Aben et al., 2016), it was always a point of concern that more empirical data was needed verify model results. This thesis aimed to provide empirical data to fill this knowledge gap, and was accomplished primarily by working with a well-studied amphibian population with nearly three decades' worth of long-term population data. With the advancement of our mark-recapture techniques, I was able to gather detailed information on individuals across all life stages. Coupled with the fact that the population was experiencing a decline due to habitat loss, the results were made even more valuable.

My results outline the complexity of density-dependence effects at multiple life stages in a pond breeding amphibian, which has been done previously to some extent (Altwegg 2003; Chelgren et al., 2006; Crespi and Warne, 2013; Tarvin et al., 2015). Chapter 2 formed the basis of the density-dependent theme, where I applied density treatments to the tadpole stage of these a population of Fowler's toads (*Anaxyrus fowleri*). By comparing two methods, one being the traditional density-by-abundance and the other being the alternate density-by-volume, I showed that the effect had similar trends with growth rate, survival and timing to metamorphosis. However, differences in size at metamorphosis revealed that there may be a benefit of large tadpole group size at a low density.

Chapter 3 and 4 focused on quantifying behavioural plasticity as a result of tadpole density. Chapter 3 compared the outcome of tadpole density conditions between the tadpole and toadlet (i.e. early post-metamorphosis) stages to detect carry-over effects. The results were unique due to the complex relationship revealed between density and activity level in tadpoles, and that toadlets behaved differently suggesting the presence of carry-over effects. To further investigate the density-dependent relationship with tadpole activity, in Chapter 4 I conducted a multifactorial experiment where temperature and density were set as controlled three-level treatments. My results pointed to a three-way interaction among density, temperature and body-size, where tadpoles put under high temperature and densities together revealed a size-dependent activity response.

In Chapter 5 I shifted my focus to the toads' terrestrial life stage and investigated their movement ability with the knowledge of their larval and post-metamorph growth experience. My results brought attention to the importance of body size on dispersal ability, in terms of both their endurance level and actual displacement in the field. Finally, Chapter 6 was where I applied the empirical data gathered from this toad population to a model that used dispersal in a metapopulation context to predict its long-term persistence. Since the population in question is known to be in decline due to a decrease in carrying capacity, I developed various scenarios of habitat loss and habitat management and compared the various outcomes. The results of the simulations revealed that habitat management must occur more than every 7 years, to allow the population enough time to respond to an increase in habitat availability, and that dispersal rates increase when carrying capacity fluctuates.

While dispersal is a major topic of discussion and study in the literature, it is still uncommon to find empirical data assessing the mechanism of realized dispersal in individuals, although it has become a popular point of discussion (Aben et al., 2016). This is because collecting detailed information on individuals across large distances and long periods of time is especially challenging (Marra et al., 2015). With the advancement of technology and refinement of techniques for recapturing individuals (Kays et al., 2015; Marra et al., 2015), it is possible to collect such detailed information. Future work should focus on filling this knowledge gap and continue to collect empirical information on an individual level with the goal of accurately informing the growing pool of predictive models.

Literature Cited

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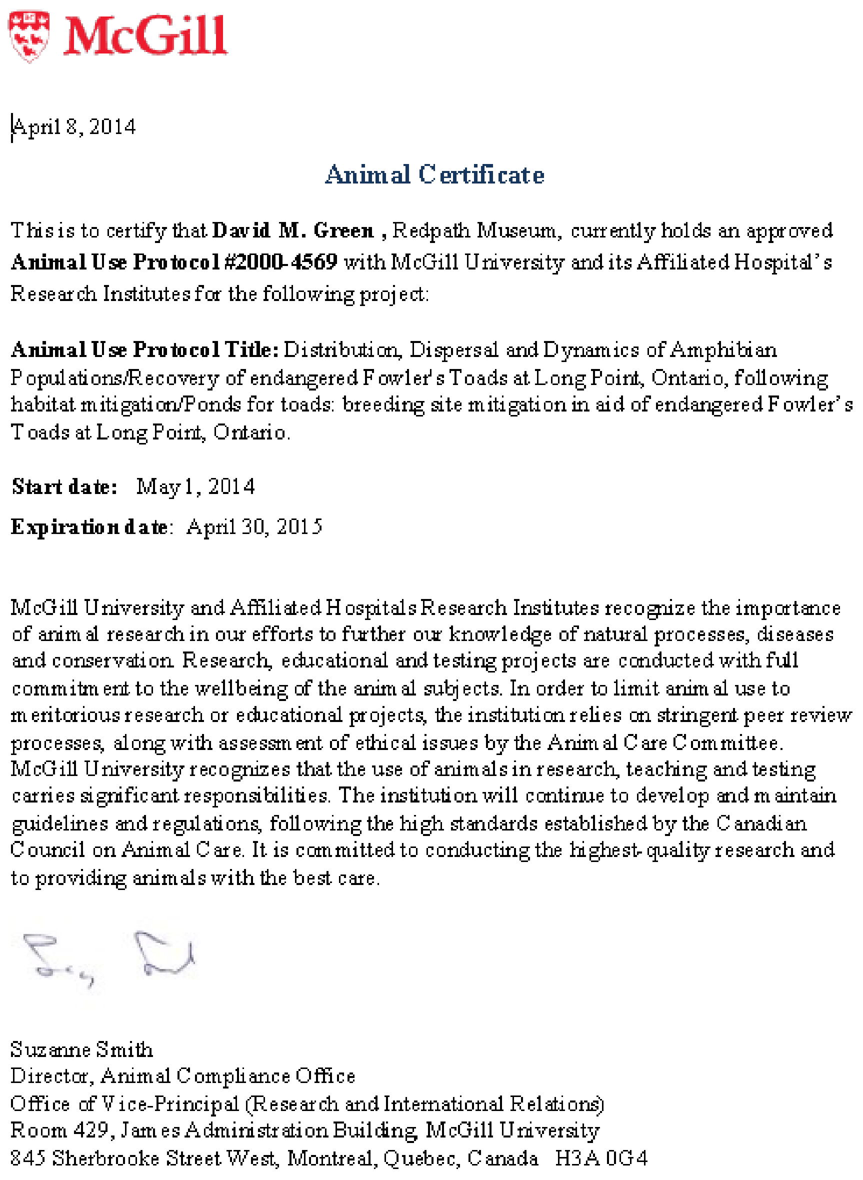
Appendices

I. McGill Animal Use Protocols



McGill

April 8, 2014

Animal Certificate

This is to certify that **David M. Green** , Redpath Museum, currently holds an approved **Animal Use Protocol #2000-4569** with McGill University and its Affiliated Hospital's Research Institutes for the following project:

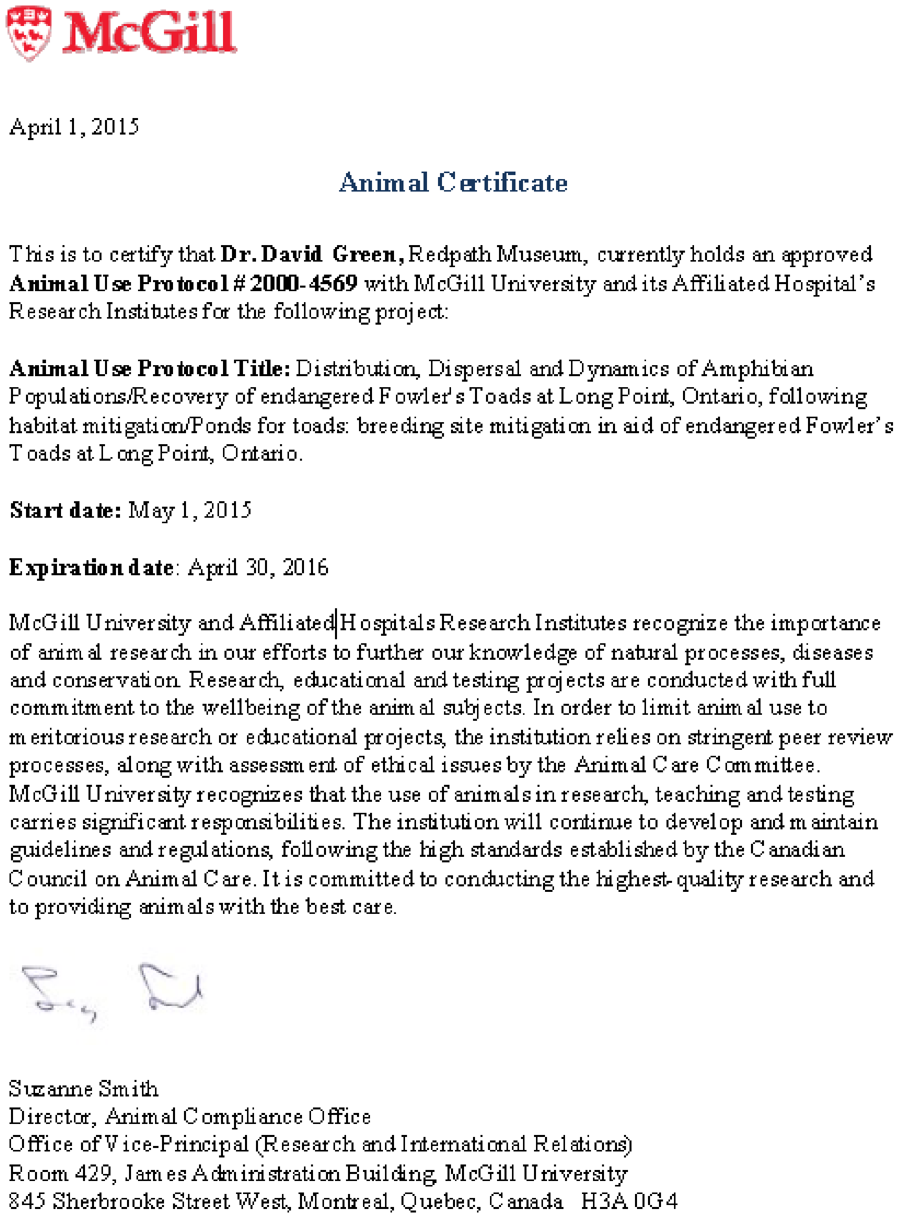
Animal Use Protocol Title: Distribution, Dispersal and Dynamics of Amphibian Populations/Recovery of endangered Fowler's Toads at Long Point, Ontario, following habitat mitigation/Ponds for toads: breeding site mitigation in aid of endangered Fowler's Toads at Long Point, Ontario.

Start date: May 1, 2014

Expiration date: April 30, 2015

McGill University and Affiliated Hospitals Research Institutes recognize the importance of animal research in our efforts to further our knowledge of natural processes, diseases and conservation. Research, educational and testing projects are conducted with full commitment to the wellbeing of the animal subjects. In order to limit animal use to meritorious research or educational projects, the institution relies on stringent peer review processes, along with assessment of ethical issues by the Animal Care Committee. McGill University recognizes that the use of animals in research, teaching and testing carries significant responsibilities. The institution will continue to develop and maintain guidelines and regulations, following the high standards established by the Canadian Council on Animal Care. It is committed to conducting the highest-quality research and to providing animals with the best care.

Suzanne Smith
Director, Animal Compliance Office
Office of Vice-Principal (Research and International Relations)
Room 429, James Administration Building, McGill University
845 Sherbrooke Street West, Montreal, Quebec, Canada H3A 0G4



McGill

April 1, 2015

Animal Certificate

This is to certify that **Dr. David Green,** Redpath Museum, currently holds an approved **Animal Use Protocol # 2000-4569** with McGill University and its Affiliated Hospital's Research Institutes for the following project:

Animal Use Protocol Title: Distribution, Dispersal and Dynamics of Amphibian Populations/Recovery of endangered Fowler's Toads at Long Point, Ontario, following habitat mitigation/Ponds for toads: breeding site mitigation in aid of endangered Fowler's Toads at Long Point, Ontario.

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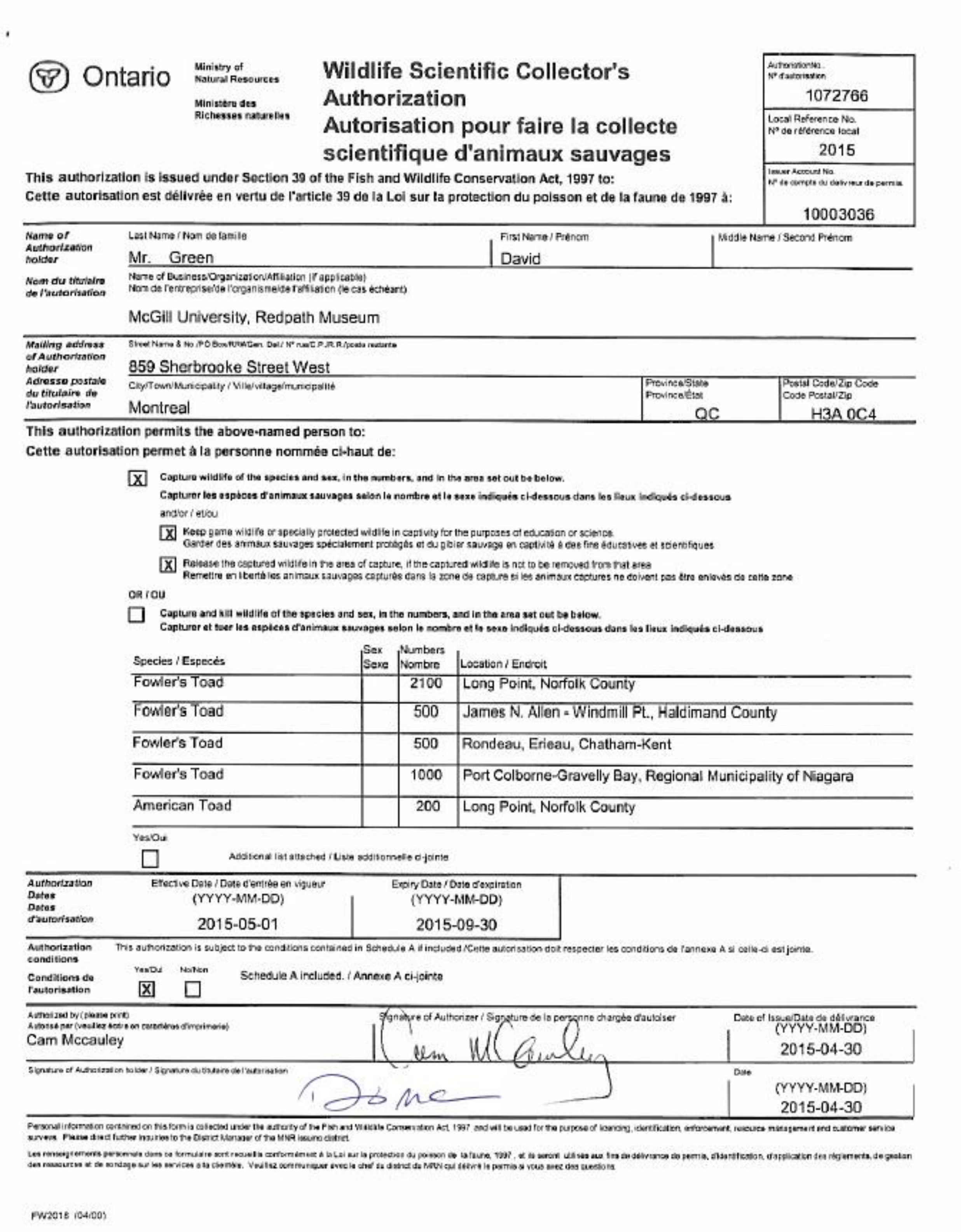
Suzanne Smith
Director, Animal Compliance Office
Office of Vice-Principal (Research and International Relations)
Room 429, James Administration Building, McGill University
845 Sherbrooke Street West, Montreal, Quebec, Canada   H3A 0G4

II. Wildlife Scientific Collector's Authorizations



Ontario

Ministry of Natural Resources
Ministère des Richesses naturelles

Wildlife Scientific Collector's Authorization
Autorisation pour faire la collecte scientifique d'animaux sauvages

Authorization No. / N° d'autorisation: 1072766
Local Reference No. / N° de référence local: 2015
Issuer Account No. / N° de compte du délivreur de permis: 10003036

This authorization is issued under Section 39 of the Fish and Wildlife Conservation Act, 1997 to:
Cette autorisation est délivrée en vertu de l'article 39 de la Loi sur la protection du poisson et de la faune de 1997 à:

Name of Authorization holder / Nom du titulaire de l'autorisation
Last Name / Nom de famille: Mr. Green
First Name / Prénom: David
Middle Name / Second Prénom:
Name of Business/Organization/Affiliation (if applicable) / Nom de l'entreprise/de l'organisme/de l'affiliation (le cas échéant): McGill University, Redpath Museum

Mailing address of Authorization holder / Adresse postale du titulaire de l'autorisation
Street Name & No./PO Box/RR#/Gen. Del./ N° rue/C.P./R.R./poste restante: 859 Sherbrooke Street West
City/Town/Municipality / Ville/village/municipalité: Montreal
Province/State / Province/État: QC
Postal Code/Zip Code / Code Postal/Zip: H3A 0C4

This authorization permits the above-named person to:
Cette autorisation permet à la personne nommée ci-haut de:

☒ **Capture wildlife of the species and sex, in the numbers, and in the area set out below.**
Capturer les espèces d'animaux sauvages selon le nombre et le sexe indiqués ci-dessous dans les lieux indiqués ci-dessous

and/or / et/ou

☒ Keep game wildlife or specially protected wildlife in captivity for the purposes of education or science.
Garder des animaux sauvages spécialement protégés et du gibier sauvage en captivité à des fins éducatives et scientifiques

☒ Release the captured wildlife in the area of capture, if the captured wildlife is not to be removed from that area
Remettre en liberté les animaux sauvages capturés dans la zone de capture si les animaux capturés ne doivent pas être enlevés de cette zone

OR / OU

☐ **Capture and kill wildlife of the species and sex, in the numbers, and in the area set out below.**
Capturer et tuer les espèces d'animaux sauvages selon le nombre et le sexe indiqués ci-dessous dans les lieux indiqués ci-dessous

Species / Espèces	Sex / Sexe	Numbers / Nombre	Location / Endroit
Fowler's Toad		2100	Long Point, Norfolk County
Fowler's Toad		500	James N. Allen - Windmill Pt., Haldimand County
Fowler's Toad		500	Rondeau, Erieau, Chatham-Kent
Fowler's Toad		1000	Port Colborne-Gravelly Bay, Regional Municipality of Niagara
American Toad		200	Long Point, Norfolk County

Yes/Oui
☐ Additional list attached / Liste additionnelle ci-jointe

Authorization Dates / Dates d'autorisation
Effective Date / Date d'entrée en vigueur (YYYY-MM-DD): 2015-05-01
Expiry Date / Date d'expiration (YYYY-MM-DD): 2015-09-30

Authorization conditions / Conditions de l'autorisation
This authorization is subject to the conditions contained in Schedule A if included /Cette autorisation doit respecter les conditions de l'annexe A si celle-ci est jointe.
Yes/Oui ☒ No/Non ☐ Schedule A included. / Annexe A ci-jointe

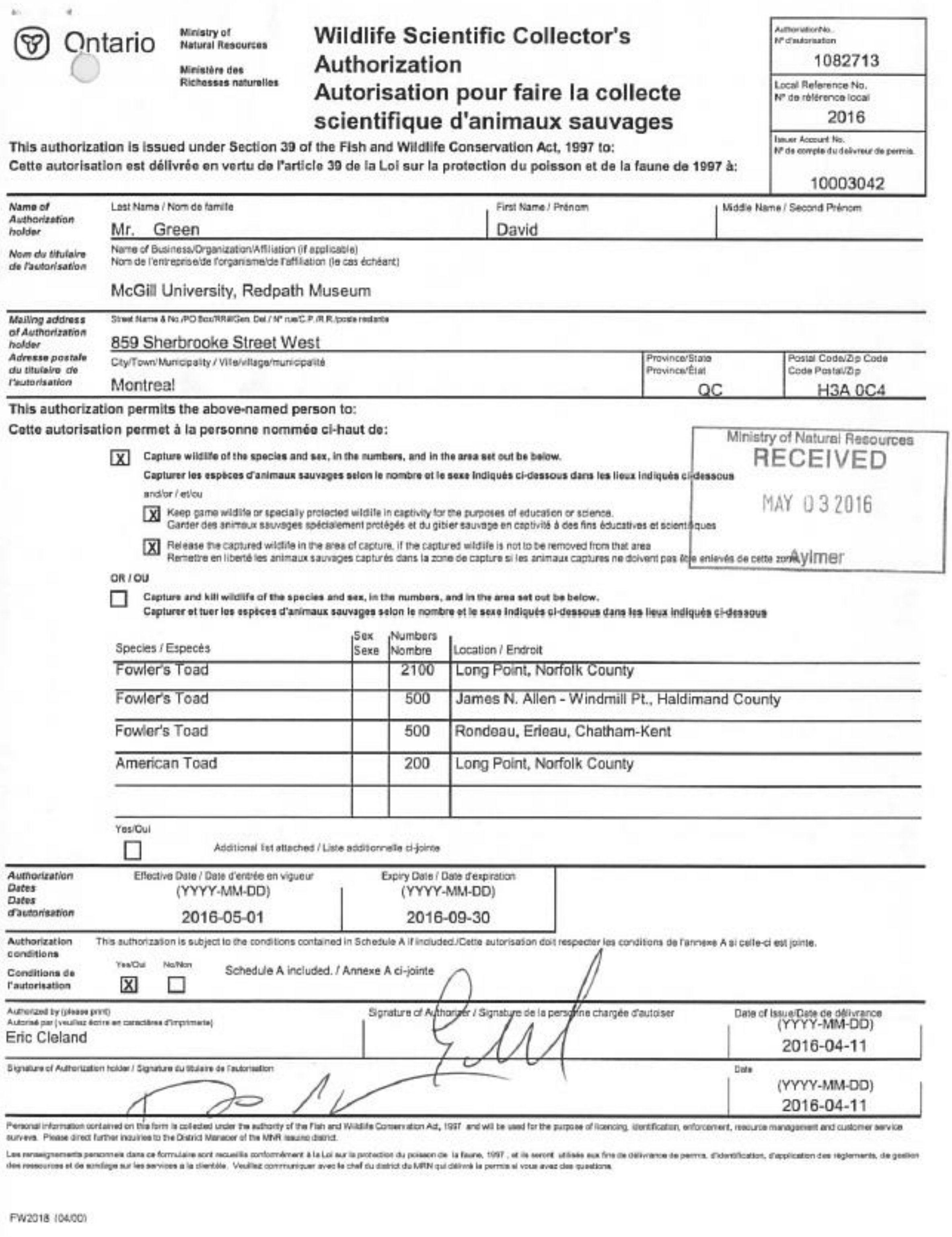
Authorized by (please print) / Autorisé par (veuillez écrire en caractères d'imprimerie): Cam Mccauley
Signature of Authorizer / Signature de la personne chargée d'autoriser
Date of Issue/Date de délivrance (YYYY-MM-DD): 2015-04-30

Signature of Authorization holder / Signature du titulaire de l'autorisation
Date (YYYY-MM-DD): 2015-04-30

Personal information contained on this form is collected under the authority of the Fish and Wildlife Conservation Act, 1997 and will be used for the purpose of licencing, identification, enforcement, resource management and customer service surveys. Please direct further inquiries to the District Manager of the MNR issuing district.

Les renseignements personnels dans ce formulaire sont recueillis conformément à la Loi sur la protection du poisson de la faune, 1997, et ils seront utilisés aux fins de délivrance de permis, d'identification, d'application des règlements, de gestion des ressources et de sondage sur les services à la clientèle. Veuillez communiquer avec le chef du district du MRN qui délivre le permis si vous avez des questions.

FW2018 (04/00)



Ontario

Ministry of Natural Resources
Ministère des Richesses naturelles

Wildlife Scientific Collector's Authorization
Autorisation pour faire la collecte scientifique d'animaux sauvages

Authorization No. / N° d'autorisation: 1082713
Local Reference No. / N° de référence local: 2016
Issuer Account No. / N° de compte du délivreur de permis: 10003042

This authorization is issued under Section 39 of the Fish and Wildlife Conservation Act, 1997 to:
Cette autorisation est délivrée en vertu de l'article 39 de la Loi sur la protection du poisson et de la faune de 1997 à:

Name of Authorization holder ***Nom du titulaire de l'autorisation***	Last Name / Nom de famille: Mr. Green	First Name / Prénom: David	Middle Name / Second Prénom:
	Name of Business/Organization/Affiliation (if applicable) / Nom de l'entreprise/de l'organisme/de l'affiliation (le cas échéant): McGill University, Redpath Museum		
Mailing address of Authorization holder ***Adresse postale du titulaire de l'autorisation***	Street Name & No./PO Box/RR#/Gen. Del. / N° rue/C.P./R.R./poste restante: 859 Sherbrooke Street West		
	City/Town/Municipality / Ville/village/municipalité: Montreal	Province/State / Province/État: QC	Postal Code/Zip Code / Code Postal/Zip: H3A 0C4

This authorization permits the above-named person to:
Cette autorisation permet à la personne nommée ci-haut de:

Ministry of Natural Resources
RECEIVED
MAY 03 2016
Aylmer

☒ **Capture wildlife of the species and sex, in the numbers, and in the area set out be below.**
Capturer les espèces d'animaux sauvages selon le nombre et le sexe indiqués ci-dessous dans les lieux indiqués ci-dessous

and/or / et/ou

- ☒ Keep game wildlife or specially protected wildlife in captivity for the purposes of education or science. / Garder des animaux sauvages spécialement protégés et du gibier sauvage en captivité à des fins éducatives et scientifiques
- ☒ Release the captured wildlife in the area of capture, if the captured wildlife is not to be removed from that area / Remettre en liberté les animaux sauvages capturés dans la zone de capture si les animaux capturés ne doivent pas être enlevés de cette zone

OR / OU

☐ **Capture and kill wildlife of the species and sex, in the numbers, and in the area set out be below.**
Capturer et tuer les espèces d'animaux sauvages selon le nombre et le sexe indiqués ci-dessous dans les lieux indiqués ci-dessous

Species / Especés	Sex / Sexe	Numbers / Nombre	Location / Endroit
Fowler's Toad		2100	Long Point, Norfolk County
Fowler's Toad		500	James N. Allen - Windmill Pt., Haldimand County
Fowler's Toad		500	Rondeau, Erieau, Chatham-Kent
American Toad		200	Long Point, Norfolk County

Yes/Oui
☐ Additional list attached / Liste additionnelle ci-jointe

Authorization Dates ***Dates d'autorisation***	Effective Date / Date d'entrée en vigueur (YYYY-MM-DD): 2016-05-01	Expiry Date / Date d'expiration (YYYY-MM-DD): 2016-09-30

Authorization conditions / **Conditions de l'autorisation**
This authorization is subject to the conditions contained in Schedule A if included./Cette autorisation doit respecter les conditions de l'annexe A si celle-ci est jointe.

Yes/Oui ☒ No/Non ☐ Schedule A included. / Annexe A ci-jointe

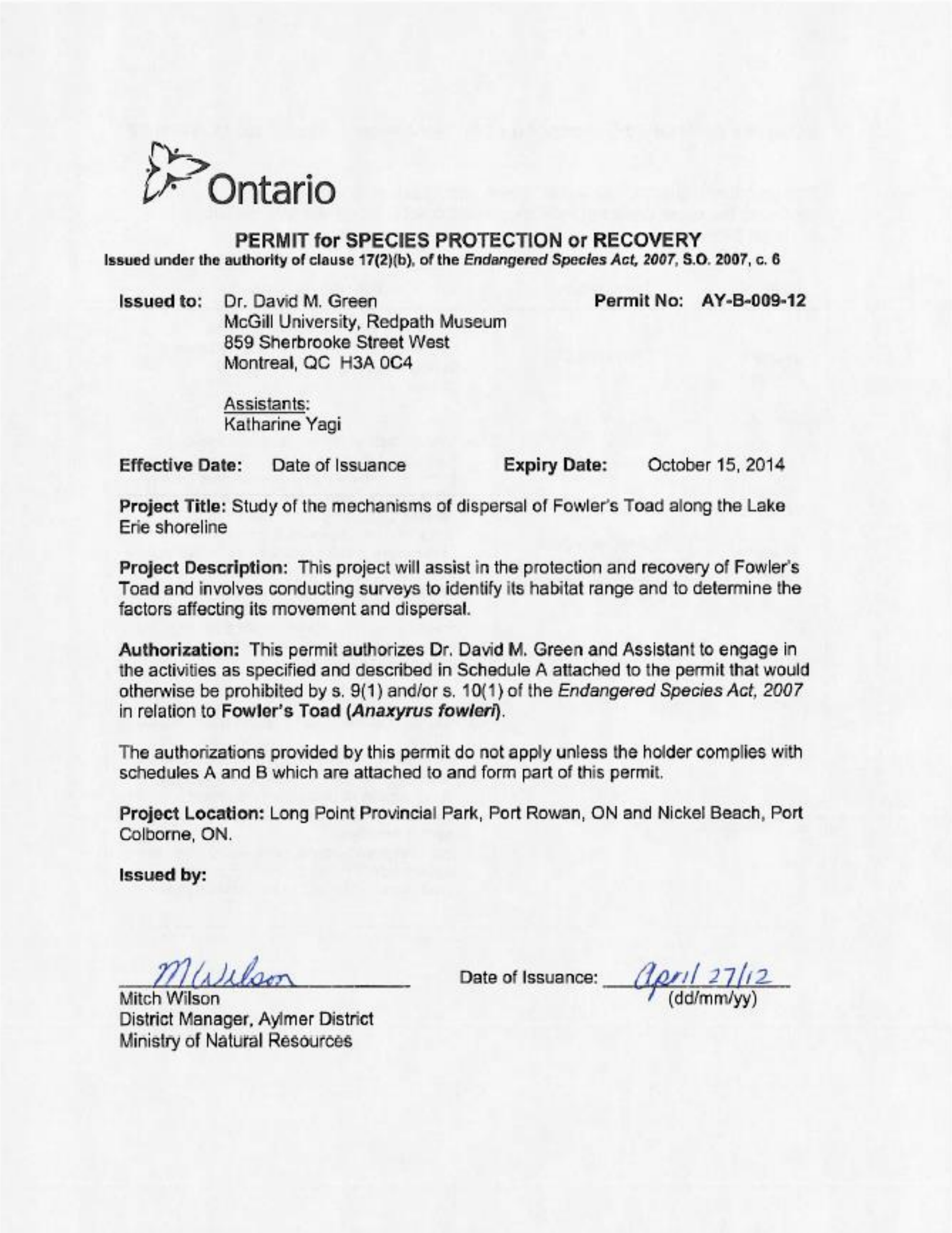
Authorized by (please print) / Autorisé par (veuillez écrire en caractères d'imprimerie): Eric Cleland	Signature of Authorizer / Signature de la personne chargée d'autoiser	Date of Issue/Date de délivrance (YYYY-MM-DD): 2016-04-11
Signature of Authorization holder / Signature du titulaire de l'autorisation		Date (YYYY-MM-DD): 2016-04-11

Personal information contained on this form is collected under the authority of the Fish and Wildlife Conservation Act, 1997 and will be used for the purpose of licencing, identification, enforcement, resource management and customer service surveys. Please direct further inquiries to the District Manager of the MNR issuing district.

Les renseignements personnels dans ce formulaire sont recueillis conformément à la Loi sur la protection du poisson de la faune, 1997, et ils seront utilisés aux fins de délivrance de permis, d'identification, d'application des règlements, de gestion des ressources et de sondage sur les services à la clientèle. Veuillez communiquer avec le chef du district du MRN qui délivre le permis si vous avez des questions.

FW2018 (04/00)

III. Ontario Endangered Species Act (2007) 17(b) permits



Ontario

PERMIT for SPECIES PROTECTION or RECOVERY

Issued under the authority of clause 17(2)(b), of the *Endangered Species Act, 2007*, S.O. 2007, c. 6

Issued to: Dr. David M. Green
McGill University, Redpath Museum
859 Sherbrooke Street West
Montreal, QC H3A 0C4

Permit No: AY-B-009-12

Assistants:
Katharine Yagi

Effective Date: Date of Issuance **Expiry Date:** October 15, 2014

Project Title: Study of the mechanisms of dispersal of Fowler's Toad along the Lake Erie shoreline

Project Description: This project will assist in the protection and recovery of Fowler's Toad and involves conducting surveys to identify its habitat range and to determine the factors affecting its movement and dispersal.

Authorization: This permit authorizes Dr. David M. Green and Assistant to engage in the activities as specified and described in Schedule A attached to the permit that would otherwise be prohibited by s. 9(1) and/or s. 10(1) of the *Endangered Species Act, 2007* in relation to **Fowler's Toad (*Anaxyrus fowleri*)**.

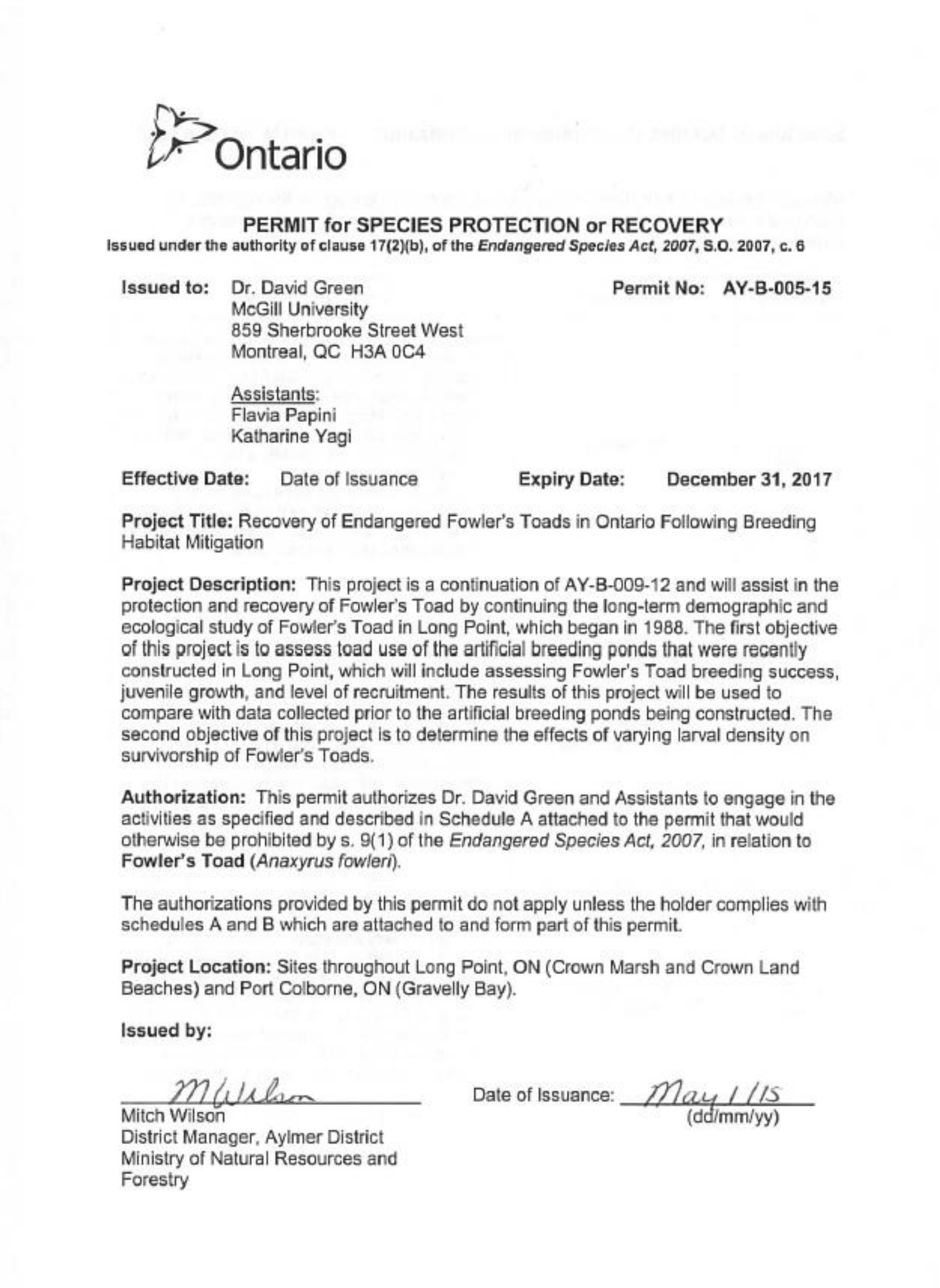
The authorizations provided by this permit do not apply unless the holder complies with schedules A and B which are attached to and form part of this permit.

Project Location: Long Point Provincial Park, Port Rowan, ON and Nickel Beach, Port Colborne, ON.

Issued by:

MWilson
Mitch Wilson
District Manager, Aylmer District
Ministry of Natural Resources

Date of Issuance: April 27/12 (dd/mm/yy)



Ontario

PERMIT for SPECIES PROTECTION or RECOVERY

Issued under the authority of clause 17(2)(b), of the *Endangered Species Act, 2007*, S.O. 2007, c. 6

Issued to: Dr. David Green
McGill University
859 Sherbrooke Street West
Montreal, QC H3A 0C4

Permit No: AY-B-005-15

Assistants:
Flavia Papini
Katharine Yagi

Effective Date: Date of Issuance **Expiry Date:** **December 31, 2017**

Project Title: Recovery of Endangered Fowler's Toads in Ontario Following Breeding Habitat Mitigation

Project Description: This project is a continuation of AY-B-009-12 and will assist in the protection and recovery of Fowler's Toad by continuing the long-term demographic and ecological study of Fowler's Toad in Long Point, which began in 1988. The first objective of this project is to assess toad use of the artificial breeding ponds that were recently constructed in Long Point, which will include assessing Fowler's Toad breeding success, juvenile growth, and level of recruitment. The results of this project will be used to compare with data collected prior to the artificial breeding ponds being constructed. The second objective of this project is to determine the effects of varying larval density on survivorship of Fowler's Toads.

Authorization: This permit authorizes Dr. David Green and Assistants to engage in the activities as specified and described in Schedule A attached to the permit that would otherwise be prohibited by s. 9(1) of the *Endangered Species Act, 2007,* in relation to **Fowler's Toad** (*Anaxyrus fowleri*).

The authorizations provided by this permit do not apply unless the holder complies with schedules A and B which are attached to and form part of this permit.

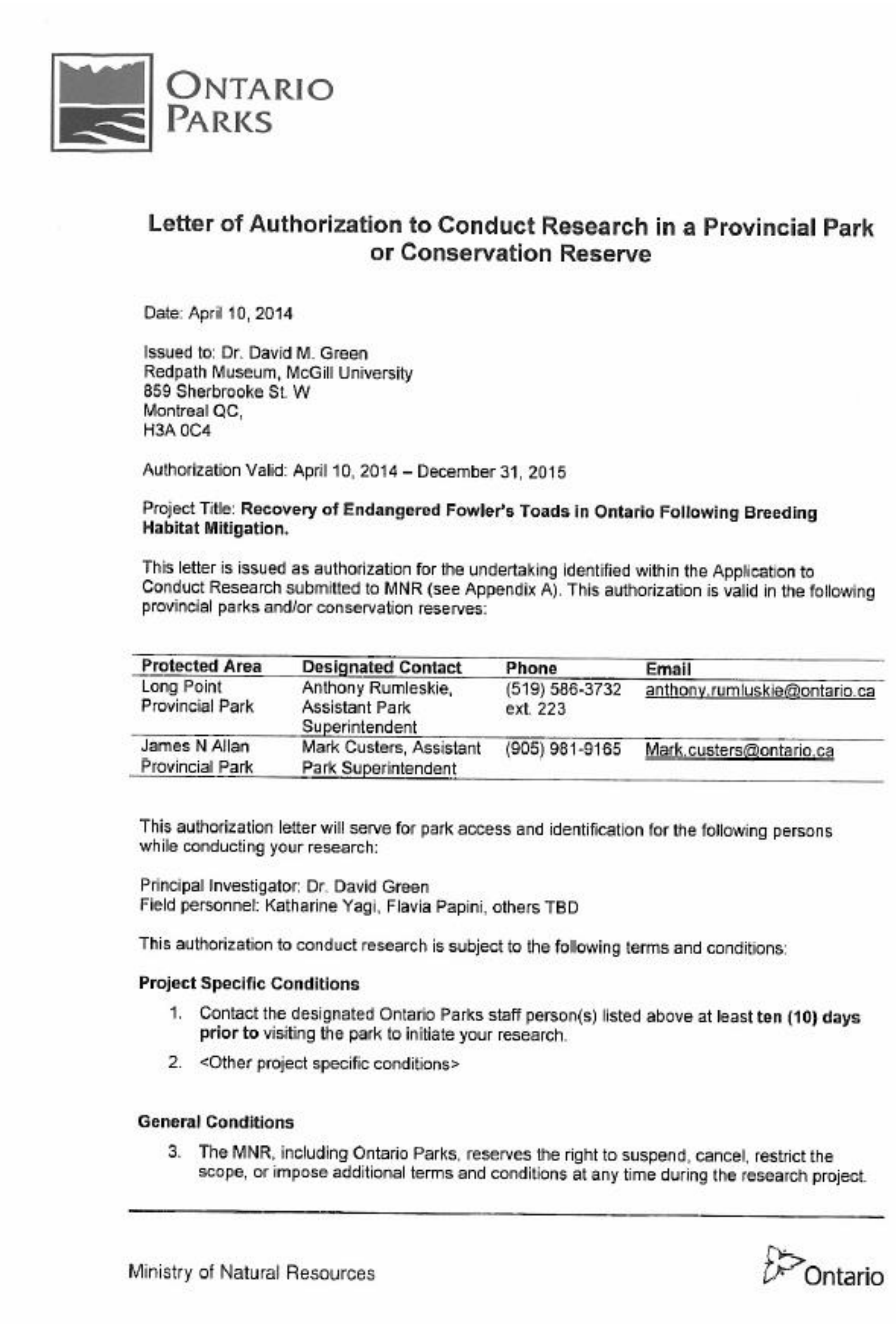
Project Location: Sites throughout Long Point, ON (Crown Marsh and Crown Land Beaches) and Port Colborne, ON (Gravelly Bay).

Issued by:

M Wilson
Mitch Wilson
District Manager, Aylmer District
Ministry of Natural Resources and Forestry

Date of Issuance: May 1/15
(dd/mm/yy)

IV. Ontario Parks permits



ONTARIO
PARKS

Letter of Authorization to Conduct Research in a Provincial Park or Conservation Reserve

Date: April 10, 2014

Issued to: Dr. David M. Green
Redpath Museum, McGill University
859 Sherbrooke St. W
Montreal QC,
H3A 0C4

Authorization Valid: April 10, 2014 – December 31, 2015

Project Title: **Recovery of Endangered Fowler's Toads in Ontario Following Breeding Habitat Mitigation.**

This letter is issued as authorization for the undertaking identified within the Application to Conduct Research submitted to MNR (see Appendix A). This authorization is valid in the following provincial parks and/or conservation reserves:

Protected Area	Designated Contact	Phone	Email
Long Point Provincial Park	Anthony Rumleskie, Assistant Park Superintendent	(519) 586-3732 ext. 223	anthony.rumluskie@ontario.ca
James N Allan Provincial Park	Mark Custers, Assistant Park Superintendent	(905) 981-9165	Mark.custers@ontario.ca

This authorization letter will serve for park access and identification for the following persons while conducting your research:

Principal Investigator: Dr. David Green
Field personnel: Katharine Yagi, Flavia Papini, others TBD

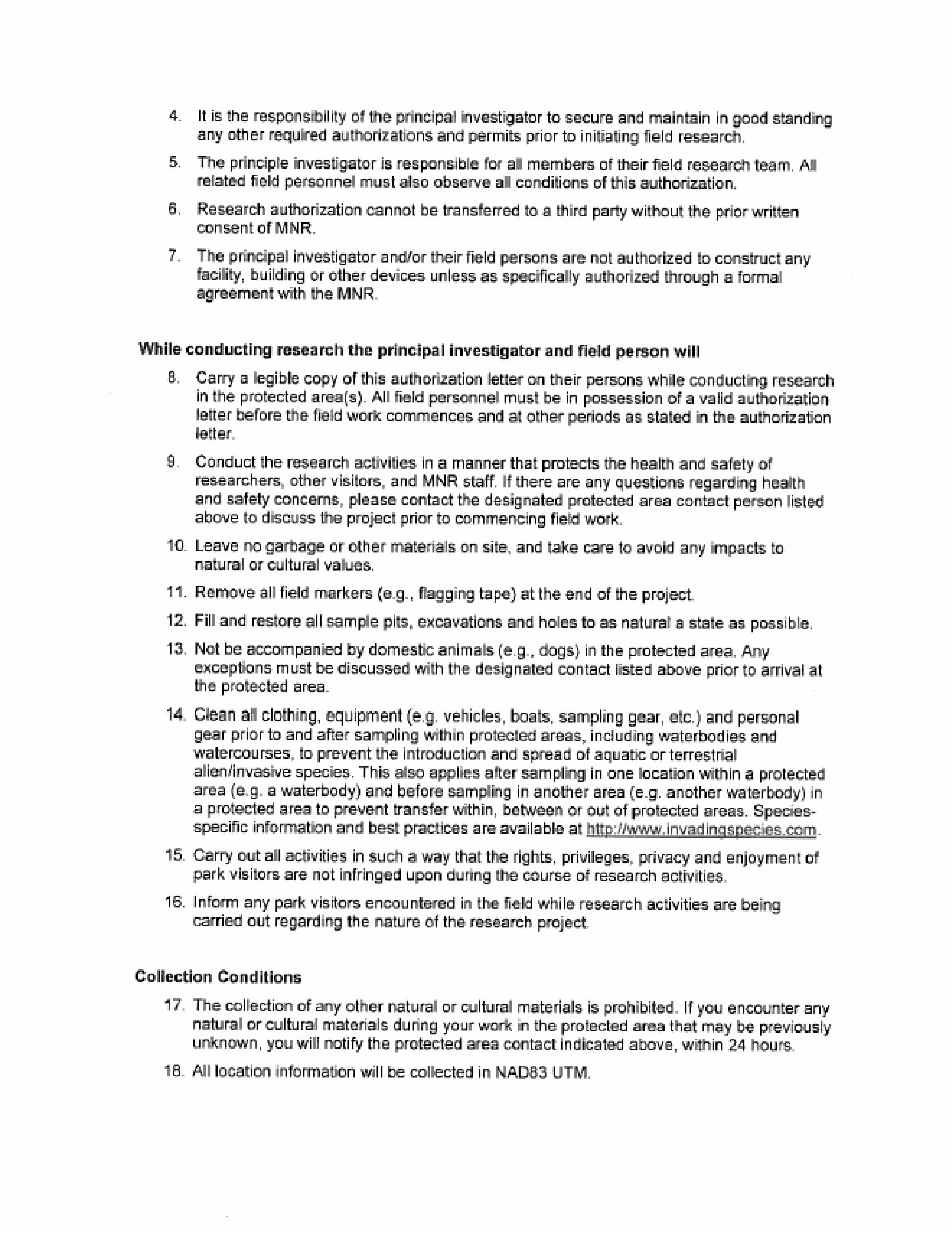
This authorization to conduct research is subject to the following terms and conditions:

Project Specific Conditions

1. Contact the designated Ontario Parks staff person(s) listed above at least **ten (10) days prior to** visiting the park to initiate your research.
2. <Other project specific conditions>

General Conditions

3. The MNR, including Ontario Parks, reserves the right to suspend, cancel, restrict the scope, or impose additional terms and conditions at any time during the research project.



4. It is the responsibility of the principal investigator to secure and maintain in good standing any other required authorizations and permits prior to initiating field research.
5. The principle investigator is responsible for all members of their field research team. All related field personnel must also observe all conditions of this authorization.
6. Research authorization cannot be transferred to a third party without the prior written consent of MNR.
7. The principal investigator and/or their field persons are not authorized to construct any facility, building or other devices unless as specifically authorized through a formal agreement with the MNR.

While conducting research the principal investigator and field person will

8. Carry a legible copy of this authorization letter on their persons while conducting research in the protected area(s). All field personnel must be in possession of a valid authorization letter before the field work commences and at other periods as stated in the authorization letter.
9. Conduct the research activities in a manner that protects the health and safety of researchers, other visitors, and MNR staff. If there are any questions regarding health and safety concerns, please contact the designated protected area contact person listed above to discuss the project prior to commencing field work.
10. Leave no garbage or other materials on site, and take care to avoid any impacts to natural or cultural values.
11. Remove all field markers (e.g., flagging tape) at the end of the project.
12. Fill and restore all sample pits, excavations and holes to as natural a state as possible.
13. Not be accompanied by domestic animals (e.g., dogs) in the protected area. Any exceptions must be discussed with the designated contact listed above prior to arrival at the protected area.
14. Clean all clothing, equipment (e.g. vehicles, boats, sampling gear, etc.) and personal gear prior to and after sampling within protected areas, including waterbodies and watercourses, to prevent the introduction and spread of aquatic or terrestrial alien/invasive species. This also applies after sampling in one location within a protected area (e.g. a waterbody) and before sampling in another area (e.g. another waterbody) in a protected area to prevent transfer within, between or out of protected areas. Species-specific information and best practices are available at http://www.invadingspecies.com.
15. Carry out all activities in such a way that the rights, privileges, privacy and enjoyment of park visitors are not infringed upon during the course of research activities.
16. Inform any park visitors encountered in the field while research activities are being carried out regarding the nature of the research project.

Collection Conditions

17. The collection of any other natural or cultural materials is prohibited. If you encounter any natural or cultural materials during your work in the protected area that may be previously unknown, you will notify the protected area contact indicated above, within 24 hours.
18. All location information will be collected in NAD83 UTM.

Reporting Requirements

19. A digital copy of all raw data collected during the 2014 field season, as well as any interim reports developed during that time, must be submitted to melody.cairns@ontario.ca with a copy to pa.science@ontario.ca by **December 31, 2014**.
    a. Raw data will be provided in the format specified by the approvers. If no format is specified, standard NHIC reporting spreadsheets shall be used.
    b. The requirement to provide raw data and reports annually as outlined is a condition of continued work in subsequent field seasons, and for future research projects by the applicant, including new personnel working under the Principle Investigator.
    c. All location information will be submitted in NAD83 UTM.
    d. Data submitted will only be used internally by the MNR for protected area management purposes.
20. After project completion, a digital copy of all final research reports will be submitted to melody.cairns@ontario.ca with a copy to pa.science@ontario.ca by **December 31, 2015**.

Some research projects may require additional permits or approvals (e.g., MNR Wildlife Scientific Collector Authorization, *Endangered Species Act, 2007* permit, a Licence to Conduct Archaeological Fieldwork). Principal Investigators and authorized field personnel must obtain and follow the terms and conditions of every permit or authorization required for the research project.

For questions regarding this authorization, please contact Melody Cairns, Southwest Zone Ecologist at melody.cairns@ontario.ca or 519-873-4632.

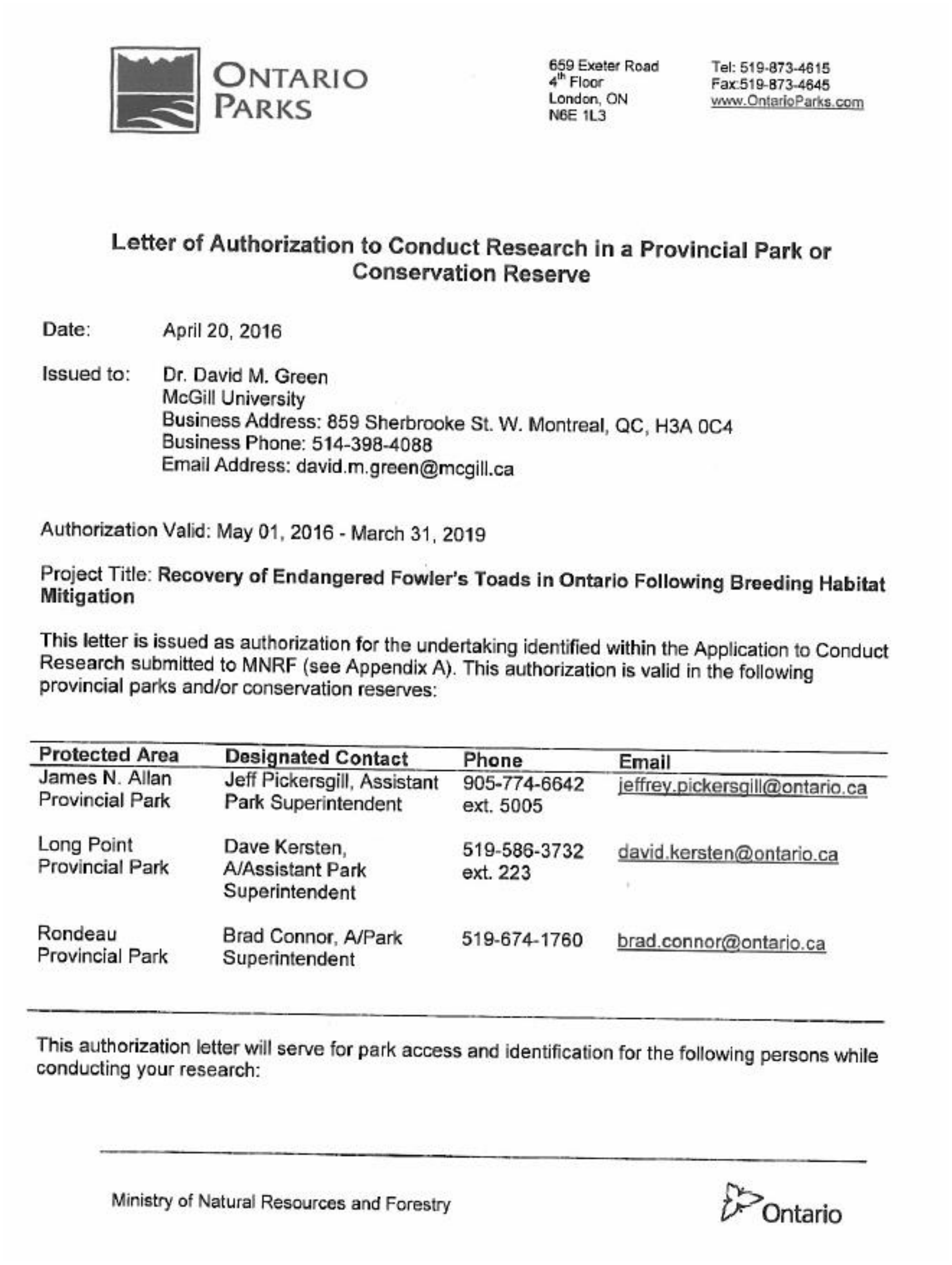
Failure to comply with the terms and conditions will result in the withdrawal of authorization and termination of the research project. The MNR reserves the right to impose additional terms and conditions at any time during the term of the research project.

Authorized by

John Salo
Manager, Southwest Zone, Ontario Parks

c.c.

Melody Cairns, Southwest Zone Ecologist
Anthony Rumleskie, Assistant Park Superintendent, Long Point Provincial Park
Mark Custers, Assistant Park Superintendent, James N Allan Provincial Park
Michelle Smith, Administrative Assistant, Southwest Zone, Ontario Parks
Adam Gryck, Protected Areas Research Analyst



ONTARIO PARKS

659 Exeter Road
4th Floor
London, ON
N6E 1L3

Tel: 519-873-4615
Fax:519-873-4645
www.OntarioParks.com

Letter of Authorization to Conduct Research in a Provincial Park or Conservation Reserve

Date: April 20, 2016

Issued to: Dr. David M. Green
McGill University
Business Address: 859 Sherbrooke St. W. Montreal, QC, H3A 0C4
Business Phone: 514-398-4088
Email Address: david.m.green@mcgill.ca

Authorization Valid: May 01, 2016 - March 31, 2019

Project Title: **Recovery of Endangered Fowler's Toads in Ontario Following Breeding Habitat Mitigation**

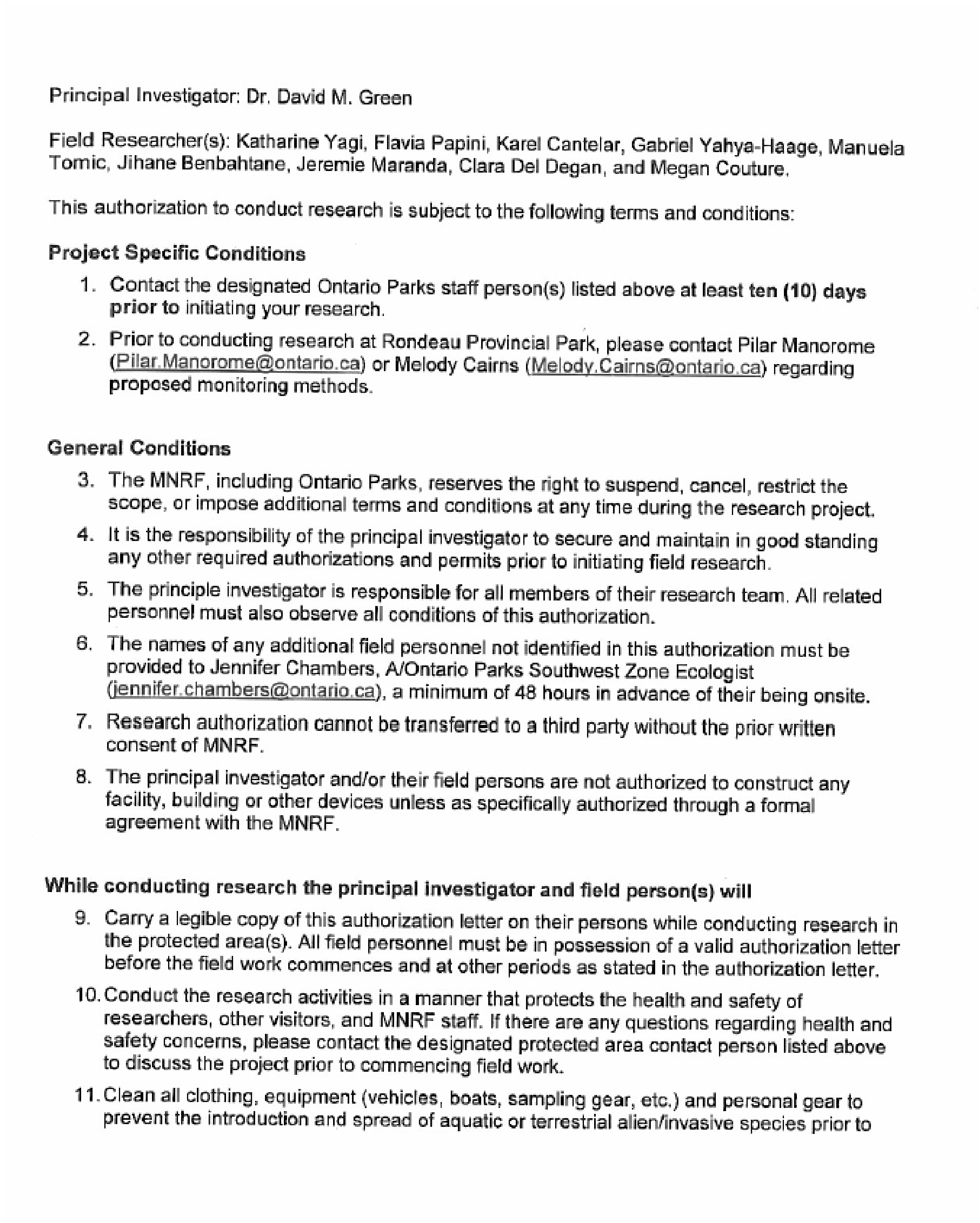
This letter is issued as authorization for the undertaking identified within the Application to Conduct Research submitted to MNRF (see Appendix A). This authorization is valid in the following provincial parks and/or conservation reserves:

Protected Area	**Designated Contact**	**Phone**	**Email**
James N. Allan Provincial Park	Jeff Pickersgill, Assistant Park Superintendent	905-774-6642 ext. 5005	jeffrey.pickersgill@ontario.ca
Long Point Provincial Park	Dave Kersten, A/Assistant Park Superintendent	519-586-3732 ext. 223	david.kersten@ontario.ca
Rondeau Provincial Park	Brad Connor, A/Park Superintendent	519-674-1760	brad.connor@ontario.ca

This authorization letter will serve for park access and identification for the following persons while conducting your research:

Ministry of Natural Resources and Forestry

Ontario



Principal Investigator: Dr. David M. Green

Field Researcher(s): Katharine Yagi, Flavia Papini, Karel Cantelar, Gabriel Yahya-Haage, Manuela Tomic, Jihane Benbahtane, Jeremie Maranda, Clara Del Degan, and Megan Couture.

This authorization to conduct research is subject to the following terms and conditions:

Project Specific Conditions

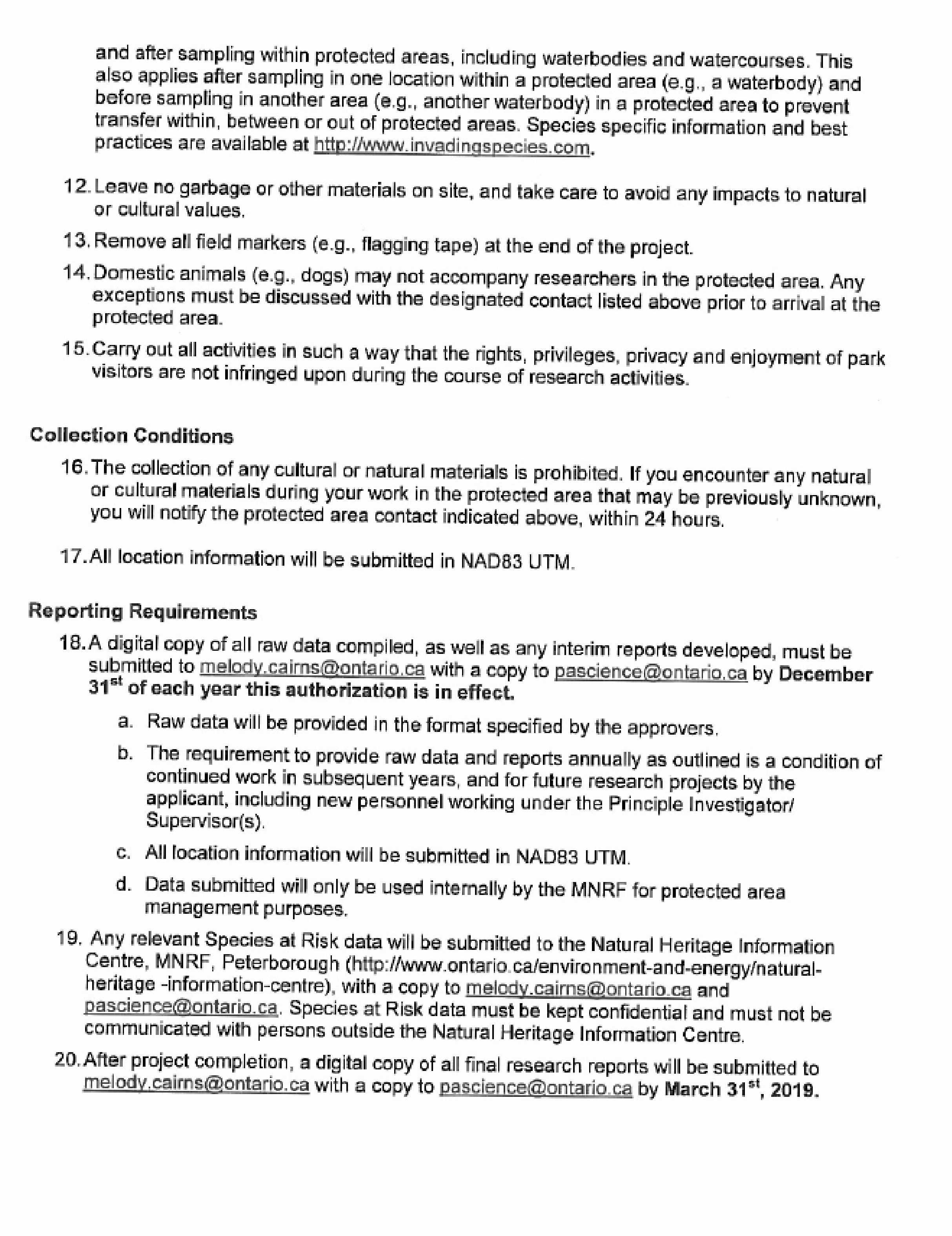
1. Contact the designated Ontario Parks staff person(s) listed above **at least ten (10) days prior to** initiating your research.
2. Prior to conducting research at Rondeau Provincial Park, please contact Pilar Manorome (Pilar.Manorome@ontario.ca) or Melody Cairns (Melody.Cairns@ontario.ca) regarding proposed monitoring methods.

General Conditions

3. The MNRF, including Ontario Parks, reserves the right to suspend, cancel, restrict the scope, or impose additional terms and conditions at any time during the research project.
4. It is the responsibility of the principal investigator to secure and maintain in good standing any other required authorizations and permits prior to initiating field research.
5. The principle investigator is responsible for all members of their research team. All related personnel must also observe all conditions of this authorization.
6. The names of any additional field personnel not identified in this authorization must be provided to Jennifer Chambers, A/Ontario Parks Southwest Zone Ecologist (jennifer.chambers@ontario.ca), a minimum of 48 hours in advance of their being onsite.
7. Research authorization cannot be transferred to a third party without the prior written consent of MNRF.
8. The principal investigator and/or their field persons are not authorized to construct any facility, building or other devices unless as specifically authorized through a formal agreement with the MNRF.

While conducting research the principal investigator and field person(s) will

9. Carry a legible copy of this authorization letter on their persons while conducting research in the protected area(s). All field personnel must be in possession of a valid authorization letter before the field work commences and at other periods as stated in the authorization letter.
10. Conduct the research activities in a manner that protects the health and safety of researchers, other visitors, and MNRF staff. If there are any questions regarding health and safety concerns, please contact the designated protected area contact person listed above to discuss the project prior to commencing field work.
11. Clean all clothing, equipment (vehicles, boats, sampling gear, etc.) and personal gear to prevent the introduction and spread of aquatic or terrestrial alien/invasive species prior to



and after sampling within protected areas, including waterbodies and watercourses. This also applies after sampling in one location within a protected area (e.g., a waterbody) and before sampling in another area (e.g., another waterbody) in a protected area to prevent transfer within, between or out of protected areas. Species specific information and best practices are available at http://www.invadingspecies.com.

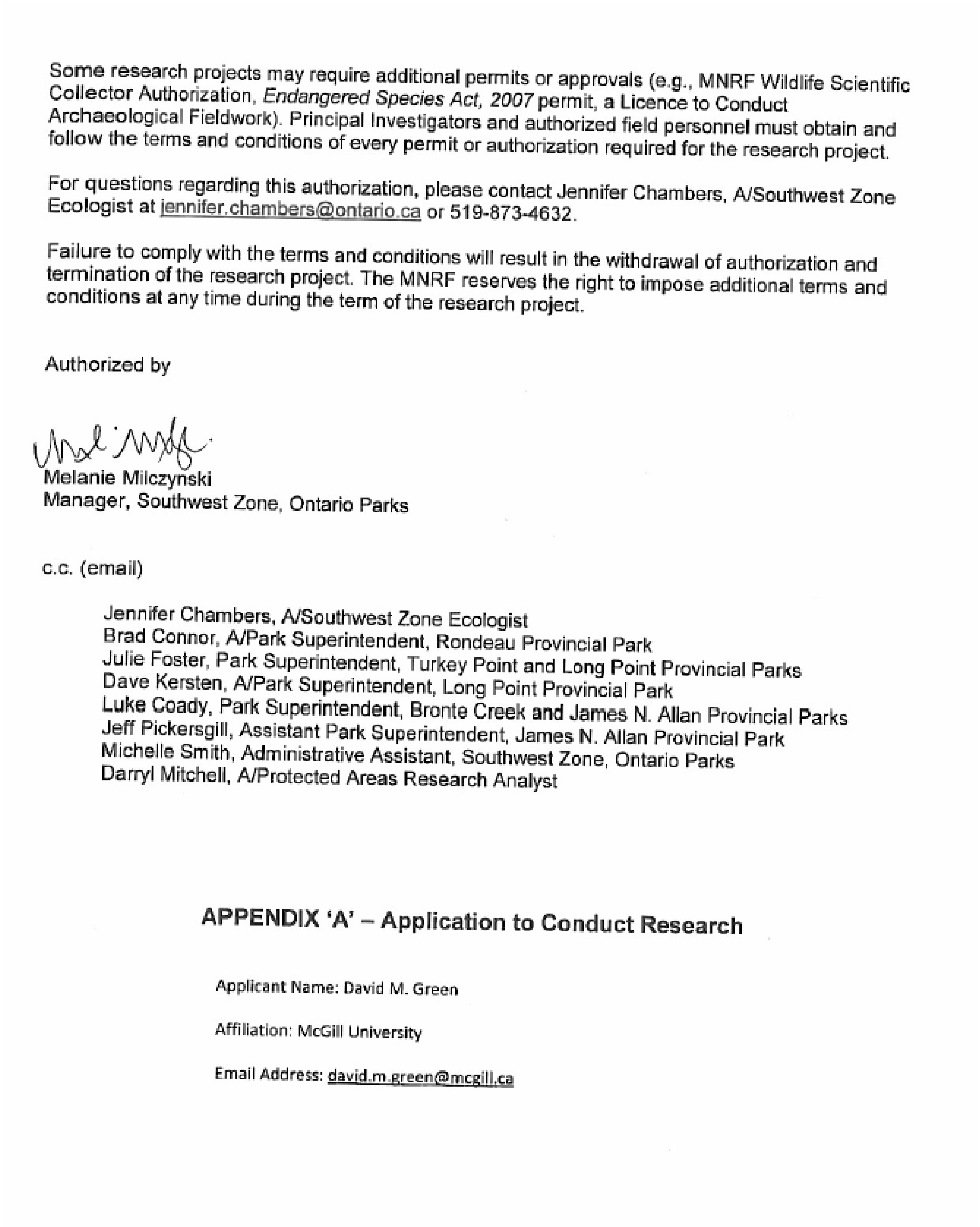
12. Leave no garbage or other materials on site, and take care to avoid any impacts to natural or cultural values.
13. Remove all field markers (e.g., flagging tape) at the end of the project.
14. Domestic animals (e.g., dogs) may not accompany researchers in the protected area. Any exceptions must be discussed with the designated contact listed above prior to arrival at the protected area.
15. Carry out all activities in such a way that the rights, privileges, privacy and enjoyment of park visitors are not infringed upon during the course of research activities.

Collection Conditions

16. The collection of any cultural or natural materials is prohibited. If you encounter any natural or cultural materials during your work in the protected area that may be previously unknown, you will notify the protected area contact indicated above, within 24 hours.
17. All location information will be submitted in NAD83 UTM.

Reporting Requirements

18. A digital copy of all raw data compiled, as well as any interim reports developed, must be submitted to melody.cairns@ontario.ca with a copy to pascience@ontario.ca by **December 31st of each year this authorization is in effect.**
    a. Raw data will be provided in the format specified by the approvers.
    b. The requirement to provide raw data and reports annually as outlined is a condition of continued work in subsequent years, and for future research projects by the applicant, including new personnel working under the Principle Investigator/ Supervisor(s).
    c. All location information will be submitted in NAD83 UTM.
    d. Data submitted will only be used internally by the MNRF for protected area management purposes.
19. Any relevant Species at Risk data will be submitted to the Natural Heritage Information Centre, MNRF, Peterborough (http://www.ontario.ca/environment-and-energy/natural-heritage -information-centre), with a copy to melody.cairns@ontario.ca and pascience@ontario.ca. Species at Risk data must be kept confidential and must not be communicated with persons outside the Natural Heritage Information Centre.
20. After project completion, a digital copy of all final research reports will be submitted to melody.cairns@ontario.ca with a copy to pascience@ontario.ca by **March 31st, 2019.**



Some research projects may require additional permits or approvals (e.g., MNRF Wildlife Scientific Collector Authorization, *Endangered Species Act, 2007* permit, a Licence to Conduct Archaeological Fieldwork). Principal Investigators and authorized field personnel must obtain and follow the terms and conditions of every permit or authorization required for the research project.

For questions regarding this authorization, please contact Jennifer Chambers, A/Southwest Zone Ecologist at jennifer.chambers@ontario.ca or 519-873-4632.

Failure to comply with the terms and conditions will result in the withdrawal of authorization and termination of the research project. The MNRF reserves the right to impose additional terms and conditions at any time during the term of the research project.

Authorized by

Melanie Milczynski
Manager, Southwest Zone, Ontario Parks

c.c. (email)

Jennifer Chambers, A/Southwest Zone Ecologist
Brad Connor, A/Park Superintendent, Rondeau Provincial Park
Julie Foster, Park Superintendent, Turkey Point and Long Point Provincial Parks
Dave Kersten, A/Park Superintendent, Long Point Provincial Park
Luke Coady, Park Superintendent, Bronte Creek and James N. Allan Provincial Parks
Jeff Pickersgill, Assistant Park Superintendent, James N. Allan Provincial Park
Michelle Smith, Administrative Assistant, Southwest Zone, Ontario Parks
Darryl Mitchell, A/Protected Areas Research Analyst

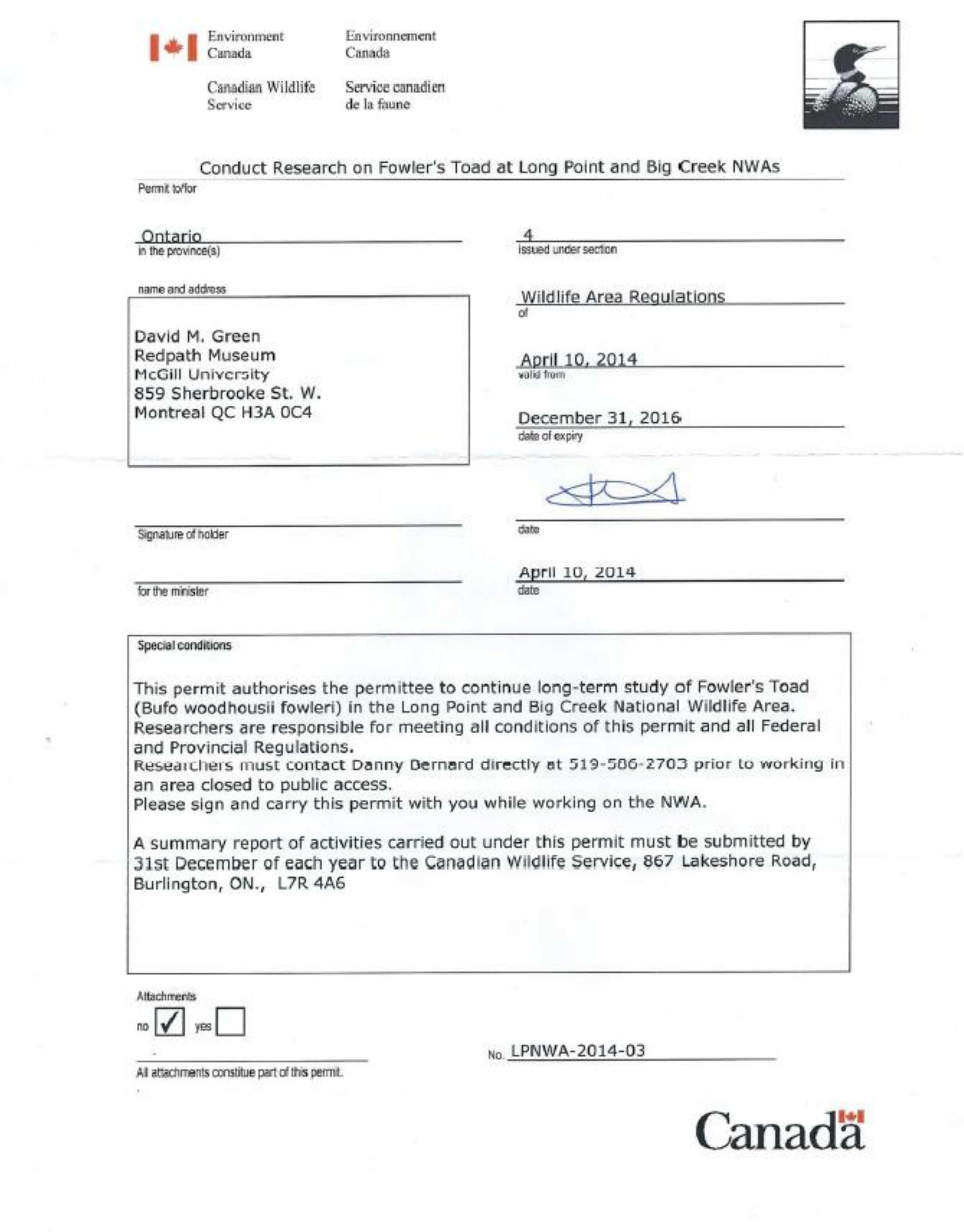
APPENDIX 'A' – Application to Conduct Research

Applicant Name: David M. Green

Affiliation: McGill University

Email Address: david.m.green@mcgill.ca

V. Canadian Wildlife Service permit



Environment Canada
Canadian Wildlife Service

Environnement Canada
Service canadien de la faune

Conduct Research on Fowler's Toad at Long Point and Big Creek NWAs
Permit to/for

Ontario
in the province(s)

4
issued under section

name and address

David M. Green
Redpath Museum
McGill University
859 Sherbrooke St. W.
Montreal QC H3A 0C4

Wildlife Area Regulations
of

April 10, 2014
valid from

December 31, 2016
date of expiry

Signature of holder

date

for the minister

April 10, 2014
date

Special conditions

This permit authorises the permittee to continue long-term study of Fowler's Toad (Bufo woodhousii fowleri) in the Long Point and Big Creek National Wildlife Area.
Researchers are responsible for meeting all conditions of this permit and all Federal and Provincial Regulations.
Researchers must contact Danny Dernard directly at 519-586-2703 prior to working in an area closed to public access.
Please sign and carry this permit with you while working on the NWA.

A summary report of activities carried out under this permit must be submitted by 31st December of each year to the Canadian Wildlife Service, 867 Lakeshore Road, Burlington, ON., L7R 4A6

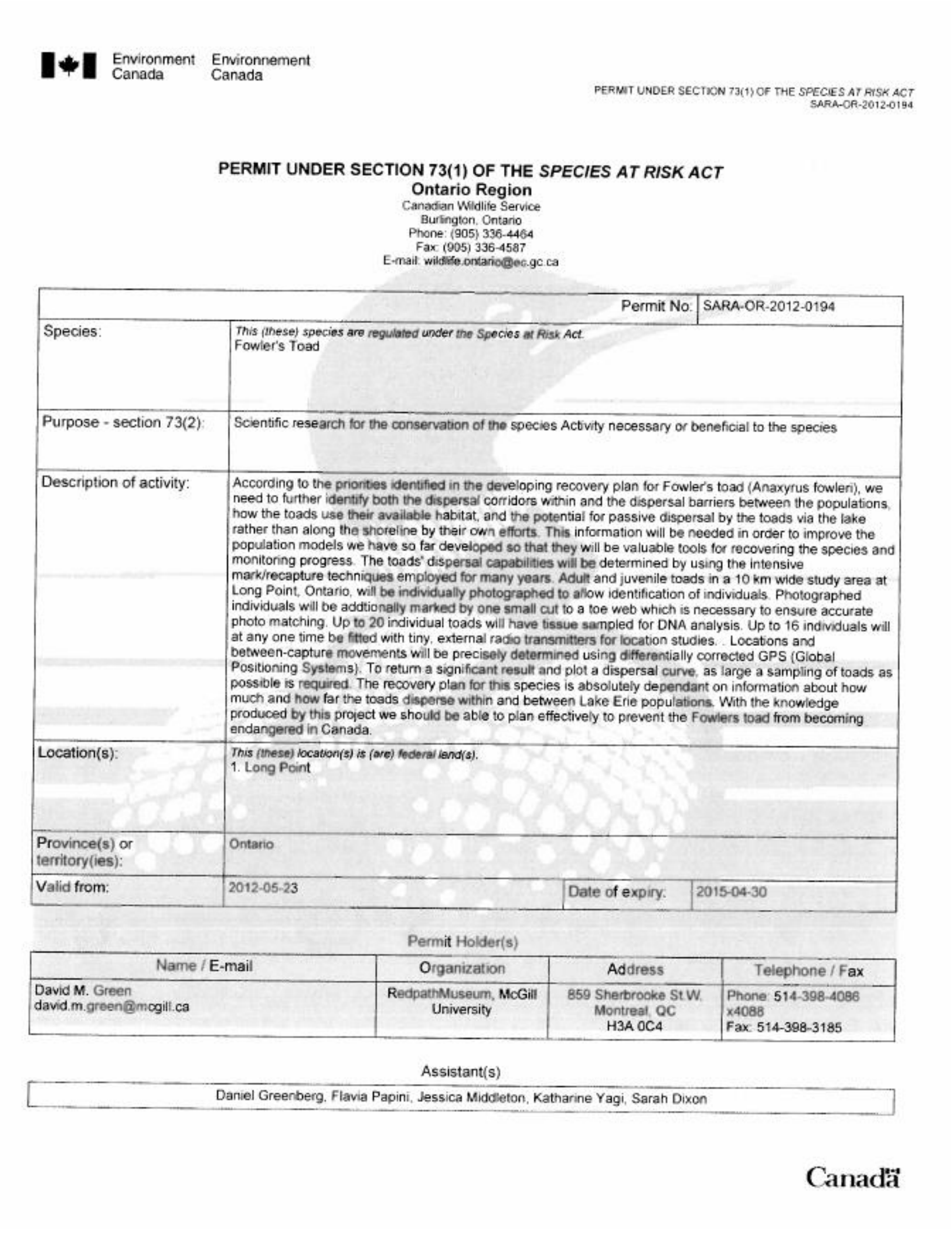
Attachments
no ☑ yes ☐

All attachments constitue part of this permit.

No. LPNWA-2014-03

Canada

VI. Species at Risk Act (SARA) permit



Environment Canada Environnement Canada

PERMIT UNDER SECTION 73(1) OF THE *SPECIES AT RISK ACT*
SARA-OR-2012-0194

PERMIT UNDER SECTION 73(1) OF THE *SPECIES AT RISK ACT*
Ontario Region
Canadian Wildlife Service
Burlington, Ontario
Phone: (905) 336-4464
Fax: (905) 336-4587
E-mail: wildlife.ontario@ec.gc.ca

		Permit No:	SARA-OR-2012-0194
Species:	*This (these) species are regulated under the Species at Risk Act.* Fowler's Toad		
Purpose - section 73(2):	Scientific research for the conservation of the species Activity necessary or beneficial to the species		
Description of activity:	According to the priorities identified in the developing recovery plan for Fowler's toad (Anaxyrus fowleri), we need to further identify both the dispersal corridors within and the dispersal barriers between the populations, how the toads use their available habitat, and the potential for passive dispersal by the toads via the lake rather than along the shoreline by their own efforts. This information will be needed in order to improve the population models we have so far developed so that they will be valuable tools for recovering the species and monitoring progress. The toads' dispersal capabilities will be determined by using the intensive mark/recapture techniques employed for many years. Adult and juvenile toads in a 10 km wide study area at Long Point, Ontario, will be individually photographed to allow identification of individuals. Photographed individuals will be addtionally marked by one small cut to a toe web which is necessary to ensure accurate photo matching. Up to 20 individual toads will have tissue sampled for DNA analysis. Up to 16 individuals will at any one time be fitted with tiny, external radio transmitters for location studies. . Locations and between-capture movements will be precisely determined using differentially corrected GPS (Global Positioning Systems). To return a significant result and plot a dispersal curve, as large a sampling of toads as possible is required. The recovery plan for this species is absolutely dependant on information about how much and how far the toads disperse within and between Lake Erie populations. With the knowledge produced by this project we should be able to plan effectively to prevent the Fowlers toad from becoming endangered in Canada.		
Location(s):	*This (these) location(s) is (are) federal land(s).* 1. Long Point		
Province(s) or territory(ies):	Ontario		
Valid from:	2012-05-23	Date of expiry:	2015-04-30

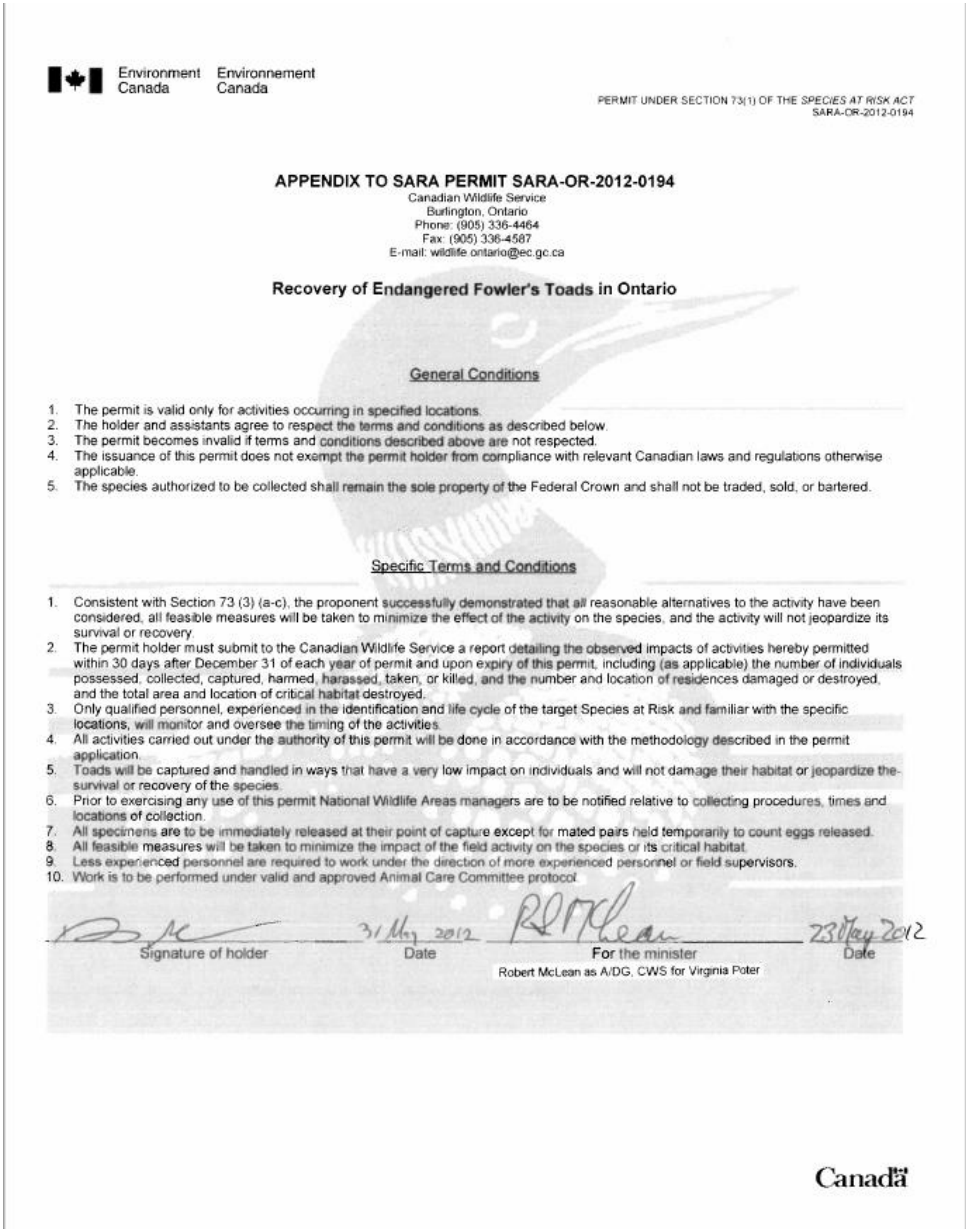
Permit Holder(s)

Name / E-mail	Organization	Address	Telephone / Fax
David M. Green david.m.green@mcgill.ca	RedpathMuseum, McGill University	859 Sherbrooke St.W. Montreal, QC H3A 0C4	Phone: 514-398-4086 x4088 Fax: 514-398-3185

Assistant(s)

Daniel Greenberg, Flavia Papini, Jessica Middleton, Katharine Yagi, Sarah Dixon

Canada



Environment Canada Environnement Canada

PERMIT UNDER SECTION 73(1) OF THE *SPECIES AT RISK ACT*
SARA-OR-2012-0194

APPENDIX TO SARA PERMIT SARA-OR-2012-0194

Canadian Wildlife Service
Burlington, Ontario
Phone: (905) 336-4464
Fax: (905) 336-4587
E-mail: wildlife.ontario@ec.gc.ca

Recovery of Endangered Fowler's Toads in Ontario

General Conditions

1. The permit is valid only for activities occurring in specified locations.
2. The holder and assistants agree to respect the terms and conditions as described below.
3. The permit becomes invalid if terms and conditions described above are not respected.
4. The issuance of this permit does not exempt the permit holder from compliance with relevant Canadian laws and regulations otherwise applicable.
5. The species authorized to be collected shall remain the sole property of the Federal Crown and shall not be traded, sold, or bartered.

Specific Terms and Conditions

1. Consistent with Section 73 (3) (a-c), the proponent successfully demonstrated that all reasonable alternatives to the activity have been considered, all feasible measures will be taken to minimize the effect of the activity on the species, and the activity will not jeopardize its survival or recovery.
2. The permit holder must submit to the Canadian Wildlife Service a report detailing the observed impacts of activities hereby permitted within 30 days after December 31 of each year of permit and upon expiry of this permit, including (as applicable) the number of individuals possessed, collected, captured, harmed, harassed, taken, or killed, and the number and location of residences damaged or destroyed, and the total area and location of critical habitat destroyed.
3. Only qualified personnel, experienced in the identification and life cycle of the target Species at Risk and familiar with the specific locations, will monitor and oversee the timing of the activities.
4. All activities carried out under the authority of this permit will be done in accordance with the methodology described in the permit application.
5. Toads will be captured and handled in ways that have a very low impact on individuals and will not damage their habitat or jeopardize the survival or recovery of the species.
6. Prior to exercising any use of this permit National Wildlife Areas managers are to be notified relative to collecting procedures, times and locations of collection.
7. All specimens are to be immediately released at their point of capture except for mated pairs held temporarily to count eggs released.
8. All feasible measures will be taken to minimize the impact of the field activity on the species or its critical habitat.
9. Less experienced personnel are required to work under the direction of more experienced personnel or field supervisors.
10. Work is to be performed under valid and approved Animal Care Committee protocol.

	31 May 2012		23 May 2012
Signature of holder	Date	For the minister Robert McLean as A/DG, CWS for Virginia Poter	Date

Canada