POPULATION DYNAMICS OF A DECLINING AMPHIBIAN

Daniel A. Greenberg

Department of Biology

McGill University, Montreal

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Abstract

With concern rising over the global decline of amphibian populations, identifying the onset of declines and the factors driving them is paramount. Amphibian populations are naturally characterized by large fluctuations in abundance, which makes separating natural fluctuations from true declines very difficult. By improving our understanding of the natural dynamics of amphibian populations, we can separate normal fluctuations from altered dynamics associated with decline. I apply this principle to an endangered population of Fowler's toads (*Anaxyrus* [=*Bufo*] *fowleri*) in Long Point, Ontario which appears to have gradually declined in abundance. With over two decades of mark-recapture data, I investigate what factors, intrinsic and extrinsic, drive growth in this population. Over this same period, there have been considerable changes to the toads' habitat, as an invasive strain of the common reed, *Phragmites australis*, has overtaken the wetlands used by toads for breeding. I show that the expansion of this reed has altered the dynamics of the toad population, causing progressive decline due to breeding habitat loss. Prior to 2002, the population of toads was driven by density-dependent growth and overwinter mortality. After 2002, at which point the reeds had eliminated most of the open water habitat, the population of toads responded only to extrinsic factors, particularly the water level of Lake Erie. I then ask whether the expansion of invasive *Phragmites* has changed not just the quantity of larval habitat, but also its quality, through the release of secondary compounds. I hypothesize that Fowler's toad larvae, as obligate gill breathers, will experience reduced survival, growth, and development in the presence of gill damaging secondary compounds from the invasive *Phragmites* and native *Typha*. In contrast, the sympatric Northern leopard frog (*Lithobates* [=*Rana*] *pipiens*) should exhibit similar performance as tadpoles in the presence of secondary compounds, as a facultative gill breather. Contrary to my expectations, I found that Fowler's toad tadpoles had a similar performance across treatments, despite the presence of secondary compounds. Furthermore, the native plant, *Typha*, but not the invasive *Phragmites,* appeared to impede growth in Leopard frog tadpoles. Based on these results, I conclude that the expansion of invasive *Phragmites* has the potential to impact species through changes to available habitat, but not by reducing larval habitat quality. By incorporating population dynamics into the study of amphibian declines we can improve our ability to infer causal links between population declines and the mechanisms that drive them.

Résumé

Alors que le déclin de la population amphibienne mondiale est de plus en plus préoccupant, il est primordial d'identifier les facteurs qui en sont la cause. Puisqu'une certaine fluctuation de population est normale, il est difficile de déterminer si une variation donnée est naturelle ou symptomatique d'un déclin réel. Une meilleure compréhension des fluctuations de la population amphibienne pourrait servir à distinguer entre les facteurs naturels de variation et une altération de dynamique associée à un déclin. Voilà l'idée directrice de cette étude d'une espèce à risque, le crapaud de Fowler (*Anaxyrus* [=*Bufo*] *fowleri*) de Long Point en Ontario, dont la population semble diminuer graduellement. À partir de l'information recueillie sur deux décennies de marquage-recapture, j'ai examiné les facteurs, internes et externes, de variation de leur population. Au cours de la période, leur habitat de reproduction a été considérablement détérioré par la présence d'une espèce envahissante de roseau commun, le *Phragmites australis*. J'explore le lien entre cette altération de dynamique et le déclin progressif des crapauds de Fowler. Avant 2002, leur population était régulée par des facteurs internes comme la croissance en fonction de la densité et la mortalité hivernale. Depuis, les roseaux communs ont éliminé la plupart de l'habitat en eau libre des crapauds de Fowler, dont la population ne répond plus qu'à des facteurs externes d'influence, en particulier le niveau de l'eau du lac Érié. Ensuite, je cherche à voir si la présence de l'espèce envahissante a influencé, non seulement la quantité d'habitat de reproduction du crapaud de Fowler, mais aussi la qualité de ce qui reste, par la production de composés secondaires. L'hypothèse explorée est que les composés secondaires nuisibles aux branchies, produits autant par l'espèce envahissante *Phragmites* que par l'espèce native *Typha*, réduisent les chances de survie, la croissance et le développement des crapauds de Fowler, qui respirent nécessairement par leurs branchies. En toute logique, l'espèce sympatrique grenouille léopard du Nord (*Lithobates* [=*Rana*] *pipiens*), qui respire par ses branchies de façon facultative, devrait être affectée de façon similaire. Cependant, ce n'est pas le cas ; j'ai remarqué que les têtards de crapauds de Fowler n'étaient pas affectés par la présence de composés secondaires. De plus, c'est l'espèce native *Typha* qui semblait nuire à la croissance des crapauds de Fowler, plutôt que l'espèce envahissante *Phragmites*. J'en conclus que l'expansion de cette dernière peut influencer plusieurs espèces par une réduction de l'habitat disponible, mais pas par la détérioration de la qualité de son habitat larvaire. En étudiant le déclin amphibien à partir de la

dynamique de population, nous pouvons améliorer notre capacité à établir des liens entre le déclin de populations d’espèces et les mécanismes qui y contribuent.

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Finally, thanks to you: the reader, for validating my time spent writing all of this. Now, on to the science.

Contributions of authors

Chapter One

I wrote this chapter with editorial support by David M. Green.

Chapter Two

I used abundance data provided by Dr. David M. Green, which was collected by Dr. Green and numerous volunteers and graduate students from 1989 to 2011, to design and perform the analyses. Dr. Green also contributed to providing parts of the environmental data and conducted the randomization test. The final manuscript was written by myself with strong editorial support from Dr. Green, and was published in the journal *Conservation Biology* with Dr. David M. Green as a co-author (DOI: 10.1111/cobi.12078, online edition May 21, 2013).

Chapter Three

I designed, conducted, and analyzed this experiment with editorial support provided by Dr. David M. Green.

Chapter Four

I wrote this chapter with editorial support by David M. Green.

CHAPTER I: Introduction and literature review – amphibian declines and population dynamics

Considerable attention has been paid to the modern biodiversity crisis (Brooks et al. 2006), which some have likened to a contemporary mass extinction event (Pimm et al. 1995). It is well recognized that globally biodiversity is threatened by anthropogenic impacts including the destruction, degradation, and fragmentation of habitat, widespread pollution, overexploitation, the increased introduction of destructive invasive species, and global climate change (Hoekstra et al. 2005; Brook et al. 2008). These factors are predicted to be negatively impacting populations and to drive further declines and extinctions into the future. Concurrent to this idea of a global biodiversity crisis are many reports of species assemblages suffering unprecedented, and often enigmatic, declines. At the forefront of this biodiversity crisis are global amphibian declines.

Amphibian declines have been documented across many different regions over the past 30 years (Stuart et al. 2004). This consensus, gleaned from many independent observations by scientists at the first World Congress of Herpetology in 1989 (Collins & Storfer 2003), was later supported with quantitative evidence suggesting declines have occurred both in the 1960's and late 1980's (Houlahan et al. 2000; Alford et al. 2001). The global amphibian decline has caused much concern for both scientists and the general public, as amphibians are often perceived as being sentinel or indicator species for environmental change (Wake & Vredenburg 2008). The global amphibian decline may therefore herald widespread environmental problems and the subsequent loss of biodiversity and ecosystems, and thus it is imperative to understand and accurately assess the causes and consequences of amphibian declines.

Amphibian Declines: Hypotheses & Evidence

There are many proposed hypotheses for the global amphibian decline, with various levels of support for each, and with some more relevant for particular regions than others. Broadly these hypotheses can be summarized as habitat loss, overexploitation, pollution, invasive species, emerging infectious disease and global change (Collins & Storfer 2003; Beebee & Griffiths 2005). Many of these hypotheses are relevant to biodiversity in general, and some are expected to disproportionately impact amphibians relative to other taxonomic groups.

Habitat loss has received much attention as a driving factor in amphibian declines and is widely to be considered one of the leading causes (Gardner et al. 2007; Gallant et al. 2007). The loss of habitat is relevant to all regions (Stuart et al. 2004), and is also closely linked to habitat fragmentation and degradation. Amphibians have typically considered being particularly vulnerable to habitat loss due to their biphasic life history (Semlitsch 2002; Cushman 2006), whereby they require both aquatic breeding habitat and suitable terrestrial habitat to survive. Fragmentation has also been hypothesized to have dire consequences for amphibians as they are considered to be, perhaps falsely (Smith & Green 2005), poor dispersers and therefore even mild fragmentation may isolate populations or interrupt migrations between breeding and terrestrial habitat (Becker et al. 2007). The impacts of habitat loss and fragmentation frequently go undocumented, however many studies have now shown negative effects on the individual (Todd & Rothermel 2006), population (Denaynadier & Hunter 1998), and community level (Lehtenin et al. 1999) for amphibians. Since wetlands, critical habitat for many amphibians, appear to suffer the highest rates of habitat loss globally (Brinson & Malvarez 2002; Gallant et al. 2007), this illustrates that while the impacts of habitat loss may be local it is certainly a global problem.

Overexploitation of amphibian populations is poorly documented and likely not a relevant factor for the majority of populations and species. Amphibians are typically collected for three purposes: for consumption, as educational models for anatomy, and for the international pet trade. Frogs legs are eaten in many cultures, and this has even resulted in attempts to farm certain species, particularly the American Bullfrog (*Lithobates catesbeiana*), which has in turn led to the introduction of this species and the pathogens it often carries (Jennings & Hayes 1985; Garner et al. 2006). The collection of wild amphibians for food is still widespread in many areas in Southeast Asia and Madagascar (Jenkins et al. 2009; Warkentin et al. 2009), both hotspots for amphibian diversity and endemism, but we still have practically no understanding of its scope and impact. The use of amphibians as models for anatomy goes back to the 19th century, and widespread collection has been documented since the turn of that century and continues to the present for several species (Jennings & Hayes 1985). The extent of collection of amphibians for the international pet trade is largely unassessed (Schlaepfer et al. 2005), but represents a potential risk for rare and declining species that are typically coveted by collectors (Courchamp et al. 2005). On the whole, overexploitation is likely highly localized but with a potential large impact on select species.

The role of industrial and agricultural pollution in global amphibian declines has remained largely elusive. For sudden and enigmatic amphibian declines it has been suspected that low levels of particular pollutants may be the culprit (Lips 1998). Pollutants can directly affect mortality of certain life stages (Blaustein et al. 2003), as well as impact reproduction by causing sterility or skewing sex-ratios (Hayes et al. 2002). No specific chemical agent has arisen as a single causal factor in declines, but increasingly research is considering the interaction of pollutants with other factors such as predation, parasites, disease and climate change (Boone & Semlitsch 2002; Rohr et al. 2008a; Hayes et al. 2010). Many studies focused on toxicological assays in the lab (Kerby et al. 2010), but some regional declines have been linked back to the use of pesticides (Sparling et al. 2001; Davidson 2004). Overall, our perception that amphibians are sensitive has probably generated more support for this hypothesis than there really is. Data suggests amphibians are no more sensitive to most chemicals than any other taxonomic group (Kerby et al. 2010), although this does not consider the synergistic role of other factors or differences in exposure risk (Hayes et al. 2010). The rise of medium wave ultraviolet radiation (UV-B) due to depletion of the ozone layer has also been of much interest due to its global nature, and capability to cause deformations, sterility, and death (Blaustein et al. 2003). It also fits with observations that amphibian populations are experiencing the most dramatic and sudden declines at high altitudes (Stuart et al. 2004), where UV-B exposure is also highest (Blumthaler et al. 1997). Much of the evidence for UV-B related impacts and declines come from controlled laboratory experiments on embryos and larvae (Blaustein et al. 2003), but many other field studies indicate a low risk of UV-B impact in natural settings (Palen et al. 2002; Licht 2003; Vredenburg et al. 2010; Palen & Schindler 2010). For both pollutants and UV-B radiation there exists a gap in our knowledge of whether strong impacts on individuals in controlled settings actually scales up to the point of causing declines in natural populations.

Invasive species can have widespread and far-reaching impacts on species and ecosystems due to their lack of shared evolutionary history with the native community. Invasive species impacts can be direct through predation and competition, or indirect through altering food webs, habitat, or acting as vectors for disease (Garner et al. 2006; Ficetola et al. 2011a; Boyce et al. 2012; McMahon et al. 2013). Amphibians are both impacted by alien species, and are notorious invasives themselves (Kats & Ferrer 2003). The introduction of the Bullfrog to western North America has been implicated in the declines of several amphibian species

(Kupferberg 1997; Lawler et al. 1999; Johnston et al. 2011). Their tadpoles have the unique trait of overwintering before metamorphosis, resulting in very large tadpoles that exert strong interspecific competitive pressure on sympatric species (Kupferberg 1997), while the large adults will wait at the edges of ponds to consume emerging metamorphs of any species (Lawler et al. 1999). Additionally, Bullfrogs are noted carriers, and an important vector, of the devastating fungus *Batrachochytrium dendrobatidis* or ‘chytrid’ fungus (Garner et al. 2006). Fish are also known to exert a major impact on amphibian populations and distributions. The frequent introduction of fish such as trout, bass, and pike for the purpose of recreational fishing has eliminated many amphibian populations from previously fishless water bodies in North America (Hecnar & M’Closkey 1997; Pilliod et al. 2010). A study by Ficetola et al. (2011b) found that invasive freshwater crayfish can strongly impact amphibian populations by predation on tadpoles, and recently it has emerged that crayfish can also act as vectors for the chytrid fungus (McMahon et al. 2013).

Introduced species can also impact species through non-trophic interactions. Several invasive plant species of Eurasian origin (Amur Honeysuckle, Purple Loosestrife, Chinese Tallow, Reed Canary Grass) have been shown to affect amphibians by altering their physical environment, including pond hydrology and microhabitats (Watling et al. 2011a; Boyce et al. 2012; Martin & Murray 2011), as well as affecting survival and larval development due to the release of secondary metabolites with decay (Maerz et al. 2005; Watling et al. 2011b). Introduction of invasive species is another factor that is highly localized but with an often large impact on the native amphibians.

The role of emerging infectious diseases in amphibian declines has been a subject of intense interest since the discovery of the disease chytridiomycosis, caused by the aforementioned *B. dendrobatidis* fungus (Berger et al. 1998). This fungus has been implicated in many declines in the Neotropics, Australia and North America (Berger et al. 1998; Pounds et al. 2006), where species in remote, undisturbed regions have suffered mysterious and enigmatic declines and apparent extinctions (Lips 1998; Lötters et al. 2009). The distribution of *B. dendrobatidis* is likely near global at this point (Lötters et al. 2009). It has been hypothesized that the fungus originated in Africa and transported via the international trade of *Xenopus laevis* for use in research (Weldon et al. 2004; Vredenburg et al. 2013). Other diseases have been implicated in amphibian declines, including ranaviruses and the water mould *Saprolegnia* (Blaustein et al. 1994; Jancovich et al. 2005), but have not received the same level of attention as *B. dendrobatidis* and therefore appear to be far less widespread and destructive. The majority of studies on chytridiomycosis have occurred in laboratory settings, where exposure typically leads to high rates of mortality (Voyles et al. 2009; Tobler & Schmidt 2010). Whether this same effect occurs in natural settings, where individuals have a greater ability to self-regulate to control infections (Richards-Zawacki 2010), has not received as much attention. Muths et al. (2011) found that while the prevalence of the *B. dendrobatidis* fungus did reduce adult survival in Boreal toads (*Anaxyrus boreas*) this was compensated by increased juvenile recruitment and as a result the overall population trajectory was unchanged despite the prevalence of infection. Similarly, Ouellet et al. (2005) found that the *B. dendrobatidis* was prevalent in North American amphibians collected as far back as the 1960's, with no evidence for catastrophic declines in this region. The wide variation in chytrid distribution and impact indicates that this emergent disease represents a complex factor in amphibian declines.

Finally, global climate change has been suspected of playing a role in global amphibian declines both by directly impacting populations and for its synergistic role with other factors (Blaustein et al. 2010). Since amphibians are reliant on often ephemeral aquatic habitat, and have low water retention compared to other terrestrial vertebrates, the impact of climate change on amphibians is expected to be severe (Donnelly 1998; Wake & Vredenburg 2008). The trend towards a warmer climate may result in widespread loss of breeding habitat and the loss of terrestrial microhabitat which is important for many species (Pounds et al. 1999; Carey & Alexander 2003; Blaustein et al. 2010). So far there has not been much evidence for a role of climate change alone in the global amphibian decline, although some impacts on populations have been noted (Pounds et al. 1999; Daszak et al. 2005; Reading et al. 2007). Climate change also has the potential to interact with other stressors including abetting the spread and outbreak of infectious disease (Kiesecker et al. 2001; Pounds et al. 2006), but evidence for this link remains equivocal (Rohr et al. 2008b). Since it is typically difficult to convincingly link broad-scale climatic changes to population declines of any species the contribution of this factor may be largely underestimated.

Since the first investigations of the amphibian declines our ability to discern declines and their causal basis has been poor. Much of the evidence for any particular factor is based on either laboratory or field experiments at the individual level (Biek et al. 2002; Licht 2003; Wilson et al. 2012), or correlative observational studies at the population level (Schmidt 2003; Reading et al. 2007; Rohr et al. 2008b). While both approaches are useful, it is hard to scale up individual level effects to the population level (Hels & Buchwald 2001; Vonesh & De La Cruz 2002; Schmidt et al. 2005) or to separate correlative evidence from causal inference (Rohr et al. 2008b). There is therefore a need to not just determine negative effects of these stressors on individual life stages, but to link these stressors to the population dynamics of the species (Vonesh & De La Cruz 2002; Schmidt et al. 2005; Wilson et al. 2012). Often a historically surveyed site will be resurveyed, and if it is found that previously occurring species are no longer present or at a reduced abundance (eg. Lannoo et al. 1994; Drost & Fellers 1996; Lips et al. 2004) this is often then related back to some particular factor, such as the presence of *B. dendrobatidis* in the environment (Lips et al. 2004; Caruso & Lips 2013). There are several issues with this approach, first is that detection probability is frequently ignored or spuriously assumed to be constant over time and space (Schmidt 2003). Additionally, amphibian populations are known to be highly variable over time (Alford & Richards 1999; Green 2003), and it has been well documented that large fluctuations in abundance are apparently a natural phenomenon in amphibian populations (Pechmann & Wilbur 1994; Alford & Richards 1999; Green 2003). This makes the task of separating natural fluctuations from decline very difficult. Therefore to confidently identify declines a clear understanding of the natural dynamics of amphibian populations is fundamental.

Amphibian Population Dynamics

Amphibian populations are subject to the same processes as any animal population, and the same equations to describe their dynamics thus apply. In all animal populations, temporal fluctuations in abundance are a result of births and deaths, and immigration and emigration (Turchin 2003). The changes in population size from year to year can be expressed with the logistic equation:

$$N_{t+1} = N_t e^r \qquad (1)$$

Where N_t and N_{t+1} are abundance in the previous and next year, respectively, and r is the annual rate of change, or population growth rate. The annual rate of change reflects the changes in a population from year-to-year and therefore encapsulates the processes of births, deaths, immigration and emigration. The magnitude of variation in this value then reflects the variability in these processes in the population, but cannot isolate whether any particular factor is driving the change in abundance.

Understanding what drives fluctuations in abundance is critical for identifying declines. Amphibians can exhibit changes in a population in the magnitude of 5 to 30-fold in a 12-year sample (Pechmann & Wilbur 1994), with this level of apparently natural variation it is exceedingly difficult to determine when a population is in decline (Pechmann & Wilbur 1994; Alford & Richards 1999). The solution is to then understand what factors drive the population dynamics of amphibians, to potentially determine whether fluctuations represent natural processes or the signature of altered dynamics due to any number of factors.

A general model of amphibian population behavior was presented by Alford & Richards (1999), which posits that amphibians are characterized by relatively constant survival rates of adults, coupled with highly variable rates of juvenile recruitment. They extend this model to suggest that amphibian populations are driven by infrequent years of high recruitment, interspersed with many years of low recruitment. Thus, amphibian populations should show punctuated dynamics of large increases followed by gradual decreases, creating a natural tendency towards "decline". This model offers both an explanation for the large fluctuations observed in amphibians, as well as suggesting that the apparent amphibian decline may not truly be a decline but a natural occurrence. The problem with this model is that it is not supported by observations of amphibian dynamics (Houlahan et al. 2001; Green 2003). The Alford & Richards model clearly predicts a distribution of many small decreases in abundance, and a peak of very large increases. Rather what we find is that the distribution of annual rates of change in amphibian populations follows a normal distribution centered close to 0, and the magnitude of fluctuation is equal when considering increases and decreases (Green 2003). Therefore, it appears this model is not suitable as a null model for amphibian population dynamics.

Rather than trying to characterize a general pattern for temporal fluctuations in amphibians, we can improve our understanding and predictive capability by attempting to characterize the general processes underlying amphibian population fluctuations. Temporal variation in our estimates of population size, assuming closure, can be attributed to the following sources: extrinsic factors, intrinsic factors, their interactions, unstable population structure and observation error.

Extrinsic factors

Extrinsic factors encapsulate all processes in the environment that act upon a population to influence births and deaths. These factors are generally stochastic, as in weather events, but can also be deterministic in the case of gradual environmental change (eg. ecological succession) or climatic factors such as periodic El Niño events (Lande et al. 2003). Amphibians, as ectotherms, should be highly responsive to extrinsic factors (Blaustein et al. 2010), but no quantitative analysis has been conducted on this subject.

Temperature and rainfall are two extrinsic factors predicted to be important for the population dynamics of many amphibians as they can influence the phenology of breeding events (Tinsley & Tocque 1995), the survival of larval offspring (Govindarajulu & Anholt 2006), the availability and hydroperiod of ephemeral water breeding habitat (Pellet et al. 2006), and adult activity, survival, and growth (Tinsley & Tocque 1995; Grafe et al. 2004). In the European tree frog (*Hyla arborea*) rainfall strongly influences temporal abundance by influencing the number of water bodies available for breeding (Pellet et al. 2006). In West African pig-nosed frogs (*Hemisus marmoratus*) rainfall has mild benefits for the survival of tadpoles and greatly increases survival of adults, which are sensitive to desiccation at high temperatures (Grafe et al. 2004). Similarly, population fluctuations of *Eleutherodactylus coqui* in Puerto Rico have been linked to extended dry periods, which increased juvenile and adult mortality due to desiccation and reducing foraging activity (Stewart 1995). Precipitation is not always beneficial however, it's been noted that stream salamanders suffer reduced adult recruitment as a result of a higher frequency of flooding driven by increasing spring and fall precipitation (Lowe 2012). Similarly a study of male European tree frogs (*Hyla arborea*) demonstrated low population growth associated with high rainfall two-years previous, corresponding to the time for juveniles to mature, suggesting a negative effect of rainfall on recruitment (Pellet et al. 2006). Finally, many populations appear to be unaffected by rainfall or temperature (Meyers et al. 1998; Salvidio 2007), as is likely the case for many amphibians occupying permanent aquatic habitats.

For temperate amphibians, winter severity can exert a great influence on annual survivorship of adults. In common toads (*Bufo bufo*) mild winters caused overwintering toads to use up energy reserves, lowering adult body condition, which subsequently reduced survivorship (Reading 2007). Similarly, in a metapopulation of great crested newts (*Triturus cristatus*) mild, wet winters reduced survivorship, putatively through depleted energy stores and water-logged soils drowning overwintering adults (Griffiths et al. 2010). For other species however, milder winters are associated with population increases as in the European tree frog (van Buggenum & Vergoossen 2012). Similarly, in a montane population of Columbia spotted frogs (*Lithobates luteiventris*) mild winters were associated with higher annual survival and fecundity (McCaffery & Maxell 2010). Differences in population responses may reflect both the overwintering behaviour of the species and their environment, as populations at their northern range limits will likely benefit from mild winters.

Any number of extrinsic factors can be included in a population model by expanding the simple logistic model for exponential growth:

$$N_{t+1} = N_t e^{\mu + \epsilon_t} \qquad (2)$$

where the drift constant, μ, indicates the trajectory of population growth and a component for stochastic noise is included, $\varepsilon_t \sim \text{Normal}(0, \sigma^2)$. Specific extrinsic factors such as temperature, rainfall, or snowfall can also be added to this model:

$$N_{t+1} = N_t e^{\mu + Z_t + \epsilon_t} \qquad (3)$$

where Z_t is some environmental variable in the present year, as many extrinsic factors can be added as we have data for and their significance evaluated through model selection (Burnham & Anderson 2002). This extrinsic variable will attempt to partition some of the variance of the noise component, ε_t, to a specific environmental factor and if the noise component is reduced then this will improve model fit. Similarly, we can make the response to extrinsic factors non-linear, for instance if it decreases population growth at extreme lows or highs, by square-transformation, or adding a scaling term to Z_t (Pellet et al. 2006).

Overall, extrinsic factors are important drivers of temporal abundance, and the response of populations to such stochastic factors greatly increases the potential for local extinction (Lande et al. 2003).

Intrinsic Factors

The influence of extrinsic factors can be largely overruled or buffered by intrinsic factors. Density dependent population growth is a widespread phenomenon in animal populations (Sibley et al. 2005; Brook & Bradshaw 2006) and can greatly influence temporal population trends. We can define density-dependent growth, or population regulation, as a change in the per-capita rate of population growth with density or abundance (Murdoch 1994; Turchin 2003; Herrando-Pérez et al. 2012). The terminology for density-dependent growth is vast and largely confusing (Herrando-Pérez et al. 2012), owing to the numerous forms that this relationship can take. Typically the term density-dependent population growth refers to a reduction in per capita growth with increasing density (Murdoch 1994), and hereafter when I refer to population regulation I am referring to this particular phenomenon. The reverse can also be true however, for instance the Allee effect is a special case of reduced per capita population growth at low densities owing to some minimum threshold of abundance required for a population to successfully recruit offspring (Courchamp et al. 1999). Density-dependent growth can be either under or over-compensatory, referring to the return rate of the population towards its carrying capacity (Turchin 2003). Under-compensatory density-dependence results in a gradual return to its carrying capacity, and therefore is associated with stable dynamics and strong population resilience to environmental perturbation (Lande et al. 2003). Over-compensatory density-dependence results in the population overshooting its carrying capacity, and typically creates periodic cycles in abundance (Turchin 2003). Density-dependence can additionally be either direct, whereby population in the present year influences growth to the next year, or lagged, whereby the abundance several years ago may influence growth to the next year (Turchin 2003). As you can see, the proliferation of confusing terminology for density-dependent growth is unsurprising given the numerous forms it can take.

Assessing the magnitude, form, and strength of density-dependence is critical for studying population dynamics. The detection of this phenomenon has received much attention, and continues to be a contentious issue. Nearly all methods have their drawbacks, including high Type I and II error rates depending on the particular test (Shenk et al. 1998) or requiring numerous years of census, beyond the vast majority of ecological time-series (Dennis & Taper 1994). Subsequently, the use of several diagnostic tests provides a more robust analysis for detecting density-dependence. It must also be noted that merely detecting density-dependence gives no indication of the mechanism or at what life history stage density is acting upon. In amphibians, numerous experimental studies indicate a strong density-dependent survival and growth in the larval stage (Wilbur 1976; Altwegg 2003; Loman 2004), and with little or no density signature at the terrestrial stage (Pechmann 1995; Altwegg 2003). However, other studies have found evidence of terrestrial regulation for both juveniles and adults amphibians (Harper & Semltisch 2007; Berven 2009). This indicates that density-dependence may act on several life stages, perhaps even in tandem, and has to be assessed on a species-by-species basis.

For amphibian populations there has been scant analysis of whether populations exhibit density-dependence. For those few populations showing population regulation, all indicate that they are governed by direct and stable density-dependence with modest return rates (Meyer et al. 1998; Pellet et al. 2006; Salvidio 2007; Salvidio 2011). Only two amphibian populations display delayed density-dependence, one of the cave salamander (*Speomantes strinatii*) and a population of the common frog (*Rana temporaria*) being lagged by 2 and 3 years respectively (Salvidio 2011). This indicates that overall density dependence in amphibians should be stabilizing regulation, and will therefore buffer against environmental stochasticity.

Several models have been developed that incorporate density-dependence into the derivation of population growth. Ricker (1954) developed a simple extension of the logistic model (eq. 1) that includes a maximum growth rate and carrying capacity that produces a pattern of density-dependence:

$$N_{t+1} = N_t e^{r_0\left(1-\frac{N_t}{K}\right)+\varepsilon_t} \qquad (4)$$

Where the ratio of present abundance, N_t, relative to the carrying capacity, K, reduces the maximum population growth, r_0. The relationship between abundance and population growth for this model is thus linear (Fig. 1). Several models describe a non-linear relationship between growth and density (Fig. 1), including the Gompertz model (Dennis & Taper 1994), eq. 5, and the Theta-Logistic model (Sæther et al. 2002), eq.6.

$$N_{t+1} = N_t e^{a-b\mathrm{Ln}(N_t)+\varepsilon_t} \tag{5}$$

$$N_{t+1} = N_t e^{r_0(1-(\frac{N_t}{K})^{\theta})+\varepsilon_t} \tag{6}$$

Where *a* and *b* in the Gompertz model represent the maximum growth and strength of density effects, from this you can calculate the carrying capacity as $e^{\frac{a}{b}}$. The Theta-Logistic is an extension of the Ricker Logistic model with a scale parameter, θ, that can create a convex (θ > 1) or concave (θ < 1) curve depending on the value of this parameter (Fig. 1). These models can be used to describe various relationships between growth and density, and can be analyzed for accuracy based on model selection criteria (Brook & Bradshaw 2006). Since population regulation greatly influences how a population responds to disturbance, detecting this phenomenon is important in the context of amphibian declines. The ideology of these models relates to this concept of carrying capacity, which can be reduced by many of the factors outlined previously including habitat loss, climate change, and pollution. As such, we can incorporate this loss of carrying capacity into our models of population dynamics for declining amphibians.

Few amphibian populations and species have sufficiently long, and accurate, time-series to assess the role of density dependence in a meaningful way, however if amphibians are characterized by direct population regulation this has large consequences for how we expect a population to behave in the face of environmental disturbance. Importantly, if a population is regulated and does not recover from perturbation this suggests a fundamental shift in the underlying dynamics of that population, which may indicate the onset of decline.

Interactions of Intrinsic and Extrinsic Factors

Both exogenous and endogenous factors can act together to influence population dynamics, and additionally these factors can interact with each other to produce multiplicative effects (Dennis & Otten 2000). For amphibians, the carrying capacity of a population may be strong influenced by extrinsic factors such as rainfall or drought (Pellet et al. 2006). During years of low rainfall, ephemeral ponds will lose volume and this may heighten intraspecific competition in the larval stage, producing a pattern of density dependence (Tejedo & Reques 1994). A mark-recapture study on Pacific treefrog (*Pseudacris regilla*) tadpole survival in natural ponds found that at low density higher temperatures were beneficial, while at high density survival decreased with temperature (Govindarajulu & Anholt 2006). This was explained by the fact that higher temperatures increase metabolic rate and therefore the energy demand of tadpoles, and as a result this would strengthen exploitative competition (Govindarajulu & Anholt 2006). Similarly, if the carrying capacity is a product of the number of high quality refuge sites, any abiotic factor that influences the abundance or quality of these sites may shift the strength of density-dependence in that population (Rudolf & Rödel 2005). Alternatively, density dependence may exacerbate the effects of extrinsic factors. This was the case for spadefoot toads (*Scaphiopus couchi*) breeding in ephemeral desert ponds, where high tadpole density slowed time to metamorphosis and subsequent pond desiccation resulted in total recruitment failure (Newman 1987). Numerous mechanisms exist whereby extrinsic and intrinsic factors can interact to affect the vital rates of individuals.

We can incorporate both intrinsic and extrinsic factors in one population growth model by combining eq. 3 and 4:

$$N_{t+1} = N_t e^{a+bN_t+ cZ_t+ \varepsilon_t} \qquad (7)$$

Whereby the extrinsic variable influences the carrying capacity by reducing or increasing a, equivalent to r_0, and therefore sets the carrying capacity at time t according to the following equation:

$$K_t = \left(\frac{-(\mathrm{a}-\mathrm{c}\mathrm{Z}_\mathrm{t})}{\mathrm{b}}\right) \qquad (8)$$

This integration of extrinsic and intrinsic factors may be a useful model for species that rely primarily on ephemeral habitat which varies according to any number of abiotic factors. The relative role of extrinsic and intrinsic factors in dictating population growth has been a subject of much debate in ecology (Turchin 2003), but determining their relative influence is paramount to understanding population dynamics and the natural patterns of population fluctuations.

Unstable Population Structure

Many population models, including those presented previously, assume a stable age, stage, or size structure in the population and lump together all individuals across classes. In reality, the structure of populations is rarely near the stable distribution in natural settings (Fox & Gurevitch 2000; Clutton-Brock & Coulson 2002), and therefore ignoring age structure greatly reduces our ability to predict future abundance (Clutton-Brock & Coulson 2002). Unstable population structure often results in 'transient dynamics' whereby population size can fluctuate greatly as it approaches the long-term asymptotic growth rate of the stable distribution (Ezgard et al. 2010). This scenario is most profound in species that exhibit high reproductive investment, long generation times, and with vital rates that vary greatly with environmental conditions (Koons et al. 2005), all attributes that apply to many amphibians. For many amphibian populations, age structure is frequently high variable over time and rarely converges onto a stable structure (Miaud et al. 1993; Kellner & Green 1995; Reaser 2000; Marunouchi et al. 2003). As a result fluctuations in some amphibians may be driven by unstable age structures.

This state of transient dynamics due to unstable age structures may not necessarily be only relevant for short-term dynamics. The importance of intrinsic and extrinsic factors frequently varies across different age and stage classes (Farand et al. 2002; Grafe et al. 2004). For amphibians, it's expected that juveniles are disproportionally affected by extrinsic factors such as desiccation, due to lower mobility and greater surface area-to-volume ratios (Rothermel & Semlitsch 2006), as well as limited energy stores for overwintering (Scott et al. 2007). The result is that stochastic variation in the environment can destabilize the age distribution and possibly create cohort effects, whereby population cohorts differ in vital rates due to differences in their early developmental environment (Lindström & Kokko 2002). These cohort effects can further increase population fluctuations and cause unbalanced age structure (Lindström & Kokko 2002). If environmental perturbation is therefore frequent enough, the population may never reach a stable age distribution and the 'transient' dynamics may proceed indefinitely.

As the quality and quantity of data on amphibian populations improves, due to long-term monitoring effort to document declines, the use of more sophisticated stage structured models may enable us to more accurately characterize population dynamics. Long term time-series on multiple age or stage classes in amphibian populations will provide greater insights into deciphering population fluctuations compared to univariate measures of adults or total abundance alone.

Observation Error

It has been well recognized for many years now that, put quite simply, counting animals is difficult. Numerous methods to accurately census populations have been developed, but over 90 % of published amphibian time-series are still based on a simple count index (Schmidt 2003). This makes detecting declines or determining dynamics exceedingly difficult, because detection probability is not constant over time and is often highly correlated with other variables such as weather (Hyde & Simons 2001; Schmidt 2003). As such, count based time-series have limited uses, as fluctuations can just as easily be due to measurement error as they can be a result of population response. While there are methods to estimate the amount of observation error using state-space models (Dennis et al. 2006), and subsequently smooth estimates based on the proportion of error and process noise, this still highly limits our ability to interpret dynamics (Schmidt 2003). Despite the limitations of count based time-series, they continued to be used to infer population dynamics in amphibians (Meyer et al. 1998; van Buggenum & Vergoossen 2012). To what degree extreme fluctuations observed in amphibian time series are a result of process noise, measurement error, or both is still unknown. Therefore, accurate censuses using mark-recapture based methods are invaluable for studying amphibian population dynamics and declines.

Incorporating Population Dynamics to Study Declines

Understanding the processes underlying population fluctuations is critical for examining declines in amphibian abundance. By characterizing how populations should change over time, on the basis of the extrinsic and intrinsic factors known to influence growth, we can then detect departures from these predictions. Similarly, these same principles can be extended to the study of changes in the number of populations over time (Green 1997), and can be used to track the dynamics of population extinctions and recolonizations over a species' range (eg. Adams et al. 2013). Outlining departures from natural dynamics will provide stronger evidence in establishing a causal basis for a putative factor to be causing decline, as well as helps us develop quantitative tools to detect early warning signals of decline in populations.

In this thesis, I will first show how long-term monitoring using highly accurate mark-recapture based census can be used to characterize the natural dynamics of an amphibian population and identify the timing and cause of a shift in dynamics due to a putative factor, the expansion of an invasive species, in Chapter Two. I hypothesize that the expansion of the invasive *Phragmites australis* has reduced the carrying capacity of the population, altering its previous dynamics around a set return point. In Chapter Three, I will examine a proximate mechanism of how this putative driver of decline may be impacting recruitment. I hypothesize that the expansion of *Phragmites australis* is degrading larval habitat through the release of secondary compounds and therefore reducing larval recruitment. I test this with a laboratory experiment examining vital rates of tadpoles exposed to various plant extracts. This will illustrate how studying population dynamics can clarify our understanding of amphibian declines and provide direct causal evidence to identify the driving agent.

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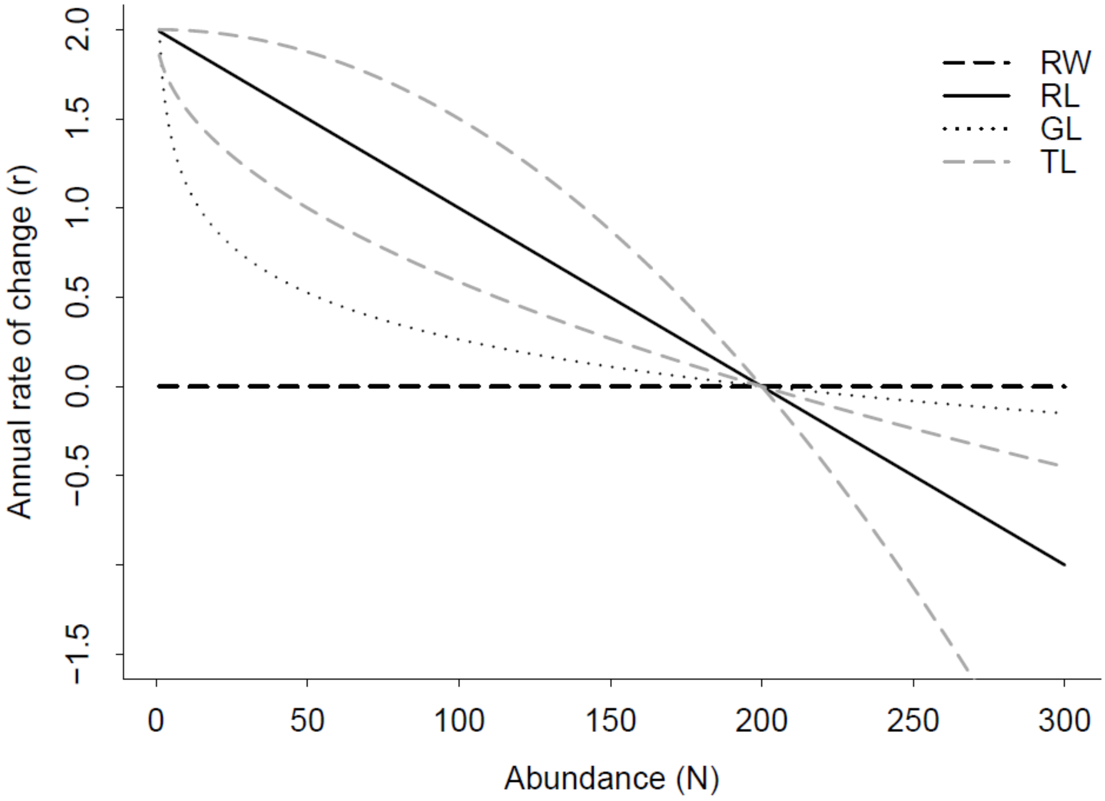

Figure 1. Relationship between the annual rate of change (= population growth rate) and abundance for a variety of density dependent models, and the density independent random walk (RW). The Ricker Logistic (RL) model is a linear relationship between r and N_t, while the Gompertz Logistic (GL) model is always a concave curve. The Theta-Logistic (TL) model can be either concave if the shape parameter < 1 or convex if > 1, as per the lower and upper lines, respectively. All of these models were parameterized with an $r_0 = 2$, and K = 200.

CHAPTER II: Effects of an Invasive Plant on Population Dynamics in Toads

Linking Statement

In order to confidently identify the onset of population declines, there must be some understanding of the dynamics underlying population growth. This is particularly true for species that exhibit naturally large fluctuations in temporal abundance, as do many amphibians. By characterizing the factors that drive growth in a population we can then determine if a populations' response shifts with some putative factor underlying decline. Here, I apply this principle to an amphibian population purported to be declining in Canada, the Fowler's toad (*Anaxyrus fowleri*). The apparent decline of Fowler's toads in both Long Point and other populations has not been specifically linked to any causal factor. While the *B. dendrobatidis* fungus has been linked to many amphibian declines, there have been no reports of mass mortality events in this region consistent with the epizooticic nature of chytridiomycosis. As such, I investigate alternative mechanisms of decline including how changes to habitat availability and climate may be driving the subsequent decline of this species.

Abstract

When populations decline in response to unfavorable environmental change, the dynamics of their population growth shift. In populations that normally exhibit high levels of variation in recruitment and abundance, as do many amphibians, declines may be difficult to identify from natural fluctuations in abundance. However the onset of declines may be evident from changes in population growth rate in sufficiently long time series of population data. With data from 23 years of study of a population of Fowler's toad (*Anaxyrus* [=*Bufo*] *fowleri*) at Long Point, Ontario (1989 – 2011), we sought to identify such a shift in dynamics. We tested for trends in abundance to detect a change point in population dynamics and then tested among competing population models to identify associated intrinsic and extrinsic factors. The most informative models for population growth included terms for toad abundance and the extent of an invasion marsh plant, the common reed (*Phragmites australis*), throughout the toads' marshland breeding areas. Our results showed density-dependent growth in the toad population from 1989 through 2002. After 2002, however, we found progressive population decline in the toads associated with the spread of common reeds and subsequent loss of toad breeding habitat. This resulted in reduced recruitment and population growth despite the lack of any significant loss of adult habitat. Our results underscore the value of using long-term time series to identify shifts in population dynamics coincident with the advent of population decline.

Introduction

The abundance of amphibians is in apparent decline around the globe (Houlahan et al. 2000; Collins & Storfer 2003). Numerous factors have been invoked to explain this, including emergent diseases, habitat loss, invasive species, climate change, and pollution (Alford & Richards 1999; Gardner et al. 2007; Pounds et al. 2006), but evidence for any particular driving factor is largely correlative and, in numerous cases, the causes of declines remain enigmatic (Stuart et al. 2004). In addition, many amphibian populations, particularly temperate pond-breeding species, may experience large fluctuations in abundance, generally driven by variation in annual recruitment (Alford & Richards 1999; Green 2003). The result is often a boom-and - bust pattern that makes it difficult to distinguish deterministic trends in abundance, due to any number of factors, from natural fluctuations (Pechmann & Wilbur 1994; Beebee & Griffiths 2005; Pellet et al. 2006). To determine whether a population is truly in decline, it is necessary to understand its dynamics under presumed normal conditions, ascertain when it departs from this regime into an uncompensated trend, and determine a probable cause for this shift.

The natural dynamics of populations, including populations of amphibians, can be strongly affected by intrinsic factors, such as population density, that influence abundance via variable population growth rates (Turchin 2003) or by extrinsic factors that directly affect births and deaths. These extrinsic factors can be stochastic, as with weather events, or deterministic, as with many forms of environmental change (Lande et al. 2003). By characterizing the effect of these various factors on populations under presumably normal conditions, it should be possible to identify deviations from these patterns that may be coincident with population decline.

Density-dependent population growth has a particularly important potential effect on population dynamics (Meyer et al. 1998; Turchin 2003). The occurrence of density-dependent regulation has been demonstrated in many animal groups (Brook & Bradshaw 2006), including some amphibian populations (Meyer et al. 1998; Pellet et al. 2006; Salvidio 2009). Density-dependent growth, assuming it is not overcompensating (May et al. 1974), implies there is an equilibrium size around which a population will naturally fluctuate (Crowley 1992; Turchin 2003). The tendency for a population to return to this equilibrium size results in fluctuations between upper and lower limits. This provides stability through bounded stochasticity (Crowley 1992). In general, amphibian populations appear to be density regulated in this way and characterized by stable, undercompensated dynamics with modest annual return rates (Salvidio 2011). As such, the detection of density-dependent growth may indicate an amphibian population is neither declining nor increasing. However, long-term time series for amphibian populations are rare (Houlahan et al. 2000; Brook & Bradshaw 2006).

Determining the underlying dynamics of a population requires accurate and unbiased estimates of abundance over many years. If the factors affecting a population's growth remain stable over time, the same underlying model of population growth should be expected to pertain throughout a data time series and during any particular period within it (Turchin 2003). If there were a trend in the time series, however, a change in model likelihood reflective of such a change in population dynamics would be expected. This may indicate a phenomenon of decline, particularly if extrinsic, rather than intrinsic, factors have a greater effect on population dynamics.

We used this principle to investigate the population dynamics of a population of Fowler`s toads (*Anaxyrus* [=*Bufo*] *fowleri*) over more than 20 years. We compared the annual abundance of these toads with various models of population growth that incorporated assorted intrinsic and extrinsic factors. Coincident with what appears to be a decline in the abundance of these toads over a decade of study has been the loss of breeding habitat attributable to the expansion of an invasive marsh plant. If this habitat loss has adversely affected the toads' population dynamics, then we expected to be able to detect a shift from the normal pattern of fluctuations in abundance to a deterministic trend of progressive decline.

Methods

Study Species and Area

We used data derived from the study of a population of Fowler's toads (*Anaxyrus fowleri*) at Long Point, Ontario, Canada. Fowler's toads are common and widespread in the eastern United States, but there are only 3 populations in Canada, all located on the northern shore of Lake Erie (Green 2005). Threats to the species' continuing existence in Canada have led to their classification as endangered under both Canadian federal and Ontario provincial legislation. The population at Long Point is of particular conservation interest due to its genetic differentiation from the other Lake Erie populations (Smith & Green 2004).

Our study was conducted along 8.5 km of the Lake Erie north shore at the western base of Long Point, Ontario (between 42°34’33” – 42°35’3” N and 80°22’15” – 80°28’24” W) (Fig. 1). The study area consisted of a system of sand dunes running parallel to sandy beach along the shore of the lake. North of the dunes were shallow marshes, where Fowler’s toads gathered to breed. Beginning in 1995, the area covered by the invasive strain of common reed (*Phragmites australis*) rapidly increased at Long Point (Wilcox et al. 2003; Badzinski 2008). This was coincident with what appeared to be a decline in the abundance of Fowler’s toads. The area invaded by the reeds included the shallow marshes historically used as breeding habitat by the toads (Green 1997). The reeds filled in these sites, after which the toads no longer used them.

Toad Abundance

We used an intensive mark-recapture method (Donnelly & Guyer 1994) to estimate abundances of adult male Fowler’s toads during each breeding season from 1989 through 2011. The protocol was designed to maximize recaptures, minimize effects of individual heterogeneity on detection probability, and obtain the high capture probabilities required for a reliable census (Freckleton et al. 2006). We identified all breeding sites each year by seeking out calling males after their emergence from winter dormancy in early to mid-May (Blaustein et al. 2002) and inspected them nightly through approximately 10 June, by which time chorus activity had waned. While considerable changes to breeding phenology have occurred over the course of the survey (Blaustein et al. 2002), in every year surveys began prior to the onset of breeding activity and are therefore unlikely to have impacted abundance estimates. We also surveyed on foot 5 km of beach adjacent to the breeding sites to capture males after the end of breeding activity (Green 1997). Animals were captured, individually marked with toe clips, and immediately released. All procedures with the animals were authorized under permits issued by the Ontario Ministry of Natural Resources and Environment Canada and Animal Use Protocol Number 4569 from McGill University.

We used the individual capture histories of all male toads encountered and the closed-capture models embodied in the program MARK (White & Burnham 1999) to estimate the number of living animals (N) each year. We assumed closure due to the intensity of the survey method, the boundaries imposed on the study area, and the short span of time over which each annual survey was conducted (Table 1). Furthermore, because toads move only very short distances (Smith & Green 2004), we considered immigration and emigration insignificant for the duration of each census.

To ensure consistency and comparability among years, all estimates of N used model M_t (Otis et al. 1978), which incorporated the assumption that capture probability ($\hat{p}$) varied only with time (Darroch 1958). This model was notated $\{N, \hat{p}(t) = c(t)\}$ by White and Burnham (1999). We chose this model from among a suite of models that incorporated terms for temporal, behavioral, or individual variability. The chosen model had the lowest value of Akaike's (1974) information criterion adjusted for small sample sizes ($\mathrm{AIC_c}$) in 20 of the 23 years of the survey and an average model likelihood of 0.87.

Environmental Variables

We obtained daily weather records for 1988 through 2010 from the National Climate Data and Information Archive (Environment Canada 2011) for the Port Colborne weather station located on the north shore of Lake Erie approximately 100 km east northeast of Long Point. This was the closest weather station to Long Point with continuous records from 1988 to the present. Like Long Point, Port Colborne is in the Eastern Lake Erie Basin climatic regime (Sly 1976), and weather patterns at the 2 stations are similar. The weather data included mean maximum summer temperatures (May 1 to September 30), summer rainfall (May 1 to September 30), and winter snowfall (January 1 to March 31. We obtained records on the average monthly water levels of Lake Erie for May through July each year from 1988 through 2010 from the Canadian Hydrographic Service (Fisheries and Oceans Canada 2011).

Common Reed

For quantitative assessments of the area covered by common reed at Long Point, we concentrated on the central 580 ha Crown Marsh region of our study area. We used the estimated area covered by common reed as calculated by Wilcox et al. (2003) and Badzinski et al. (2008) for 1985, 1995, 1999, and 2006. For 2010 we used ArcMap (version 9.2, ESRI, Redlands, California) to map the extent of the reed beds visible on aerial orthophotographs and calculated their total area within the marsh boundaries. The fine texture and exceptional height of the monospecific common reed stands were readily distinguishable from surrounding vegetation on the photographs (Rice et al. 2000). We derived year-by-year values of common reed extent for the purpose of analyses by assuming linear change between known data points.

Change-Point Analyses

To determine whether and when there might have been a change point in the dynamics of the population over the course of the 23-year time series, we used binary segmentation as implemented in the "changepoint" package (Killick & Eckley 2012) in R (version 2.13, R Development Core Team 2011). This analysis breaks the series into subsets and tests the hypothesis that the means are the same in each subset with a likelihood ratio test (Scott & Knott 1974). We tested for change points in both the mean and variance of the annual rate of increase in the population (r_t), where $r_t = \ln(N_t/N_{t-1})$ and N_t and N_{t-1} represent the abundance of toads in the current and previous year, respectively.

Density-Dependent Population Regulation and Trends in Abundance

Because periodic cycles in abundance may be the result of complex density-driven dynamics (May et al. 1974), we used an autocorrelation function (ACF) in R (version 2.13) to test the possibility of long-term cycles in the time series. The ACF was estimated through the correlation between pairs of abundance data at multiple time lags, N_t and N_{t-x}, where $x = 1\ldots22$, with a significant correlation at lag x indicating the period of abundance cycles (Turchin 2003). We used the Dickey-Fuller test, implemented in the "urca" package (Pfaff 2008) for R (version

2.13), to determine whether the population oscillated in abundance in a manner consistent with stabilizing density-dependent regulation (Dickey & Fuller 1979). We used this test with either a drift constant for the mean or a deterministic trend constant to examine an autoregressive model of the time series with the test statistic, τ, for the presence of a unit root. The unit root, if present, indicates the population was not regulated around a return point.

We used randomization tests on the toad abundance data to determine whether the average annual rate of increase (r_t) significantly deviated from zero, which would mean the null hypothesis of no trend was rejected. We used the whole data set ($n = 23$ years) and subsets of varying duration. Using a custom program written in BASIC, we randomized the abundance values in each of these data sets, without substitution, 1000 times and computed the average r_t for each randomization. We computed the mean and variance of these 1000 random average r_t values and computed the one-tailed probability that the average r_t obtained from the real temporal sequence of data was not more extreme than any value in this distribution. To test for the absence of a trend in the data, we also performed the two-part test for a return point developed by Schmidt and Meyer (2008). In this test, the presence of a return point indicates there is no trend.

Model Selection and Evaluation

We employed both an information theoretic approach (Akaike 1974; Burnham & Anderson 2002) and leave-one-out cross-validation (Turchin 2003), implemented in the package 'DAAG' (Maindonald and Braun 2011) in R (version 2.13), to evaluate candidate models of the population dynamics of the Fowler's toad population. The models variously incorporated terms for environmental variables, the extent of common reed in the marshes and the intrinsic factor of population density, which we modeled on the basis of our estimates of abundance either as N or $\ln(N)$ according to the Ricker (1954) or Gompertz (1825) logistic models of population growth, respectively. Density-dependent models were included on the basis of Dickey-Fuller test results. Each model represented the hypothesis that its particular combination of factors most strongly influenced the annual rate of increase of the population. As such, the null model contained only a stochastic term (ε_t) and a drift constant (i.e., $r_t = a + \varepsilon_t$). To avoid the unnecessary proliferation of uninformative models, we did not investigate multiyear time lags or complex interactions among factors that would not have been expected *a priori*. For each model, we calculated AIC_c scores, $\Delta\mathrm{AIC}_c$, and Akaike weights, which we used to identify the most informative model(s). From cross-validation, we calculated ΔCV (mean square error across all folds) and R^2_{pred}, which estimates the ability of the model to predict the test value relative to the mean of the series (Turchin 2003). Using the same set of models each time, we applied both methods of model selection for the entire 23-year time series and identified subsets of the time series to investigate those factors related to shifts in the dynamics of the population.

Results

Toad Abundance

In 909 evening surveys over 23 years of data collection, we made 3729 captures of 1957 individual male Fowler's toads. The number of toads captured each year ranged from 15 to 259, and individual capture probabilities ($\hat{p}$) ranged from 0.035 to 0.218 (Table 1). The estimated abundance of male toads went through a series of peaks and troughs over the 23 years of our study (Table 1 & Fig. 2). The highest of 5 peaks in abundance were in 1991 and 1993. The lowest abundances were in 1997 and 2011, when $N = 15$ (SE 0) individual males. From 1989 to 2002, there was a pattern of fluctuating abundance (Fig. 2). During this time, 6 of 13 changes (46.2%) were increases and 7 (53.8%) were decreases. However, after 2002 only one increase in abundance, in 2004, was recorded out of 9 population changes (11.1%).

Environmental Variables and Common Reed

Of the extrinsic environmental variables (Supporting Information), only Lake Erie water level exhibited a discernible trend over the study period. Although water levels averaged 174.4 (SE 0.2) m above mean sea level overall, all records prior to 1999 were above that level and all records thereafter, except for 2009 and 2011, were below. Summer air temperature averaged 22.9 ºC (SE 1.0) and increased 0.05 ºC/year. Summer rainfall ($\bar{x}$ = 419.6 mm/year [SE 23.0]) and winter snowfall ($\bar{x}$ = 85.5.6 cm/year [SE 37.0]) showed no patterns or trends.

The area of the Crown Marsh covered by common reeds remained <1 ha. until the advent of the invasive strain around 1995 (Fig. 2 & Supporting Information). After 1995 the extent of common reeds increased by approximately 11.1%/year. By 2010, common reeds covered 85.4 ha (15% of the total marsh area). The reeds were concentrated primarily adjacent to the toads' overwintering habitat and where they had been known to breed.

Change Point

There was a significant change in the mean and variance of r_t in the toad population in 2002. From 1989 to 2002, mean r_t was 0.136 and year-to-year variance in r_t was 0.920. Thus, overall abundance was increasing, but it was highly variable. After 2002, however, mean r_t was -0.308, and its variance was 0.110, indicating abundance was consistently decreasing. Accordingly, our subsequent analyses examined both the entire 23-year time series and the 2 consecutive subsets, 1989 – 2002 and 2002 – 2011.

Density Dependence and Trends in Abundance

There were no significant correlations in the autocorrelation function beyond a 1-year time lag in both the complete 23-year data set ($\text{ACF}_{[1]} = 0.572$) and the 2002–2011 subset ($\text{ACF}_{[1]} = 0.629$). These results indicate no evidence of long-term cycling or periodicity in the population during these periods. For 1989 – 2002, there were no significant correlations in the autocorrelation function.

Results of the Dickey-Fuller test showed the null hypothesis of the presence of a unit root could not be rejected when the test included a drift constant for the mean ($\tau = -2.46$, $p > 0.10$). However, the inclusion of a deterministic trend constant returned marginal evidence that abundance was regulated around a shifting return point ($\tau = -3.46$, $0.05 < p < 0.10$). Results of this analysis indicated that an initial carrying capacity of 176 male toads (SE 59) in 1989 declined by an average of 8 toads/year over time.

Trends in Abundance

Over the entire time series, the average r_t of the male toad population was -0.046. Randomizing the abundance data produced a distribution of average r_t values with a mean of -0.002 and variance of 0.003. The one-tailed probability that the actual average r_t of the data set was not more extreme than any value in this distribution was $p = 0.226$. When we partitioned the data into 2 subsets and repeated the randomization test for each, the distribution of average randomized r_t values for 1989 – 2002 had a mean of -0.004 and variance of 0.010. This result was not much different from actual average r_t of 0.043for the same period ($p = 0.350$). For the period 2002 – 2011, the distribution of average randomized values of *rt* was -0.005 with a variance of 0.020; however, the actual average r_t was -0.308 ($p = 0.015$). Thus, we rejected the null hypothesis of no trend.

The Schmidt-Meyer test returned a low probability that there was no trend ($p = 0.037$) over the entire time series. However, the results of this test differed significantly for the population on either side of the 2002 change point. The probability of no trend during the period 1989 – 2002 was $p = 0.96$, and the return point for abundance of male toads was 111 individuals (CI 68 to 181 individuals). But the probability of no trend during the subsequent period, 2002 – 2011, was $p < 0.01$ and there was no return point for the abundance of the toads.

Model Selection and Evaluation

The 10 most informative models for the entire data set included ln(N), and the top 8 of those also included the term for the extent of common reed (Supporting Information). For all models better than the null model, those with ln(N) had a cumulative $\mathrm{AIC_c}$ weight of 0.967 and those incorporating common reed had a cumulative weight of 0.938. The model with the lowest $\mathrm{AIC_c}$ value (Table 2) included the 4 terms ln(N), common reed, spring rainfall, and winter snowfall and had a significantly greater predictive capability compared to the null model ($R^2_{\mathrm{pred}} = 0.27$). Several models differing only in their inclusion of particular environmental variables provided a similar level of information ($\Delta\mathrm{AIC_c} < 2$). The simplest model in which all terms were significant (i.e., the most parsimonious model) contained only ln(N) and the term for common reed, which was always negative. Comparing the predictions of models parameterized on the basis of all years of data (Fig. 3a), the model containing only ln(N) performed poorly mainly because it predicted positive growth in the population after 2002 instead of the actual negative growth. Adding common reed to this model increased the accuracy of predictions relative to the realized negative values of r_t after 2002. The environmental variables such as rainfall and snowfall increased the variance in r without markedly changing this pattern, a result that suggests these variables did not add substantially to model likelihood.

All models better than the null model that explained variation in r_t from 1989 to 2002 included ln(N) (Table 2). Environmental factors, which performed relatively poorly as single-factor models, added significantly to population growth models that also accounted for toad abundance. The most informative model ($w = 0.682$) included ln(N) and winter snowfall and predicted observed r_t significantly better than the null model ($R^2_{\mathrm{pred}} = 0.33$). However, the next most informative model ($\Delta\mathrm{AIC_c} = 1.78$) included only ln($N$). When we compared the 2 models of density-dependent growth, ln(N) provided a better fit than N ($\Delta\mathrm{AIC_c} = 2.74$, evidence ratio = 2.61). Models parameterized on the basis of data from 1989–2002 all performed poorly in predicting r_t after 2002 (Fig. 3). This result is a further indication that the dynamics of the population changed at the that time and that it no longer responded to ln(N), common reed or the environmental factors that had held sway previously.

The only model more informative than the null model to explain variation in r_t following 2002 incorporated only Lake Erie water level ($w = 0.78$, $R^2_{\mathrm{pred}} = 0.21$) (Table 2). Even so, $\Delta\mathrm{AIC_c}$ for the null model was 1.51 ($w = 0.17$), indicating that defining r_t as a function of lake level between 2002 and 2011 was not a substantial improvement over simple stochastic growth. Models that included ln(N) or common reed all had values of $\Delta\mathrm{AIC_c} > 3$, a result that indicated the population was responding largely to extrinsic, stochastic factors.

Discussion

Population decline is the invariable prelude to population loss. Decline may be catastrophically rapid or so slow as to be nearly imperceptible but, in all cases, populations will decline in response to extrinsic causes of unfavorable environmental change. They shift from their previous, regulated dynamics into a period of adjustment to whatever levels of abundance the new environmental conditions may support. On the basis of over 2 decades of accurate census data, we have successfully identified this shift in the Fowler's toads at Long Point as they were affected by a major environmental perturbation: the loss of breeding habitat due to expansion of invasive common reeds.

The dynamics of the Fowler's toad population at Long Point from 1989 to the early 2000s is characterized by considerable fluctuations in abundance, which is entirely as expected for a pond-breeding anuran (Green 2003) and consistent with a hypothesis of density dependence. Such undercompensating regulation would be stabilizing, thereby increasing population persistence (Crowley 1992; Turchin 2003). It would also counteract stochastic environmental factors that would serve to increase variance in the rate of increase, exaggerate fluctuations in abundance, and foster higher probabilities of extinction (Lande et al. 2003). Yet density-dependent regulation is exceeding difficult to demonstrate (Dennis & Taper 1994), even with time series of data longer than ours, and we are unable to prove it conclusively despite the precision of our data. Detection is confounded when time series are short and highly variable, and rendered nearly impossible when changes in environmental carrying capacity induce trends in abundance. Thus, we used a battery of tests to investigate the dynamics underlying our 23-year time series of abundance data, including the Dickey-Fuller test, which is conservative but lacks statistical power for short time series, and model selection, which may be overly liberal in detecting density dependence. Nevertheless, it is not entirely necessary to prove that the population of Fowler's toads at Long Point, Ontario, is density regulated for our results to show quite strongly that the population shifted at about the year 2002 from a dynamic regime of fluctuating abundance to a condition of uncompensated decline.

The change in the dynamics of the toad population appears strongly linked to the uncontrolled spread of common reeds and the corresponding decline in the extent of open water in the Long Point marshes (Wilcox et al. 2003; Badzinski et al. 2008). The change point in our data in the year 2002 corresponds to a transition between an earlier period before common reeds dominated the marsh and a later period when common reeds effectively eliminated all shallow, sparsely vegetated, aquatic breeding habitat used by the toads. Common reeds proliferate especially during periods of low water (Whyte et al. 2008), thus Lake Erie water levels also played a significant role in this process. Once toads' breeding habitat had all but disappeared, any further expansion of common reeds may have been largely irrelevant for the toads, whose subsequent downward trend in abundance can be interpreted as regulation toward the new, lower carrying capacity of the altered environment. This would explain the strong effect of common reed and ln(*N*) on toad abundance over the full data set but the lack of significant effect of either variable when only abundance of the toads from 2002 on was considered.

Although the current outlook for this population of Fowler's toad may appear bleak, there is the potential for a successful recovery. The toads appear to be well-adapted to the dynamic nature of their marshland and sand dune environment (Breden 1988) and have high reproductive potential; adult female Fowler's toads can lay clutches of 2,000 to 10,000 eggs (Green 2005). Their natural resilience to environmental perturbations indicates that mitigation of breeding habitat loss through control of common reeds should result in a fairly rapid, positive response by the population. Although no effective method for controlling the invasive common reed has yet been devised (Tewksbury et al. 2002), temporary artificial breeding ponds could rescue this population of toads until effective strategies can be implemented to limit the extent of common reeds and restore breeding sites in wetland complexes.

Our results underscore the importance of long-term time series of data for identifying declines in vertebrate populations and their causes. Understanding of the natural dynamics of species, particularly those of conservation concern, is a necessity for identifying patterns consistent with changes to those dynamics (Pechmann & Wilbur 1994) and the onset of population decline (Lande 2003). The establishment and maintenance of long-term monitoring projects are therefore crucial for accurate assessment of population status, recovery of populations in decline, and mitigation of the causes of population loss.

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Table 1. Capture statistics, capture probabilities, and estimated abundances of male Fowler's toads (*Anaxyrus fowleri*) over 23 years within the study area at Long Point, Ontario.

Year	Abundance (N, SE)	Survey period (nights)	Capture occasions	Animals captured and marked	Total captures	Capture probability ($\hat{p}$)
1989	41, 1	48	20	39	107	0.130
1990	156, 40	35	9	58	68	0.041
1991	393, 24	40	23	253	365	0.035
1992	307, 16	39	18	222	367	0.064
1993	394, 23	36	20	259	389	0.046
1994	93, 4	38	13	83	166	0.144
1995	43, 1	37	19	43	141	0.170
1996	39, 2	39	16	37	95	0.151
1997	31, 1	31	13	31	90	0.218
1998	191, 4	32	28	180	494	0.092
1999	74, 4	35	17	15	45	0.176
2000	63, 5	44	22	50	92	0.065
2001	69, 4	40	21	60	125	0.084
2002	240, 23	46	22	139	197	0.035
2003	134, 14	46	19	85	128	0.049
2004	174, 6	41	30	152	336	0.064
2005	89, 5	40	20	77	162	0.090
2006	48, 4	39	17	38	72	0.090
2007	42, 3	40	18	39	87	0.110
2008	42, 6	40	13	32	51	0.085
2009	37, 2	41	22	34	79	0.095
2010	20, 3	41	16	16	28	0.085
2011	15, 0	41	17	15	45	0.176

Table 2. Summary of the most informative models predicting the annual rate of change (r_t) in the abundance of Fowler's toad (*Anaxyrus fowleri*) at Long Point, Ontario[a].

Period		Model: $r_t =$ [b]	ΔAIC$_c$	w	ΔCV	R^2_{pred}
1989 - 2011						
	1	$2.521 - 0.551\ln(N_{t-1}) - 0.0159P_{t-1} + 0.0023R_{t-1} - 0.0078S_t + \varepsilon_t$	0.00	0.412	0.432	0.27
	2	$3.392 - 0.547\ln(N_{t-1}) - 0.0152P_{t-1} - 0.0073S_t + \varepsilon_t$	0.68	0.209	0.472	0.20
	3	$2.749 - 0.550\ln(N_{t-1}) - 0.0140P_{t-1} + \varepsilon_t$	1.50	0.092	0.531	0.10
	4	$1.905 - 0.554\ln(N_{t-1}) - 0.0146P_{t-1} + 0.002R_{t-1} + \varepsilon_t$	1.76	0.071	0.491	0.17
	5	$191.4 - 0.578\ln(N_{t-1}) - 0.0193P_{t-1} - 0.0080S_{t-1} - 1.076L_{t-1} + \varepsilon_t$	1.87	0.063	0.657	-0.11
	null	$-0.0457 + \varepsilon_t$	4.62	0.004	0.590	-
1989 - 2002						
	1	$4.258 - 0.683\ln(N_{t-1}) - 0.0116S_t + \varepsilon_t$	0.00	0.682	0.551	0.33
	2	$2.837 - 0.591\ln(N_{t-1}) + \varepsilon_t$	1.78	0.115	0.771	0.06
	3	$0.652 - 0.0037N_{t-1} + \varepsilon_t$	2.74	0.044	0.813	0.01
	4	$9.100 - 0.622\ln(N_{t-1}) - 0.314T_{t-1} - 0.0108S_t + \varepsilon_t$	3.01	0.034	0.630	0.24
	5	$3.091 - 0.630\ln(N_{t-1}) + 0.002R_{t-1} - 0.0104S_t + \varepsilon_t$	3.04	0.033	0.649	0.21
	null	$0.0846 + \varepsilon_t$	3.86	0.014	0.824	-
2002 – 2011						
	1	$522.75 - 3.00L_{t-1} + \varepsilon_t$	0.00	0.811	0.077	0.21
	null	$-0.308 + \varepsilon_t$	1.51	0.179	0.098	-

[a] For each period, the 5 most informative models are presented, except for 2002 – 2011 for which only one model had an AIC$_c$ value less than the value for the null model. [b] Abbreviations: *N,* toad abundance; *P*, extent of common reeds; *R,* spring rainfall; *S* , winter snowfall; *T*, summer temperature; *L*, lake level.

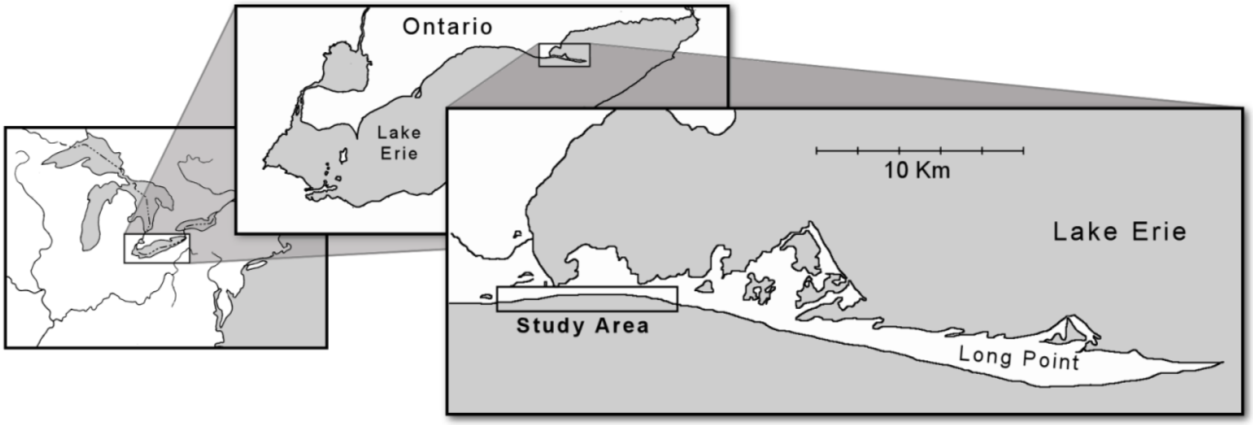

Figure 1. Location of the study site on the Long Point peninsula on the north shore of Lake Erie in Ontario, Canada.

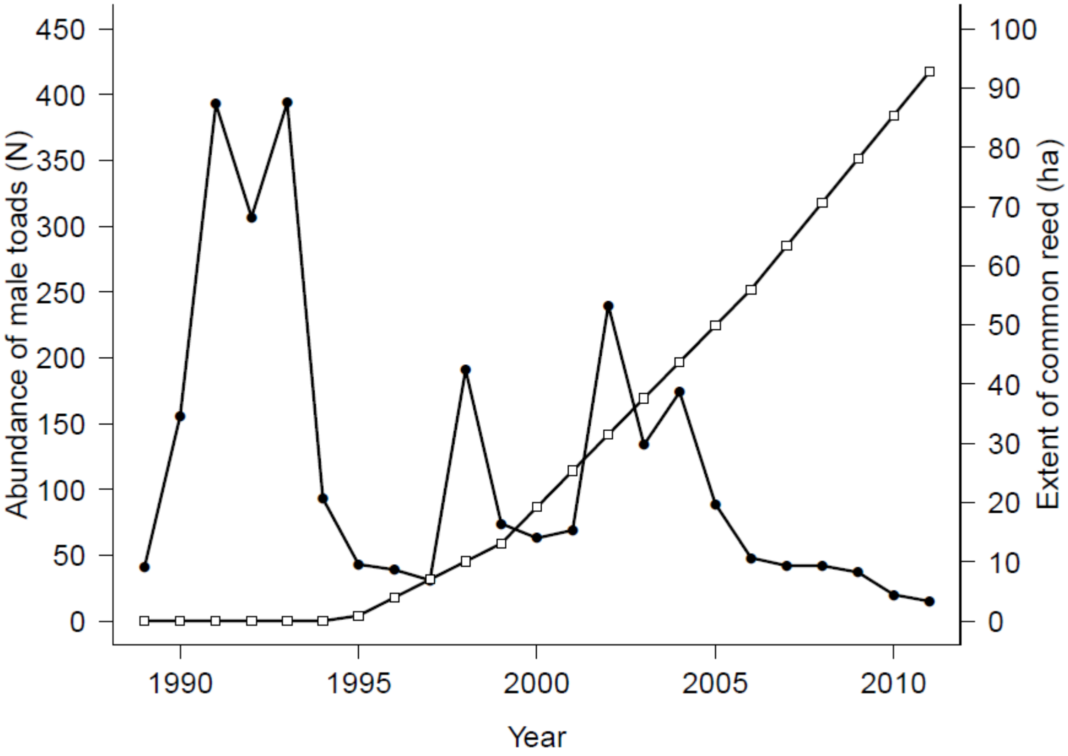

Figure 2. Estimated abundance of male Fowler's toads (shaded circles) and extent of invasive common reed (open squares) over 23 years, 1989 – 2011, at Long Point, Ontario.

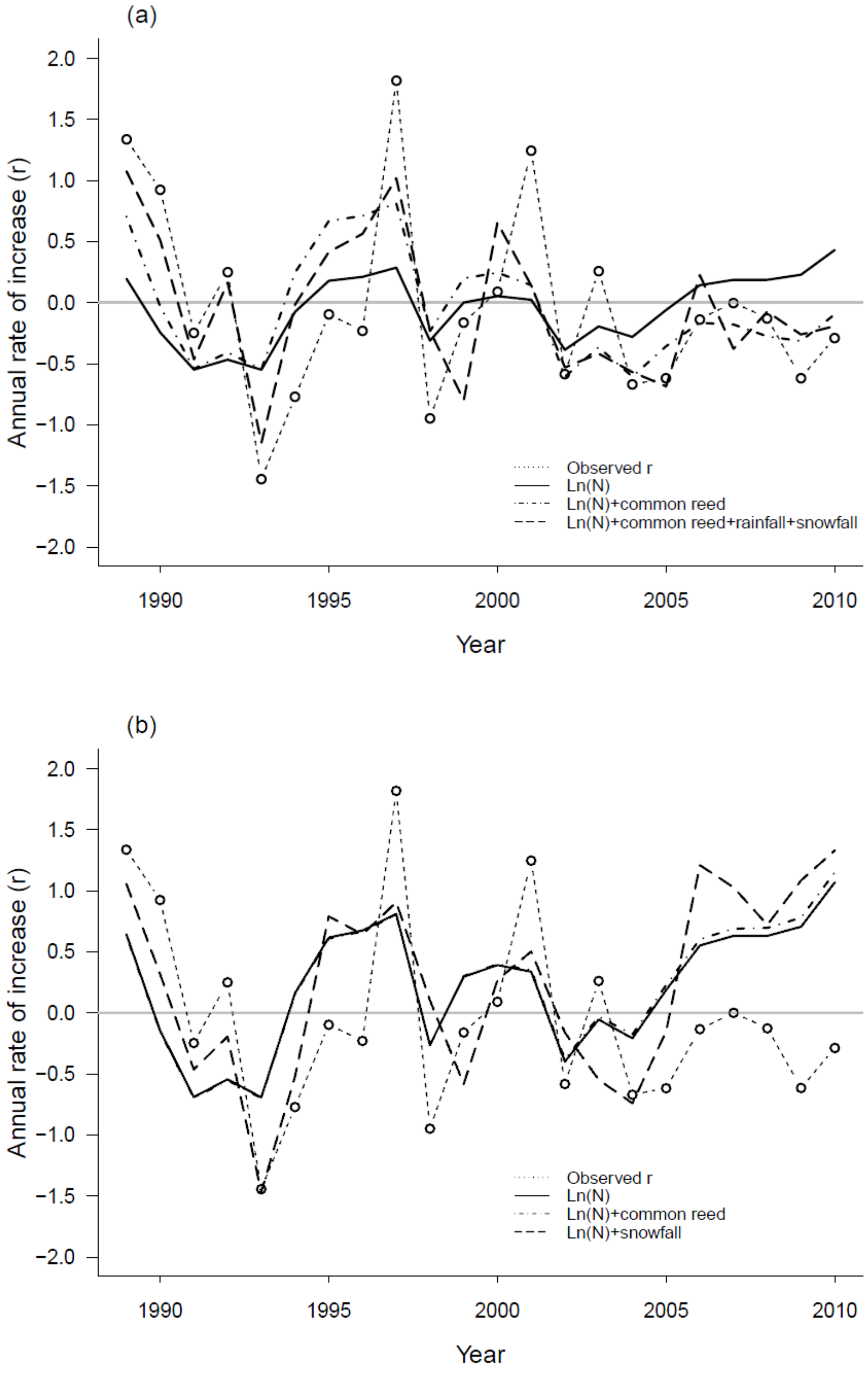

Figure 3. Variation in observed annual rate of change (*r*) in Fowler's toad abundance relative to predictive models of abundance (Table 2): (a) models parameterized on the basis of data from 1989 - 2011 and (b) models parameterized on the basis of data from 1989 - 2002.

CHAPTER III: Assessing proximate mechanisms of decline – degradation of larval habitat by plant secondary compounds

Linking Statement

Identifying agents of decline is merely the first step towards understanding the problem. Subsequently, the next task is to determine the mechanistic basis by which those agents are driving a population to decline. Here, I investigate whether the causal agent of decline in the Fowler's Toads at Long Point, the invasive strain of *Phragmites australis*, is impacting recruitment by degrading larval habitat via plant secondary compounds.

Abstract

Changes to the plant communities can precipitate numerous changes to the abiotic environment, including the introduction of novel chemicals: plant secondary compounds. Plant compounds are ubiquitous in many aquatic environments, but we still lack clarity in our understanding of how they impact the organisms within those communities. Here, I investigate how the expansion of two emergent marsh plants, the invasive *Phragmites australis* and native *Typha latifolia*, in the wetlands of Long Point may affect the larval performance and development of two anurans, the Fowler's toad (*Anaxyrus fowleri*) and Northern leopard frog (*Lithobates pipiens*), through the release of secondary compounds. These anuran species differ in some key larval traits, particularly the timing of lung development, which is expected to influence their sensitivity to gill damage caused by plant secondary compounds. I hypothesized that *A. fowleri*, as an obligate aquatic respirator, would experience reduced larval fitness when exposed to either plant compound, while *L. pipiens* larvae would experience similar performance across treatments, due to early development of lungs. Individual tadpoles were raised in water inoculated with senescent tissue of *Phragmites*, *Typha*, or a no plant control. Tadpoles were monitored daily for survival, with weekly measurements of body size, and final developmental stage after 21 days. I found that contrary to my expectations, *A. fowleri* tadpoles experienced no significant differences in survival, growth, or development in the presence of plant secondary compounds. As well, *L. pipiens* experienced a significant initial reduction in growth in the native *Typha*, and performed best in the presence of *Phragmites* compounds. This reiterates the point that non-native species do not always exert a negative impact on native species by virtue of their origin. This also illustrates that the expansion of *Phragmites* in the wetlands of Long Point is unlikely to significantly degrade larval habitat in these two species, and indeed may even benefit the Northern leopard frog.

Introduction

Population declines are frequently precipitated by environmental change, be it natural or anthropogenic (Blaustein & Kiesecker 2002; Brook et al. 2008), but disentangling the various factors that may be driving declines has remained a difficult task in conservation biology. Environmental change can be accelerated by the introduction of species that are considered ecosystem engineers (Crooks 2002; Byers et al. 2010), those that alter the availability of resources, typically through modifying habitat, with subsequent effects on abiotic and biotic conditions (Jones et al. 1994). For example, many plant species directly or indirectly affect various aspects of their environment, including surface temperatures, microclimates, soil and sediment dynamics, hydrology, and soil or water chemistry (Vitt & Chee 1990; Breshears et al. 1998; Madsen et al. 2001). Changes to plant communities, through either natural processes such as succession or the introduction of non-native species, can thus precipitate numerous changes to the environment that may subsequently cause the decline of native species (Maerz et al. 2009). Understanding how these changes impact species is paramount to preventing the gradual erosion of biodiversity.

Many wetlands in North America have undergone significant environmental change due to pollution, drainage changes, human development and the introduction of non-native species (Brinson & Malvarez 2002). In the wetlands at Long Point, Ontario considerable changes to the landscape have occurred since the onset of modern development. These wetlands historically consisted of grasses, sedges and hummocks where succession, water level fluctuations and periodic storm events produced a temporally dynamic plant community (Leahy et al. 2005). However, over the past 20 years these wetlands have been progressively changing to a more consistent, uniform landscape dominated by large stands of emergent vegetation, particularly the invasive genotype of *Phragmites australis* and the native *Typha latifolia* (Wilcox et al. 2003; Leahy et al. 2005).

The invasive strain of *Phragmites australis* was introduced to the Long Point wetlands in 1995 (Wilcox et al. 2003), and the area it occupies has expanded exponentially (Wilcox et al. 2003; Greenberg & Green 2013). The success of the invasive *Phragmites* in wetlands across North America has been attributed to its tolerance of disturbance and its capability to modify the hydrology, food webs, and community dynamics of invaded environments (Minchinton et al. 2006). When *Phragmites* colonizes wetlands it produces dense monotypic stands of ramets, which can begin to overtake the landscape (Rice et al. 2000). One trait hypothesized to give *Phragmites* an advantage over native marsh plants is its ability to actively secrete a concentrated phenolic secondary compound, gallic acid (3,4,5-trihydroxybenzoate), which has been shown to substantially inhibit the growth of competitors (Rudrappa et al. 2007).

The native cattail, *Typha latifolia*, also produces dense clonal stands around permanent water bodies (Dickerman & Wetzel 1985), but the gradual replacement of *Typha* in the Long Point wetlands by the invasive *Phragmites* suggests that it is losing ground to the novel invader (Wilcox et al. 2003). Like *Phragmites*, *Typha latifolia* has also been shown to produce numerous secondary compounds (Ozawa & Imagawa 1988), but it is unclear whether these phenols exhibit the same level of toxicity as those produced by *Phragmites*.

The aquatic larvae of many amphibians may be exposed to secondary compounds released by senescing plant material (Maerz et al. 2005; Earl et al. 2012), but what effect the expansion of *Phragmites* and *Typha* in wetlands will have on larval recruitment is still largely unknown. The release of plant secondary compounds into the aquatic environment has been recognized as a significant influence on larval habitat quality for certain amphibian species (Maerz et al. 2005; Watling et al. 2011b; Earl et al. 2012; Cotton et al. 2012). Impacts of plant secondary compounds on larval anurans include reduced survival, growth, mass at metamorphosis, and delayed development, with the effects varying significantly across different species (Maerz et al. 2005; Earl et al. 2012; Stephens et al. 2013). The mechanism by which phenols impact amphibian larvae is thought to be through damage to the gill epithelium (Maerz et al. 2005; Watling et al. 2011b), as has been found to occur in fish (Kirk & Lewis 1998), which may impede oxygen uptake. As a result, interspecific differences in sensitivity to phenols may be the result of differences in the timing of lung development (Maerz et al. 2005) as some species only develop functional lungs shortly before metamorphosis (Savage 1952; Feder 1984) and thus lack an alternative mechanism to acquire oxygen if gill function is impeded.

Over the past two decades at Long Point, at the same time as considerable changes to the wetlands have occurred there has been a progressive decline in abundance ofan endangered population of Fowler's toad (*Anaxyrus* [=*Bufo*] *fowleri*). This has been related to breeding habitat loss associated with the expansion of the invasive *Phragmites* (Greenberg & Green 2013). Fowler's toads are known to prefer shallow, oligotrophic wetlands with low levels of emergent vegetation and organic materials (Tupper & Cook 2008). Similar to other toads, *A. fowleri* tadpoles only develop lungs shortly before metamorphosis (Feder 1984), and are thus limited to aquatic respiration through most of their development. This suggests they may be susceptible to gill damage incurred by the presence of phenol compounds. Concurrently, several species of ranid frogs have maintained a high abundance and distribution in the wetlands of Long Point despite changes to the plant community (Archer & Jones 2008). Ranid frogs develop lungs as early as Gosner stage 29 in their larval development (Feder 1984), and thus may be able to supplement oxygen through aerial respiration and potentially abate the negative effects of phenol compounds (Maerz et al. 2005). Therefore, it appears that changes to the plant communities in these wetlands may be influencing the population viability of resident anurans by altering larval habitat quality for some, but not all species.

The goal of this study is to assess how the presence of *Typha* and *Phragmites* in wetlands alters the quality of habitat for larval anurans through the release of secondary compounds. We examine this by comparing larval fitness in both an obligate gill-breathing species, Fowler's toad (*Anaxyrus fowleri*), and a facultative gill-breathing species, the Northern Leopard Frog (*Lithobates pipiens*), raised in the presence of secondary compounds from either of these two plants. If obligate gill-breathing tadpoles are sensitive to plant compounds from emergent vegetation, then *A. fowleri* tadpoles should have significantly reduced survival, growth, and development in both *Typha* and *Phragmites* extracts relative to a control. Conversely, *L. pipiens* tadpoles old enough to breath with their lungs should be relatively unaffected by the presence of *Phragmites* or *Typha* secondary compounds.

Methods

Study Species and Area

Egg masses of both the Fowler's toad (*Anaxyrus fowleri*, N = 2) and the Northern leopard frog (*Lithobates pipiens*, N = 8) were collected opportunistically from wetlands in Long Point, Ontario, Canada. Leopard frog egg masses were collected from shallow ponds (N = 4) and channels (N = 4) in the Crown Marsh, with the channels being heavily inundated with common reeds, while the ponds had limited exposure to reeds. Toad egg masses were collected from shallow beach pools with no emergent vegetation. Egg masses for each species were pooled together and kept in buckets filled with water from the collection site until tadpoles hatched. All collection of animals was authorized under permits issued by the Ontario Ministry of Natural Resources and Environment Canada.

Experiments

To test the effect of plant phenols on larval development aged tap water was inoculated with senesced plant material from either *Phragmites australis*, *Typha latifolia*, or in a no-plant control. Inoculation consisted of suspending plant material in nylon stockings in aged tap water for a period of 48 h at a level of 2 g/L, representing a slightly higher value than previous studies (Maerz et al. 2005; Watling et al. 2011b; Earl et al. 2012) but reflective of an ecologically realistic biomass for *Phragmites australis* in invaded wetlands (Chun & Choi 2009). Control water batches consisted of an empty nylon stocking suspended in aged tap water for 48 h. I collected water samples from each batch of treatment water and used the Folin-Ciocalteu method to assay the total phenol concentration via spectrophotometry according to standard procedures (Clesceri et al. 1998). The absorbance of each sample was measured with a Vernier spectrophotometer and Logger Pro v.3.8.4, and the concentration of total phenols was estimated from a standard curve of known concentration of Gallic acid, ranging from 1 to 25 ppm. Additionally, I collected water samples from 14 current or previous amphibian breeding sites in Long Point in May 2013, and performed the same assay to determine the range of phenol concentrations in a natural context.

Beginning at Gosner stage 25 (Gosner 1960), each tadpole (n = 90 for *L. pipiens*, n = 75 for *A. fowleri*) was randomly assigned to one of the three treatments, consisting of 700 mL of extract water in a 1 L polypropylene cup. Tadpoles were kept on a 14 h: 10 h dark: light cycle and fed 0.1 g of boiled kale every two days, at which point uneaten food and waste was removed. Every four days I performed a 50% water change to maintain the concentration of plant phenols and remove nitrogenous wastes. Each experiment ran for a total of 3 weeks, with Leopard frogs starting on May 15 and Fowler's Toads on June 18, owing to differences in breeding phenology, with individual tadpoles being monitored for daily survival.

Prior to being randomly assigned to their respective treatments, each tadpole was photographed top-down against a standardized 6 mm grid. From this image I calculated the snout-to-vent length of each individual by digitally measuring the straight-line distance from the snout to the base of the tail using the software ImageJ v.1.63 (Rasband, NIH, 2009). Photographs were subsequently taken at 1 week intervals to compare growth over time. At the end of the experiment, each tadpole was identified to its final Gosner developmental stage (Gosner 1960), and was euthanized in a 1g/L solution of buffered MS-222. All procedures complied with basic animal use policy and were authorized by McGill University according to Animal Use Protocol Number 4569.

Analyses

To determine the effect of phenol extracts on tadpole vital rates I compared daily survival across each treatment with a Cox proportional hazard model of survival over time for each species, with treatment (*Phragmites*, *Typha*, control) as a covariate, using the 'survival' package (Therneau 2012) in R v.2.15. Significance of treatment on mortality was determined by the Wald chi-square statistic and the calculated hazard ratio for each treatment. I compared the concentration of total phenolics across sampling sites and batches with a one-way analysis of variance and Tukey's post-hoc tests to determine where, if any, significant differences occurred. Similarly I compared mean Gosner stage across treatments with a one-way ANOVA. Differences in body size (SVL, mm) over time were determined using linear mixed effects models including time and treatment as independent variables with random slopes and intercepts for tadpole identity using the 'nlme' package (Pinheiro et al. 2012). All analyses were performed in the software R v.2.15 (R Core Development Team, 2012).

Results

Phenol Concentration

The concentration of total phenols was significantly different across treatments ($F_{2,\,13}$ = 14.9, $p < 0.001$). The average phenol concentration in water inoculated with *Phragmites* was

2.74 ± 0.91 ppm (mean ± SD), significantly higher than control water at an average concentration of 0.54 ± 0.33 ppm ($p < 0.001$). Water from the *Typha* treatments similarly had a higher concentration at 2.50 ± 0.71 ppm ($p = 0.001$), and was not significantly different from the concentration of total phenolics in *Phragmites* extract water ($p = 0.837$).

From 14 potential anuran breeding sites the mean concentration of total phenols was 1.94 ± 0.86 ppm, with sites ranging from 1.02 ppm to 4.04 ppm. Sites variously contained common reeds and bullrushes surrounding the sites, and those water bodies with emergent plants ($n = 8$) had a significantly higher mean concentration of total phenols, at 2.32 ppm, than sites with no emergent vegetation ($n = 6$), at 1.43ppm ($t_9 = 2.45$, $p = 0.037$).

Survival

For larval leopard frogs, survival varied significantly between different plant extract treatments ($X^2 = 15.12$, $p < 0.001$; Fig. 1a). Leopard frog tadpoles in the *Phragmites* water experienced significantly lower mortality compared to either control or *Typha* groups ($z = 2.95$, $p = 0.003$ and $z = 3.87$, $p < 0.001$). Tadpoles in the control and *Typha* treatments exhibited hazard ratios of 2.987 (95% CI: 1.443, 6.181) and 4.094 (95% CI: 2.003, 8.369) compared to tadpoles reared in *Phragmites* extract, indicating a much greater likelihood to die in these treatments. There was no significant difference in mortality between tadpoles raised in no extract control water and those in *Typha* extracts ($z = 1.08$, $p = 0.282$). Mortality in the control tadpoles appeared to exceed what would be expected naturally, with 74.3% of individuals expiring by the 12th day (Fig. 1a), suggesting issues with water quality or conditions in this experiment.

Fowler's toad tadpoles experienced no significant difference in survival across the three treatments ($X^2 = 0.21$, $p = 0.92$; Fig. 1b). Mortality was slightly higher in *Phragmites* and *Typha* inoculated water, with hazard ratios of 1.33 (95% CI: 0.38, 4.73) and 1.13 (95% CI: 0.3, 4.21), respectively, but this slightly higher mortality was not significant ($z = 0.447$, $p = 0.655$ and $z = 0.184$, $p = 0.85$).

Growth

Larval *L. pipiens* exhibited significant differences in size across treatments with both *Phragmites* and *Typha* being significantly smaller than control tadpoles after the initial week ($t_{66} = 7.1$, $p < 0.001$ and $t_{66} = 5.06$, $p < 0.001$; Fig. 2a). Additionally, the growth of individuals was different between treatments, as those raised in *Phragmites* experienced accelerated growth compared to tadpoles in both control and *Typha* water ($t_{72} = 5.11$, $p < 0.001$). There was no difference in growth between tadpoles raised in control and *Typha* water ($t_{72} = 0.90$, $p = 0.37$).

A. fowleri tadpoles exhibited no significant differences in size or growth (Fig. 2b). After the initial week tadpoles in *Typha* treatments were slightly smaller, but not significantly ($t_{72} = 1.78$, $p = 0.0798$), and growth throughout the experiment was not different than the controls ($\mathrm{t}_{129} = 0.79$, $p = 0.431$). Similarly, those raised in *Phragmites* were indistinguishable from controls in both size and growth over time ($\mathrm{t}_{72} = 0.49$, $p = 0.625$ and $\mathrm{t}_{129} = 0.67$, $p = 0.505$).

Development

For larval Fowler's toads, after 21 days there was no significant difference in the mean Gosner stage across all treatments ($F_{2, 52} = 0.83$, $p = 0.44$), the mean stage across all treatments was 35. Similarly, it was also found in Leopard frog tadpoles that developmental stage did not differ across treatments ($F_{2, 26} = 0.92$, $p = 0.41$) at Gosner stage 27.

Discussion

Survival, growth, and development of tadpoles did not appear to be negatively affected by the presence of emergent plant secondary compounds as hypothesized. Both the obligate (*A. fowleri*) and facultative (*L. pipiens*) gill breathing tadpoles appeared to cope, or even perform better, in the presence of *Phragmites* secondary compounds at ecologically realistic levels, while only *L. pipiens* appeared to exhibit reduced growth in *Typha* extracts. This indicates that changes to the plant community in the wetlands of Long Point are unlikely to have degraded the larval habitat of these anurans through the release of secondary compounds.

The response of both species exposed to *Phragmites* secondary compounds was contrary to expectation. The lack of effect on *A. fowleri* tadpoles is surprising, given the high sensitivity to phenolics demonstrated by the closely related *Anaxyrus americanus*, which has been a focal species in several experiments on plant secondary compounds (Maerz et al. 2005; Watling et al. 2011b; Cohen et al. 2012; Earl et al. 2012). Putatively, the sensitivity of *A. americanus* has been explained by its late lung development (Maerz et al. 2005), a trait that is shared with *A. fowleri* (Feder 1984), suggesting that *A. fowleri* should be similarly sensitive to plant secondary compounds.

This lack of an effect can be explained in several ways. First, considerable intraspecific variation exists in the amount of secondary compounds produced in both native and introduced *Phragmites australis* populations (Martin & Blossey 2013). As such, the population of introduced *Phragmites* at Long Point may simply produce less secondary compounds than other populations, which could be expected due to the relative lack of a previously established plant community, and therefore low competition for space and resources in this area (Leahy et al. 2005). However, the concentration of total phenolics from *Phragmites* plant material wasn't exceptionally low at 2.5 ppm, and other plant species have had a noted negative impact on *A. americanus* fitness at lower concentrations (Watling et al. 2011b). As such, it seems to indicate that the secondary compounds produced by *Phragmites* are simply not particularly toxic to these anuran larvae, as has been found with several other species (Rogalski & Skelly 2012; Martin & Blossey 2013; Perez et al. 2013).An alternative explanation for this result is that prior experience to the invasive or native *Phragmites* has resulted in either a plastic or adaptive response of tadpoles to mitigate the negative effects of its secondary compounds. No studies that have examined tadpole origin, with regards to prior experience with *Phragmites*, have found differences in fitness consistent with adaptation to plant compounds (Rogalski & Skelly 2012). However, it is possible that even with only two decades of exposure to these compounds there has been selection for resistant larvae, as the Fowler's toad exhibits a very rapid generation time (Kellner & Green 1995). Similarly, it should be recognized that since *Phragmites* is merely an invasive genotype, rather than a completely novel species, the lack of a strong impact of its phenols may reflect the prior evolutionary history these amphibians share with this plant. Certainly, the role of adaptation in mitigating the effect of plant compounds on amphibian larvae warrants further investigation.

The response of *L. pipiens* tadpoles was similarly unexpected, although the low survivorship of individuals in the control group invites some caution in interpreting the results of this experiment. The positive effect of *Phragmites* compounds on survivorship and growth is certainly context dependent, as this may be better explained as an amelioration of the potential negative effects experienced by the control group. Significant mortality in the controls appeared to follow several water changes, indicating issues with water quality, and consistent with events of municipal water contamination (Canadian Broadcasting Corporation 2013). As such, the inoculation of water with *Phragmites* may have balanced pH, or introduced peptides that bind to heavy metals (Steffens 1990), remediating water quality and giving the appearance of a positive effect on larval fitness, but if this was the case a similar pattern should also be found in the *Typha* treatment. The accelerated growth of *L. pipiens* in the *Phragmites* treatment after the first week may indicate an initial impact of *Phragmites* compounds on some individuals, after which individual growth appeared to be compensatory. This pattern may be explained by heterogeneity in individual response to these compounds and the increased mortality of initially small individuals that may have greater difficulty in coping with gill damage from secondary compounds. This apparent positive effect may however reflect an advantage for larval development with invasive *Phragmites* for this species, as similar results were found with the bullfrog, *Lithobates catesbeiana*, which exhibited increased survivorship and growth when reared with *Phragmites* detritus (Rogalski & Skelly 2012). Other species of ranids seem to exhibit a neutral response to *Phragmites*. A study across varying concentrations of *Phragmites* phenols found no negative effects on survival, size, or development even at 25 ppm for *Lithobates palustris* (Martin & Blossey 2013). Although not all ranids appear entirely resilient to *Phragmites* compounds, as Perez et al. (2013) found that the density of *Phragmites* reeds did not impact survivorship, but it did slow development for the wood frog, *Lithobates sylvatica*. Therefore, there is still considerable uncertainty over how *Phragmites australis* may influence larval habitat quality for different anurans.

The *Typha* extracts also elicited an unexpected response from both *L. pipiens* and *A. fowleri* tadpoles, as the impact on body size in *L. pipiens* was not predicted based on the fact that larvae can supplement oxygen with aerial respiration yet no impact was found for the obligate aquatic respirator *A. fowleri*. Since our method of determination for phenol concentration only considered total concentration of all phenols, but not the number or identity of the phenol compounds, it is possible that a specific phenol compound has a negative effect on *L. pipiens*. Eleven distinct phenolic compounds have been isolated from the flowers of *T. latifolia* (Ozawa & Imagawa 1988), and many fatty acids have also been isolated from the plant tissue of this species (Aliotta et al. 1990). These fatty acids have noted phytotoxic properties (Aliotta et al. 1990), and similar compounds in other plants can act as chemical appetite suppressants to limit herbivory (Walling 2000). Therefore the potential to inhibit feeding and thus growth in tadpoles exists. However, why this only appeared to affect *L. pipiens* but not *A. fowleri* is not certain and may reflect differences in sensitivity or behaviour that limit the exposure and impact on *A. fowleri*. Similarly, Maerz et al. (2005) found no negative effects of *T. latifolia* leachate on either *Anaxyrus americanus* or *Hyla versicolor* larvae, a finding consistent with what I found in *A. fowleri* larvae. Stephens et al. (2013) raised larval wood frogs, *Lithobates sylvatica*, on detritus from different native and invasive plant species and found that individuals consuming *T. latifolia* leaf litter were significantly smaller at metamorphosis than those raised with different plant leaf litter, including non-native *Phragmites*, consistent with the reduced growth of *L. pipiens* larvae found in this study. This may indicate a particular sensitivity of larval ranid frogs to the compounds found in cattails.

Overall, the prevalence of secondary compounds of both native and invasive plant species in larval habitat appears to have little consequence for the Fowler's toad or Northern leopard frogs at Long Point. However, the transformation of wetlands in this region by the expansion of invasive *Phragmites* and native *Typha* still has many potential consequences for these species. Alteration to larval habitat quality may be affected by the expansion of these species into wetland water bodies through the alteration of hydrology, lowering pH, reducing sunlight and thermal heterogeneity, or by altering phytoplankton assemblages (Minchinton et al. 2006; Perez et al. 2013). While this experiment addressed solely the impact of plant secondary compounds on larval anurans, in a natural context multiple stressors are likely to occur during larval development simultaneously. Plant secondary compounds may interact synergistically with other stressors including low oxygen conditions, predators, and pond drying to impact developing amphibians. Additionally, regardless of impacts to larval habitat quality, the progressive loss of open water habitat to *Phragmites* is still a continued threat to these species (Greenberg & Green 2013). As the invasive genotype of *Phragmites australis* continues its now continental invasion (Saltonstall 2002), understanding the myriad impacts on floral and faunal communities is a top priority.

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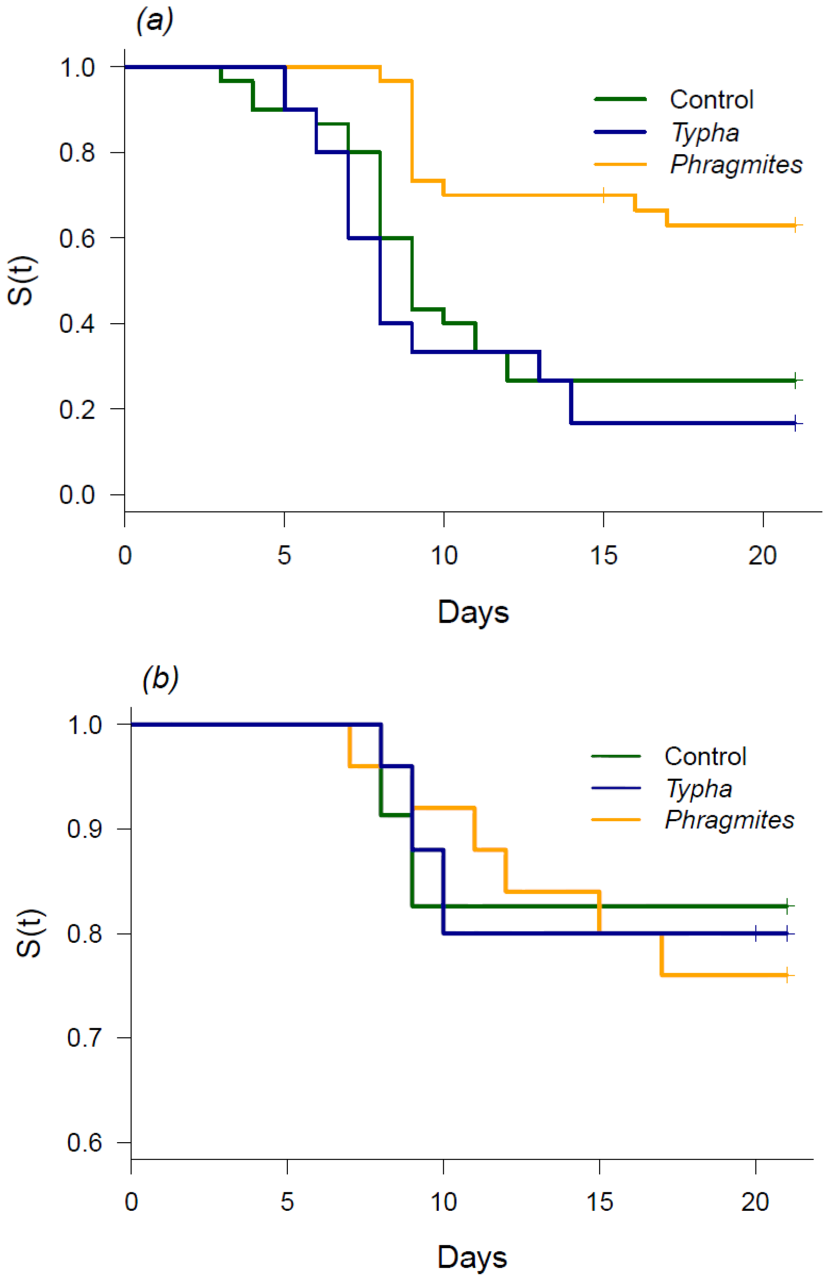

Figure 1. Survival as a function of time, $S(t)$, for *Lithobates pipiens* tadpoles (*a*) and *Anaxyrus fowleri* tadpoles (*b*) raised in water inoculated with plant material from the invasive *Phragmites australis*, the native *Typha latifolia*, or a no plant control treatment. Only Leopard frogs exhibited a significant difference in survival between treatments, with those in *Phragmites* experiencing a higher survival than those in control or *Typha* water.

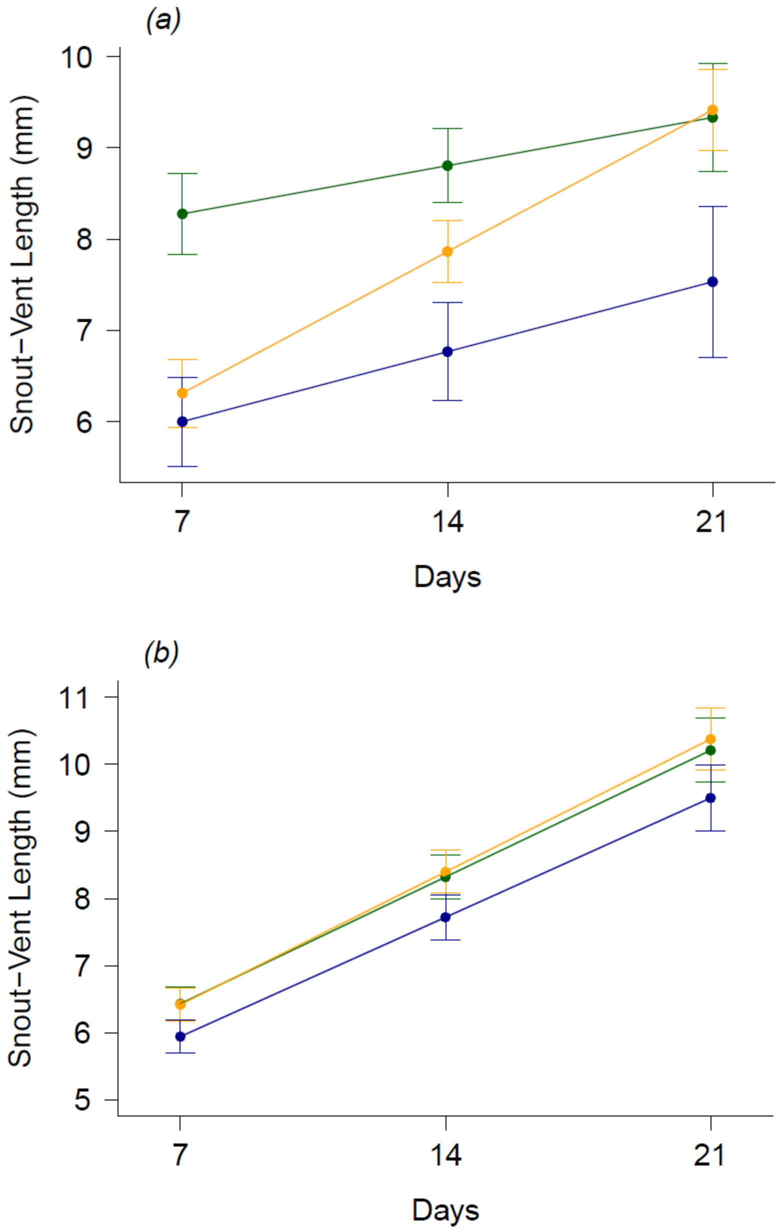

Figure 2. Change in body size over time for larval Northern leopard frogs (*a*) and Fowler's toads (*b*) raised in water inoculated with either *Phragmites* (orange), *Typha* (blue), or a no plant control (green). Leopard frogs significantly differed in size and growth across treatments, with growth being greatest in those raised in *Phragmites* after the initial week, approaching the same size as control tadpoles by the end of the experiment. Tadpoles in the *Typha* treatment were significantly smaller, but growth was similar to those in the control treatment. Fowler's toad tadpoles did not differ in size or growth between any of the treatments.

CHAPTER IV: CONCLUSIONS AND IMPLICATIONS

In order to confidently identify the onset of decline in amphibian populations we require a better understanding of their temporal dynamics (Pechmann & Wilbur 1994; Alford & Richards 1999). As population monitoring in response to the global amphibian decline generates an increased quantity and quality of long-term amphibian population time series we will be able to better characterize the factors that drive population fluctuations (Blaustein et al. 1994). This will provide us with an increased capability to predict how populations should respond to climatic fluctuations, changes in habitat, emergent disease and other environmental changes (Alford & Richards 1999; Keith et al. 2008).

In Chapter Two, I show how the Fowler's toad population at Long Point was regulated by stabilizing density-dependence prior to the expansion of the invasive marsh plant, *Phragmites australis*. This adds to the growing evidence that stabilizing density-dependence is the norm, at least in temperate pond breeding amphibians (Salvidio 2011). This property of population dynamics is very important when interpreting population persistence and response to environmental fluctuations and stressors (Lande et al. 2003). Since many amphibian species appear to be regulated by stabilizing density-dependence, monitoring for declines could be improved by setting quantitative guidelines based on this property of their population dynamics. Annual return rates predict how fast a population should return to equilibrium after any perturbation (Sibley et al. 2007), and therefore provide a good basis on which to create a "time-frame" for recovery. Rather than the traditional method of regressing log-abundance against year, which requires lots of time and data, instead a population would be considered to be in decline if a specific time-threshold has passed with no apparent recovery (eg. no positive growth in 3 years).

Following the methods of Sibley et al. (2007), the Fowler's Toads at Long Point from 1989 to 2002 have a return rate of 0.663 year^{-1}, corresponding to a return to carrying capacity after perturbation in 1.51 years. Rounding to that to the annual census interval would predict a return to carrying capacity 2 years after a perturbation. In the long-term data-set on Fowler's toads, only one period deviates from this prior to 2002, whereby negative growth was experienced for 4 consecutive years (1994 to 1997), and after 2002 whereby a period of 7 consecutive losses was observed (2005 to 2011). A probabilistic model could thereby indicate the probability of observing such a phenomenon naturally based on a population's return rate and the degree of environmental stochasticity, providing a rigorous test of decline with only minimal data required. Setting guidelines for identifying declines based on this property of amphibian dynamics could improve efficiency of monitoring and expedite conservation response to decline.

The results of this thesis already have direct consequences for the continued management of Fowler's toads at Long Point. As evidenced in Chapter Two, the invasive strain of *Phragmites australis* has had a considerable impact on this population, by eliminating shallow, open water habitat used by the toads for breeding. In response to this finding, 12 ponds were excavated in the wetlands east of Long Point Provincial Park in the fall of 2012 by the Canadian Wildlife Service in order to facilitate the recovery of the Fowler's toad population in this area. Whether the population will respond positively as it recovers to a new carrying capacity, as would be consistent with our original hypothesis, has yet to be demonstrated. Future success of this restoration project requires a full understanding of how *Phragmites* impacts this population, as recolonization of these ponds by *Phragmites* will occur.

Chapter Three addresses one mechanism by which *Phragmites* may impact recruitment in the Fowler's toad population, through the release of secondary compounds into larval habitat. However, as was demonstrated this is unlikely to cause any negative impacts on larval recruitment and therefore the presence of *Phragmites* around these ponds is unlikely to impact larval habitat quality, at least through this pathway, for Fowler's toads. Whether other effects of *Phragmites*, for example on the survival, movement or growth of metamorphs, may impede population recovery is still unknown. As is clear, many questions on how the expansion of *Phragmites* may impact both this species and other amphibians still remain.

While *Phragmites* is recognized as one of the most ubiquitous and important invasive species in wetlands, our understanding of its impacts on native fauna is surprisingly low (Able & Hagan 2000; Meyer et al. 2010).Understanding how this novel invader effects native wildlife is essential as it continues its expansion throughout North America (Saltonstall 2002). Wetlands are already some of the most threatened, and degraded of temperate habitats (Brinson & Malvarez 2002), and host an abundance of unique and rare biodiversity (Junk et al. 2006), therefore understanding and responding to this continental invasion is of the utmost importance.

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