POPULATION ECOLOGY OF A DECLINING AMPHIBIAN IN RELATION TO DENSITY

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ABSTRACT

In species with complex life cycles such as amphibians, population regulation can occur in one or multiple stages. Density effects in the larval, aquatic stage of the amphibian life cycle have been widely documented, leading many researchers to assume that this is the stage in which amphibian populations are regulated. However, recent evidence suggests that factors affecting survival and reproduction in the terrestrial stage may play a greater role in the growth and decline of amphibian populations than previously thought. I review the evidence for density-dependent population regulation in the terrestrial stage of amphibians and argue that variation in body size may be an important mechanism linking population density to changes in population growth rate (CHAPTER ONE). I used data from a 23-year population survey at Long Point, Ontario and conducted summer growth-rate surveys to examine the body size-abundance relationship in Fowler's Toad (*Anaxyrus fowleri*). Over the past 10 years of the population survey at Long Point, we observed a significant increase in body size ($R^2 = 0.874$, $p = <0.001$) that was coincident with a previously detected negative trend in abundance. The age structure of this population is highly unstable, but overall the average age is getting older ($R^2 = 0.325$, $p = < 0.01$) (CHAPTER TWO). Somatic growth rates in Fowler's toad toadlets are also highly variable and are significantly inversely correlated with the density of adults ($R^2 = 0.98$, $p = 0.010$) (CHAPTER THREE). The single most important factor influencing average body size on a long time-scale is abundance ($R^2 = 0.713$, $p = <0.001$) (CHAPTER FOUR). Therefore, body size variation in these toads is likely related to density-dependent resource availability for growth in the terrestrial stage.

RÉSUMÉ

Pour des espèces ayant des cycles de vie complexes tels les amphibiens, la régulation de leur population peut se produire dans un ou plusieurs stades. Les effets de la densité au stage larvaire aquatique du cycle de vie de l'amphibien ont été largement documentés amenant plusieurs chercheurs à assumer que c'est à ce stage que la régulation de leur population se produit. Toutefois, des preuves récentes suggèrent que des facteurs affectant la survie et la reproduction au stage terrestre peuvent jouer un plus grand rôle dans la croissance et le déclin de la population des amphibiens contrairement à ce que l'on pensait auparavant. Je fais la revue de la preuve de la régulation de la population qui dépend de la densité au stage terrestre des amphibiens et j'argumente que la variation de la masse corporelle pourrait être un mécanisme important reliant la densité de la population aux changements du taux de croissance de la population (CHAPITRE UN). J'ai utilisé les données d'un relevé de la population sur une période de 23 ans à Long Point, Ontario et mené des relevés du taux de croissance durant l'été afin d'examiner la relation entre la dimension et la masse corporelle du crapaud de Fowler (*Anaxyrus fowleri*). Durant les 10 dernières années du relevé de la population à Long Point, nous avons remarqué une augmentation significative de la masse corporelle ($R^2 = 0.874$, $p = <0.001$) qui coïncidait avec une tendance négative de leur abondance détectée précédemment. La structure de l'âge de cette population est très instable, mais dans l'ensemble la moyenne d'âge est plus vieille ($R^2 = 0.325$, $p = < 0.01$) (CHAPITRE DEUX). Les taux de croissance somatiques des petits du crapaud de Fowler sont hautement variables et inversement corrélatifs à la densité des adultes ($R^2 = 0.98$, $p = 0.010$) (CHAPITRE TROIS). Le seul facteur d'importance qui influence la dimension corporelle moyenne sur une longue période c'est l'abondance ($R^2 = 0.713$, $p = <0.001$) (CHAPITRE QUATRE). Ainsi, la variation de la dimension corporelle de ces crapauds est vraisemblablement reliée à la croissance somatique laquelle dépend de la densité au stage terrestre.

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I am also pleased to acknowledge my fellow lab mates—Jay Ploss, Morgan Boenke, Trond Sigurdsen, Daniel Greenberg, Katharine Yagi, David O’Connor, Elizabeth McCurry and Alan Schoen-- for all of the discussions that helped inform, inspire, and entertain me during my time at McGill. As the lab membership grew, our space under the Redpath auditorium and in the cottages became rather tight; but it was worth it to gain your companionship and benefit from collaboration. In addition to the members of the Green Lab, several individuals from the Redpath Museum and Biology department added to different aspects of this thesis. Eric Pedersen is especially notable for his generosity in helping me with the age structure analyses for Chapter 2.

The data collection for this thesis was definitely a group effort. Long before I started at McGill, past graduate students of the Green Lab and numerous volunteers went to Long Point each spring to catch toads for David’s long-term population study. I thank them for their contributions and efforts to keep comprehensible records. To the toad crews in more recent years, thank you for your dedication and for sharing with me the many challenges and rewards of studying a wild population. Rory Lattimer, Alice Cheffins, Kevin Jepson and Justine Latremouille assisted in the lab to slice hundreds of teeny toe bones for Chapter 2, and Ivan Lange, Marianne Meyer and Erwin Meyer accompanied me on several road trips across Lake Erie to measure toadlets for Chapter 3. Anne Yagi of the OMNR welcomed me to her crew and home in Niagara, and shared data that was essential for Chapter 3. Emily Slavic and Scott Taylor introduced me to Rondeau Provincial Park and shared in the search for Fowler’s toads there.

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CONTRIBUTIONS OF AUTHORS

CHAPTER ONE

I surveyed the literature with advice from Dr. Green.

CHAPTERS TWO AND FOUR

I used the data from Dr. Green's long term population survey of Fowler's Toad at Long Point, Ontario, which have been collected by Dr. Green and numerous graduate students (including myself for the years 2009-2012) and volunteers beginning in 1989. I analyzed the results. The final versions of the manuscripts have been prepared with editorial support from Dr. Green.

CHAPTER THREE

I collected the data with the field assistance of volunteers and analyzed the results. The final version of the manuscript was prepared with editorial support from Dr. Green.

CHAPTER FIVE

I conclude the thesis with advice from Dr. Green. The final version of the manuscript was prepared with editorial support from Dr. Green.

CHAPTER ONE: Introduction and Literature Review

Understanding the means by which numbers of individuals are determined in populations (population regulation) is a central goal in ecology (Royama 1977, 1992, Berryman 1989, 1999). Density-independent factors, such as fires and the changing of seasons, cause populations to fluctuate, while density-dependent processes, such as competition for resources, tend to have a stabilizing effect (Royama 1977). Thus, animal populations grow and decline, but they do not increase indefinitely and they seldom become extinct (*e.g.* Newton, 1998). And yet, populations of some species are more prone to larger fluctuations than others. For example, many amphibians that breed in temporary ponds experience cycles of "boom and bust", with a few good years often followed by several bad years (e.g. Semlistch et al. 1996). Because the probability of local extinction generally increases with increasing variance in population size, the strength of density-dependence has important consequences for the long term persistence of populations (Ginzburg et al. 1990; Stacey and Taper 1992; Diamond 1984; Pimm *et al.* 1988; Bengtsson & Milbrink 1995).

Larval amphibians, or tadpoles, have widely been used as model organisms in population and community ecology. This is because they are readily available (at least for common species), are sensitive to numerous stressors, and can be easily maintained in both laboratory and outdoor enclosures or mesocosms (e.g. reviewed in Duellman and Trueb 1994; Hopkins 2007). Much of our knowledge about population regulation in amphibians comes from the studies of Dr. Henry Wilbur and colleagues, who championed the experimental approach to studying ecology in the 1970's and 1980's (Semlitsch and Boone 2010). What started out as relatively simple experiments in which larvae were raised in cattle tanks and subjected to different densities of competitors (Wilbur 1976; Wilbur 1977), evolved into large-scale, full-pond manipulations that addressed hypotheses about the control of structure and function of natural communities (Wilbur 1997). The results of these and other studies have led to a consensus that neither competition nor predation alone determine the structure of temporary pond communities, but rather they interact to determine the different consequences of the desiccation of a pond to the success of multiple species (Wilbur 1997).

A new wave of interest in amphibian population biology began with the First World Congress of Herpetology in 1989. It was at this time that widespread reports of amphibian population declines and extinctions were acknowledged as a global problem. Thus, where previous research objectives focused on advancing ecological theory, they now aim to include results with implications for conservation. Effective conservation depends on our ability to analyze population viability and target the best ways to improve population growth (Mills et al. 1999). Population modeling assists in these efforts by providing a quantitative link between population-specific demographic rates and population growth, and has been attracting increasing attention from amphibian conservation biologists (e.g. Biek et al. 2002; Vonesh and De la Cruz 2002; Schmidt et al. 2005; Harper and Semlitsch 2007; Salice et al. 2011). The accuracy of models to make predictions depends on the extent to which they reflect the natural processes operating in real populations. This, in turn, depends on an understanding of the factors regulating abundance at all stages of the life cycle.

The majority of amphibian populations are not restricted to larval individuals. Eventually, the typical larva undergoes a series of sudden ontogenetic changes, termed metamorphosis, and emerges from the aquatic habitat as a four-legged, terrestrial pre-adult. The result is a complex, biphasic lifecycle with stage-specific differences in survivorship, growth and fertility. Consequently, the population dynamics of amphibians may be regulated in the larval stage, the terrestrial stage, or both (Wilbur 1980).

In comparison to studies of amphibian larvae, studies of the terrestrial life-history stage are extremely rare. This is, in part, due to the greater logistical difficulties of studying postmetamorphic individuals; terrestrial amphibians are generally difficult to track outside of the breeding season, and are not as amenable to experimental manipulations. Another reason for the paucity of studies on postmetamorphic amphibians is due to an old assumption that, because larval vital rates are so variable, amphibian population dynamics are primarily determined by population regulation operating in the larval life-history stage (e.g. Wilbur and Collins 1973; Kiesecker et al. 2001). However, recent population models incorporating amphibian demographic data have shown that overall population growth is more affected by reductions in juvenile and adult survival than similar reductions in larval survival (Biek et al. 2002; Vonesh and De la Cruz 2002). Therefore, our lack of understanding of the factors affecting postmetamorphic individuals could severely limit our ability to develop effective conservation strategies (Dodd 2010).

Several aspects of amphibian ecology suggest that density-dependence may be an important factor limiting abundance in the terrestrial stage. Many species have high fecundities and synchronized breeding, resulting in large numbers of emerging metamorphs. Although some individual amphibians have been observed dispersing long distances, the majority display high site fidelity and a low vagility (Smith and Green 2005). The amount of habitat that is suitable for amphibians is further limited by physiological restrictions associated with their permeable skin (Wyman 1988). Therefore, the density of postmetamorphic amphibians may often exceed the carrying capacity of the terrestrial environment. In addition, the habitat requirements for many species' oviposition sites are very particular (e.g. Heying 2004), resulting in limited reproduction opportunities when adult densities are high. Under these situations density-dependence might limit population size.

The few experimental studies that have manipulated population density in terrestrial habitat all demonstrate some extent of effects on postmetamorphic vital rates. Density-dependent growth in body size has been demonstrated for *Bufo marinus* (Cohen and Alford 1993), *Gastrophyne carolinensis* (Pechmann 1994), *Bufo bufo* (Goater 1994), *Rana lessonae* (Altwegg 2003), *Rana sylvatica*, *Bufo americanus* (Harper and Semlitsch 2007), and *Rana arvalis* (Loman and Lardner 2009). In addition to density-dependent growth rates, Harper and Semlitsch (2007) found that juvenile *Rana sylvatica* and *Bufo americanus* raised in high density treatments experienced lower survival and showed minimal evidence of reproductive development after one year. Although these experiments cannot account for the complexity of interacting variables in nature, they do provide a causative link between poor terrestrial performance and high population densities.

Perhaps the best evidence of density-dependent population regulation in the terrestrial stage of an amphibian comes from Dr. Keith Berven's long-term study of a natural population of wood frog, *Rana sylvatica*, in Michigan (Berven 2009). Every active season from 1985-2005, Berven estimated adult and juvenile population sizes by physically marking adults and juveniles captured along a fence surrounding an isolated pond. Berven measured the body size of all captured individuals, determined reproductive traits of a number of females in the laboratory, and estimated age-specific survivorship and time to sexual maturity. By correlating these vital rates with other variables, including annual population densities, average body size at metamorphosis, and precipitation, Berven found that the single most important factor explaining year-to-year variation in juvenile and adult vital rates was indeed population density. In addition, Berven found that as juvenile population size increased, the number of surviving adults and total biomass plateaued, supporting that population size was constrained by a carrying capacity in the terrestrial environment.

Long-term studies of amphibian populations are still rare, and few document the level of detail on demographic rates that Berven (2009) did. Accurate estimates of survivorship depend on assumptions that are often difficult to meet (e.g. that one is studying a closed population), and determining fecundity requires that egg masses are readily observable. An alternative way to discover density-dependent population regulation is to look for situations where population density is inversely related to body size. Unlike survivorship and fecundity, body size in amphibians is easily measurable. Although not itself a "vital rate", the average body size of individuals composing a population has strong implications for the population growth.

In general among amphibians, body size is positively correlated with physical performance; larger individuals exhibit greater jumping ability, stamina (John-Alder and Morin 1990), metabolic rate, sprint speed, endurance (Beck and Congdon 2000) and tolerance to dehydration (Ray 1958; Newman and Dunham 1994) than smaller individuals. In addition, larger individuals have a greater range of prey sizes which they can consume than their smaller conspecifics (Clarke 1974; Flowers and Grayes 1995). Larger individuals also tend to be more toxic (Phillips 2006) and may be unsuitable prey to gape-limited predators (Brodie and Formanowicz 1983). Body size is also positively correlated with lifetime reproductive success; larger females have larger eggs, lay more eggs per clutch (Tejedo 1992; Gibbons and McCarthy 1986), and are more likely to lay multiple clutches per breeding season (Krupa 1986) than smaller individuals. Larger individuals of both sexes are more likely to be sexually mature at a younger age (Smith 1987) and be successful in securing a mate (Wilbur et al. 1978; Fairchild 1981; Ramer et al. 1983). All of these results suggest that amphibian populations composed of larger individuals should experience greater survivorship, reproduction and recruitment.

Intraspecific variation in body size of amphibians is widely documented (e.g. Sullivan 1987; Elmberg, 1987; Neveu 2009). The majority of studies examining the causes of variation are interested in large-scale spatial patterns such as Bergmann's rule, and therefore focus on environmental variables such as elevation and temperature (e.g. Angilletta et al. 2004; Cvetkovic et al. 2008; Bidau et al. 2011). Of those studies that consider the effect of population density, all have found significant, negative relationships (Beebee 1983; Oldham 1985; Gray and Smith 2005; Middleton and Green 2011). Many factors influence body size in amphibians, and these can be both density-dependent and density-independent (Halliday and Verrel 1988; Hota 1994). Despite the potential for body size to regulate population growth, no one has examined the body size-abundance relationship in an amphibian population over time and attempted to link it to density-dependent processes operating in the terrestrial stage.

Fowler's Toad, *Anaxyrus fowleri,* is an ideal model organism for studying density dependence in the terrestrial stage of amphibians. Because adults congregate conspicuously in breeding choruses and on sandy shorelines, populations lend themselves well to long-term data acquisition. Like most North Temperate anurans, Fowler's toads lay large clutches of eggs, have high early life mortality, and have short life spans (Breden 1988; Green et al. 2007). These life history traits result in population sizes that can vary considerably from one year to the next (Berven 1990; Green 2005). In Canada, Fowler's Toad is at the North extent of the species' range, so these populations may be especially sensitive to environmental stochasticity (Green 1997).

Beginning in 1989, an intensive population survey at Long Point, Ontario, has been documenting population size and the body length of Fowler's toads along a 10 km stretch of habitat every spring. My research is motivated by an observation from this study that annual population size is strongly negatively correlated with average annual body size (Middleton and Green 2011) (Figure 1). If body size is in some way density-dependent, populations may have a greater capacity to return to equilibrium abundance following stochastic events. Therefore, changes in average body size could improve population persistence. The aim of my research is to elucidate the underlying demographic process involved in the temporal body size-abundance relationship. This knowledge could allow for more accurate predictions of the numerical responses of populations to changes in the terrestrial environment.

In CHAPTER TWO we reconstruct the age structure of the Long Point Fowler's Toad population from 1992-2011. We test the hypothesis that the temporal age structure is unstable. If recruitment and survivorship are highly variable, then the shapes of the age distributions should differ from year to year. We also test the hypothesis that there should be a noticeable shift in population age structure pre- and post- invasion of a habitat-modifying marsh plant. If the loss of aquatic habitat affects recruitment, then the average age of the population should increase.

In CHAPTER THREE we test the hypothesis that somatic growth in postmetamorphic toads is dependent on population density in the terrestrial habitat. If growth rates are density-dependent, then toadlets from locations of higher density should grow at a slower rate than toadlets from a location of lower density.

In CHAPTER FOUR we identify the most likely models to explain variation in the average body size of adults in relation to abundance, age structure, and environmental variables, including temperature. If density-dependent growth rates affect the average body size of populations, then average body size should vary in concert with abundance over time as an inverse power or lognormal relationship.

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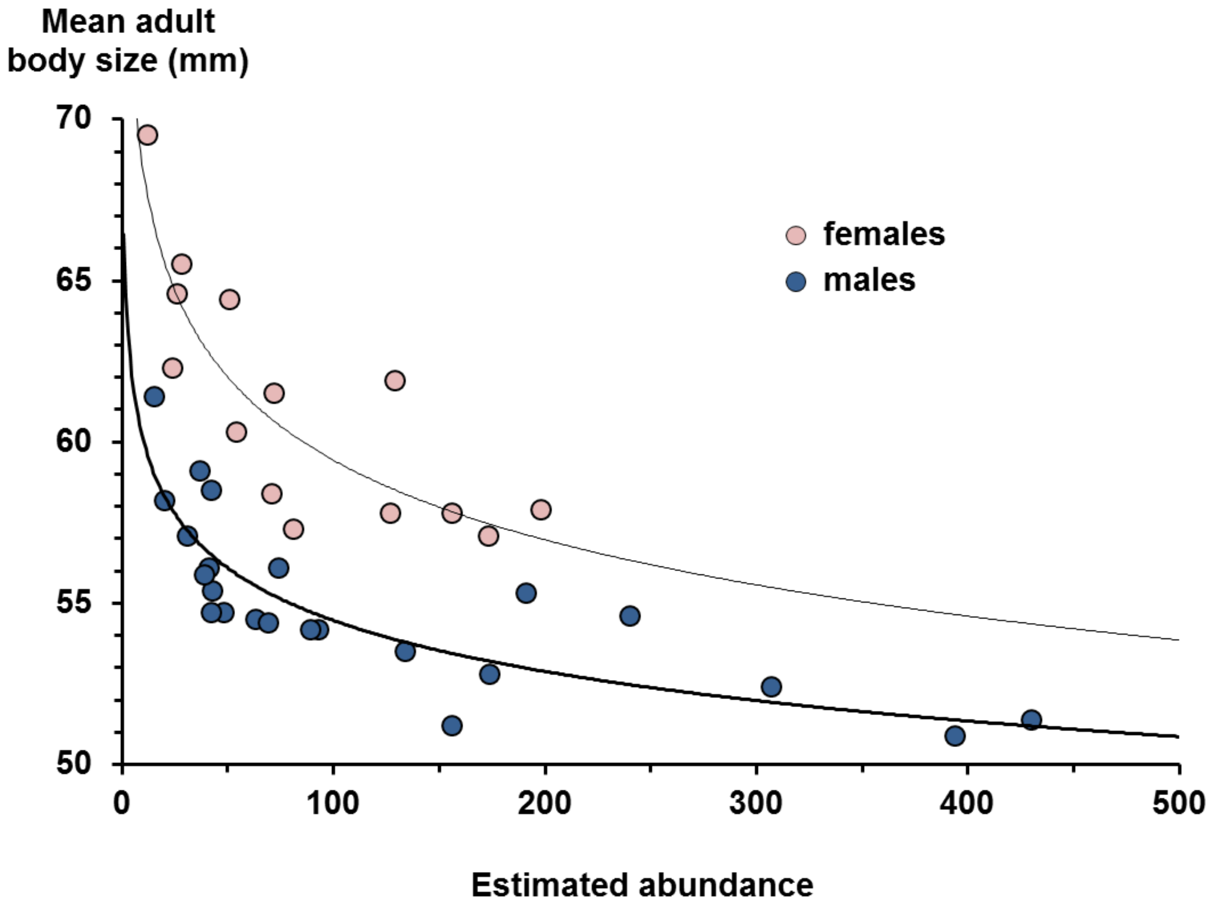
Figure 1. Preliminary evidence for density-dependent growth among postmetamorphic Fowler's Toads. The relationship is a power function with $r = 0.708$ for males and $r = 0.541$ for females.



CHAPTER 2: Age Structure Variability in a Declining Amphibian Population

CHAPTER TWO LINKING STATEMENT

Body size is a function of age-- especially in species with indeterminant growth. Therefore, temporal variation in the average body size an amphibian population could be caused by changes in age structure. Knowledge of the age structure of a population also provides valuable demographic information about the magnitude of natural variability in vital rates and how vital rates respond to specific stressors. I reconstructed the age structure of the Fowler's toad population at Long Point from 1992-2011 to measure the magnitude and patterns of variation in recruitment and survivorship over periods of natural fluctuations and decline. These results are presented in Chapter 2.

ABSTRACT

Knowledge of population age structure and its variation between years can provide valuable insight into the demographic processes underlying changes in population size, including declines in species at risk. Populations of amphibians normally fluctuate in response to environmental stochasticity but many may be experiencing unusually high age-specific mortality rates due to the loss or degradation of habitat used during the larval aquatic and/or terrestrial stage of the life cycle. Skeletochronology was used to reconstruct the age structure of a population of Fowler's Toad, *Anaxyrus fowleri*, from 1992 to 2011. The age structure of the population was highly variable, with 7 years having unstable age distributions. These were primarily driven by variation in juvenile recruitment, which fluctuated from approximately 13 to 1,112 individuals. Variable adult survivorship and a greater survival probability for 2 year-olds may also contribute to unstable age structure. Over time, there was a significant increase in average age of the population ($R^2 = 0.325$, $p < 0.01$) ($n = 17$ years) concurrent with the loss of breeding habitat due to the expansion of an invasive marsh plant, *Phragmites australis*. Due to the naturally high variability in the age structure of pond-breeding amphibians, long time series are necessary to differentiate natural declines in the sizes of specific age classes from unusually high mortality rates resulting from introduced stressors in the environment.

INTRODUCTION

Animal population dynamics are underpinned by recruitment and mortality which, in turn, are governed by vital rates such as somatic growth, maturation, fertility and longevity (Caswell 2000). If there would be constant recruitment and survivorship, the sizes of age classes within a population should decrease exponentially from the youngest to oldest. Therefore deviations from this stable age distribution indicate changes to the underlying vital rates that are difficult to see in census data alone (Doak & Morris 1999; Monson et al. 2000). This makes knowledge of population age structure, and its changes between years, very valuable for understanding the demographic factors responsible for population changes, including declines in species at risk, and for prioritizing management plans for populations affected by habitat loss or degradation (Driscoll 1999).

Changes in age structure are common in nature and should be expected in populations living in unpredictable environments, such as North Temperate amphibians (Kellner and Green 1995). Especially for species that breed in temporary ponds, juvenile recruitment is highly variable (Alford and Richards 1999). This is, in part, due to *r*-selected life histories with high reproductive rates (up to 10,000 eggs per clutch in some species) and high early-life mortality (Green 2003). Thus, the number of new metamorphs in amphibian populations frequently varies by several orders of magnitude from one year to the next (Berven 1990; Pechmann and Wilbur 1994; Semlitsch et al. 1996; Taylor et al. 2006). Adult survivorship in amphibians is generally considered to be relatively constant, but it too can be variable (Alford and Richards 1999). For example, occasional catastrophic events such as floods or severely cold winters can have sudden and dramatic effects on postmetamorphic abundance (Manion and Cory 1952; Jaeger 1980).

Deterministic changes in the factors influencing populations can result in long term changes in age structure. For example, heavily exploited fish populations often exhibit a truncation of age structure (i.e. fewer age classes and a younger average age) due to a preference by fisherman for bigger, and hence older, individuals (Longhurst 1998; Hutchings and Baum 2005). Many of the stressors that negatively affect amphibian populations are specific to habitats used during either the larval (aquatic) or terrestrial stage of the life cycle. For example, invasive trout have been implicated in the declines of some tree frogs, and these fish are unlikely to prey on metamorphosed individuals (Gillespie and Hines 1999; Gillespie 2001). Therefore, habitat-specific threats to amphibians also tend to be age-specific threats, and may result in shifts in age structure (Gillespie 2010).

The Fowler's toad population at Long Point, Ontario, provides an interesting opportunity to study temporal variability in age structure in pond-breeding amphibians. Fowler's Toads are at the North extent of the species range in Canada, so conditions here might be expected to approach the minimum requirements for the toads' survival (Kellner and Green 1995). Adults congregate to breed in the marshes in early- to late spring. Eggs hatch within one week, and larvae metamorphose 40 to 60 days later (Green et al. 2008). Most toads reach sexual maturity at two years of age, but a small and variable proportion of toads reach sexual maturity in their first or third year (Breden 1988; Kellner and Green 1995). The potential life span of Fowler's toads is 5 years, but annual mortality is high at all life stages (Green 1999b). These life history traits contribute to age structures that vary from one year to the next (Kellner and Green 1995; Green 1997).

Fowler's toad is an early successional species which depends on changeable and dynamic habitat (Green 2005). Populations in Ontario fluctuate naturally in response to Lake Erie storm events and water level cycles (Green 2011). In recent years, however, a more deterministic change has affected abundance; the invasion of a marsh plant, *Phragmites australis* (Badzinski et al. 2008). Concurrent with the invasion of *Phragmites* at Long Point, the dynamics of the population shifted from exhibiting regulated fluctuations to a sustained downward trend (Greenberg and Green, submitted). Such a shift should be evident in the patterns in age structure as well, and may provide insights into the demographic causes of the population's decline.

Using data from the long-term survey at Long Point, I reconstruct the age structure of a Fowler's Toad population from 1992 to 2011. By measuring changes in the representation of successive age classes, I break down population growth into its two main components: recruitment and survival. If recruitment and survivorship are temporally variable, then the shapes of the age distributions should differ from year to year. If the cause of the population decline is related to declining recruitment, then average recruitment rate should decrease significantly over time. If the decline is related to decreasing postmetamorphic survival, then the average survivorship of 2 year-olds (the largest sexually mature age class) should decrease significantly over time.

METHODS

Study area

We used data from the annual mark-recapture survey of Fowler’s Toad at Long Point, Ontario (Middleton and Green 2011, unpublished), from the years 1992 to 2011, inclusive. The site of this study is an 8.4 kilometer long area at the western base of Long Point, located in the UTM zone 17 N between 543295 and 551465 (m) Easting and 4713766 and 4714734 (m) Northing (NAD 83 Datum). In general aspect, the study area is a system of sand dunes running parallel to sandy beach along the shore of the lake. North of the dunes are shallow marshes where the toads breed. In this study area, there are few discrete breeding sites where the animals chorus consistently from year to year. Long Point is a highly dynamic environment and the animals are opportunistic in selecting breeding sites, which often are ephemeral. Surveys of adult Fowler’s Toads were conducted at breeding sites and on the Lake Erie beaches where non-breeding animals can easily be located as they forage at night (Green 2005). Aside from having a greater human presence (including cottages and a few roads), this area is typical of the rest of the Long Point peninsula.

Abundance estimates

The basic method used to survey the toads was to find, hand-capture, and mark every adult toad located within this area every night of the breeding season (approximately May 1st to June 10th). New individuals were uniquely marked by clipping terminal phalanges, using an additive numbering scheme (Green 1992). Toe-clipping of toads has been shown not to significantly impair the animals if done with adequate care (van Gelder and Strijbosch 1996). Adult population sizes for males and females were estimated from the nightly capture/recapture data, yielding an estimated abundance, $\hat{N}$, for each breeding season based on probability of first capture, *p,* and probability of recapture, *c,* using the closed capture model, $\{N, p(t) = c(t)\}$, embodied in the program MARK (White and Burnham, 1999). All estimates of $\hat{N}$ were normalized via log-transformation for analysis. Juvenile population sizes were roughly estimated from a “mini” mark-recapture survey along a 1 km section of beach over 3 or 4 nights at the end of each breeding season. All animals in this part of the survey were marked with an annual web-clip, and the abundance of juveniles in the main study area was estimated from the ratio of juveniles to adults. All procedures were conducted under the auspices of research permits and letters of authorization issued by the Ontario Ministry of Natural Resources and Environment Canada in accordance with the Ontario Endangered Species Act and Canadian Species at Risk Act, as well as McGill University Protocol No. 4569.

Age determinations

From 1992 onwards, chronological ages of adult toads were determined by skeletochronology, following the methods of Leclair and Castanet (1987). The use of skeletochronology as a method for aging amphibians is based on the assumption that layers of new bone are added annually throughout the lifetime of individuals. This is typically true in temperate climates, where growth is usually active throughout the warmer seasons and slows during the colder seasons. The rings created during periods of reduced growth, commonly referred to as Lines of Arrested Growth (LAGs), are visible when cross-sections of bones are stained with hematoxylin and viewed under a microscope. Age can then be determined by counting the number of LAGs (Gibbons and McCarthy 1983, Smirina 1972). Skeletochronology is a reliable method for estimating age in Fowler’s toad because this species experiences distinct annual cycles of growth and dormancy, without any period of aestivation (Clarke 1974; McDougall, unpublished). Furthermore, resorption of the inner bone, which often complicates age determination in older animals, does not occur in this short-lived species (Kellner and Green 1995).

Toes were stored in 10% neutral buffered formalin. Prior to slicing, the second phalange was isolated, cleaned of surrounding tissue, and decalicified in 3% nitric acid for 2h. The diaphyseal portion of each bone was sliced into 20 μm thick cross-sections at -25°C using an Ames Lab-Tek Cryostat Freezing Microtome. Sections were immediately stained for 15 min in Ehrlich's hematoxylin, and then rinsed with distilled water. The smallest diameter sections were mounted in glycerol on glass microscope slides. A minimum of 3 clearly legible sections from each toe were photographed at 40X total magnification. Age in years was assigned according to the number of LAGs in the sections. Because the toads were captured shortly after emergence from hibernation, a LAG was always presumed at the periphery of each bone. Skeletochronological results were not available for the years 1993 and 2001. Only one toad was recaptured in 1994, so this year was salvaged for analysis by assuming that the age of the recap was 3 years. This assumption is reasonable because it is known that the individual was originally captured one year prior, when it was most likely 2 years of age.

Age structure reconstruction

For any one year, we used a random sampling of newly marked toads, as well as recaptured toads marked and aged in previous years. Toads that were aged in a given year represented the new captures of that year. Ages of recaptured toads were determined by adding the appropriate number of years from when an individual was first aged to the time that it was recaptured. Because of differing sample sizes each year, the contributions of newly captured animals to estimations of the age structure were adjusted so that the ratio of aged new animals to aged recaptured animals matched the ratio of new to recaptured animals for the given year. The total numbers of individuals in each adult age class were estimated from the proportion of animals in each age class in the samples.

Adult survival probabilities

Age-specific survival probabilities of adults were estimated from long term age structures by comparing the number of animals aged x in a given year with the number aged $x + 1$ in the subsequent year, as in Caughley (1977) method 4. This method allows for migration of individuals, but assumes that movement into and out-of the study area is balanced. We used the survival rates of 2 year olds as a standard to compare annual survivorship between years because this is the age where the best data was available. Survival rates for 2 year olds were not available for the years 1992, 1993, 1994, 1995, 2001, 2002, and 2003.

Analysis of age structure

The annual age distributions of the entire population (males plus females) were compared to a stable age distribution constructed from 1000 replications of annual age frequency distribution by the non-parametric Kolmogorov-Smirnov two-sample test. Differences in survival probabilities between age classes were compared by ANOVA on arcsine square root transformed data.

Variation in recruitment

The hypothesis that the population decline is related to reduced recruitment success was tested by comparing average annual recruitment rates between the intervals 1992-2001 and 2002-2011 using two-sample t-tests. The years 1992-2001 represent the fluctuation phase of the toad population dynamics at Long Point, while 2002-2011 encompasses the period of population decline. Although the density of *Phragmites* at Long Point increased approximately linearly over the 23 years of study at Long Point, it was around 2002 that the plant reached a critical density to begin impacting the toad population (Greenberg and Green, submitted).

Variation in survivorship

The hypothesis that the population decline is related to a decrease adult survivorship was tested by comparing the average survival probabilities for 2 year-olds between the intervals 1992-2001 and 2002-2011 using two-sample t-tests.

All statistical tests were performed with the programs R version 2.13.0. Graphs were made in R and in Microsoft Excel for MS Windows 7. All *P*-values given are two-tailed with $\alpha = 0.05$.

RESULTS

Over the years of the population survey, the abundance of toads fluctuated dramatically at all stages of the life cycle. The number of adult males ranged from an estimated minimum of 15 ± 0 in 2011 to a maximum of 515 ± 62 in 1993. The ratio of juveniles to adults varied from 0.28 to 4.66, resulting in juvenile population estimates that ranged from 13 to 1112 (Fig. 1).

Over 800 bones were processed by skeletochronology, 767 of which were aged with certainty. Typical cross-sections for each age class are shown in Figure 2. Numerous toads for which ages had been determined were recaptured in subsequent years, resulting in a total of 869 age estimates. Adult toads ranged in age from 1 to 5 years. The number of males that reached sexual maturity at 1 year of age was small and variable ($\overline{X}$ = 4.07 %; range: 0-16.1%). The maximum age recorded for either sex was 5 years. No significant difference was found between the number of males and females within any age class. Therefore, sexes could be combined for subsequent analysis.

The age structure of the entire adult population is shown in Figure 3. The average age for the 17 years of study between 1992 and 2011 was 2.38 ± 0.028 (S.E.) years. A trend of increasing average age by 0.02 years/year was statistically significant ($R^2 = 0.346$, $p = 0.03$) (Fig. 4). The majority of annual age structures were characterised by high numbers of 2 year-olds and declining numbers of adults with increasing age, however comparisons of age frequency distributions to the stable age distribution revealed highly significant differences for several years (Kolmogorov-Smirinov test, $p < 0.05$ for 1998, 1999, 2003, 2006, 2007, 2008 and 2011) (Fig. 3). The year 1998 was unstable due to an unusually large proportion of 2 year-olds (90%), and neither 1998, 1999, nor 2003 had any toads older than 3 years. In 1999, 2006 and 2011, 3 year olds represented the dominant age class. Three year-olds outnumbered 2 year-olds in 2006 (Fig 5).

Adult Survivorship

The proportion of toads surviving from the first age class decreased approximately exponentially with age, producing an overall Type III survivorship curve (Fig. 6). Age-specific survival for males and females combined varied significantly by age class ($F_{3,44} = 6.40$; P < 0.05), with 2 year-olds experiencing the highest probability of surviving another year (Fig. 6).

Annual survival estimates were highly variable ($\overline{X}$ =0.47, range= 0.20 to 1.00) (Table 1). The actual survival estimate for 1999 was 1.82 (corrected to 1.00), indicating that the assumption for balanced movement into and out of the study area was violated. Between intervals 1992-2002 and 2002-2011, average annual survivorship rates declined from 0.50 to 0.45. This difference, however, was not significant. The average survival rate of 1 year-olds increased in the second half of the study period from 0.15 to 0.49 (P = 0.031).

DISCUSSION

Overall, the age structure of this population of Fowler's toads was highly variable, with 7 out of the 16 years with complete age data having unstable age distributions. Much of the variation in the age structure of our population can be explained by variation in juvenile recruitment, which could fluctuate by up to one order of magnitude from one year to the next. All of the observed unstable distributions were preceded by a year of unusually high or low recruitment, and large juvenile cohorts could occasionally be followed through the age structure for successive years (as in 2004) (Fig. 5). The overall correlation between the number of 1 year-olds and the number of 2 year-olds the next year was weak, however, indicating that other factors also influenced the unstable age structure. This is in contrast to a study by Tinsley and Tocque's (1995), who found peaks in the age structure of a population of *Scaphiopus couchii* were correlated with high precipitation when those cohorts were born (i.e. recruitment success), and that these peaks could persist for up to 4 years before being replaced by the next dominant cohort.

Another source of the unstable age structure in our population was variability in survivorship. Occasionally, severe winter storms destroy the sand dunes in which the toads hibernate, causing dramatic population declines such as those of 1986 and 1994 (Green et al. 1994). Because the probability of surviving is also age-specific, such high-mortality events can disproportionately affect juvenile and older age classes, resulting in increased proportions of 3 year-olds the next year. Age-specific survivorship has also been associated with changes in the size of adult age classes in *Rana japonica* (Marunouchi et al. 2002), where construction around a breeding pond one winter resulted in a significant decrease in the proportion of breeding frogs the next year. Age-specific mortality may also help explain variation in the size of postmetamorphic age classes in populations of *R. catesbeiana*, *R. clamitans*, and *R. septentrionalis*, which are known to experience occasional catastrophic mortalities (Shirose and Brooks 1995). Contrary to our finding of age-specific survivorship in Fowler's toad, Clarke (1977) suggested that mortality rates of Fowler's toads vary little after the first month post-metamorphosis. This population, however, inhabited entirely different habitat (a golf course in Connecticut) and so may not have experienced the same environmental stressors that disproportionately affected the age classes in our population.

Although neither survival nor recruitment significantly changed between the time periods of low and high density of *Phragmites*, there was a significant trend of increasing average age in the population. This suggests that *Phragmites* could be affecting multiple stages of the toad's life cycle. Recruitment rates in this study were measured as the number of yearling toads per female toad in the previous year. Therefore, poor recruitment could occur through reduced breeding effort, greater egg and larval mortality, and/or greater mortality of newly emerged metamorphs. Reductions in postmetamorphic survivorship are restricted to individuals age 2 and older, which corresponds with sexual maturity and the use of the wetland habitat by adult toads for breeding. Greater egg and larval mortality could be the result of premature desiccation of breeding ponds, while reductions in breeding effort, postmetamorphic survivorship and survivorship of new metamorphs would be due to any number of *Phragmites*-induced changes in the habitat between breeding ponds and the beach. Telemetry studies are needed to examine the movement behaviour of breeding adults in naturally vegetated versus *Phragmites*-dominated wetlands. These results would aid in the design of dispersal corridors to the proposed artificial breeding ponds, which are an important consideration for the conservation of wetland-breeding amphibians (Dodd and Cade 1998).

The role of dispersal, both around the study area and along the entire Long Point peninsula, deserves some discussion. For the purpose of estimating survival rates in this study, we assumed that movement across the eastern and western boundaries of the traditional study area is negligible, or at least that the number of toads entering and exiting the study area was balanced. However, a few survival rate estimates with values greater than 100% serve as evidence for years where there is a net inward movement of individuals. Although generally sedentary, about 2% of Fowler's toads travel over 10 km a year (Smith and Green 2006). If dispersal is density-dependent, years in which the population size is small may have been ascribed inflated survival rate estimates due to immigration. As a result, postmetamorphic survival may be lower than our estimates suggest. Therefore, with the declining abundance in our study area, immigration of individuals from presumably more pristine areas of Long Point may be playing an increasingly important role in local population persistence.

With large annual variability in recruitment and survivorship, as well as age-specific survivorship and plasticity in the age at sexual maturity, Fowler's toad populations may be exceptionally dynamic. Because the lifespan of Fowler's toads is short, changes in recruitment can have a significant influence on the next year's total population size. Thus, for example, if 2004 was followed by a year of good overall postmetamorphic survivorship (e.g. a 50% for all postmetamorphs), the population size in 2005 could have increased by over 230%. The high variance in population size may put Fowler's toad populations at increased risk of local extinction due to demographic or environmental stochasticity (Leigh, 1981; Lacy 1993; Doak et al. 1994). But given that Fowler's toads evolved in early successional habitats, this life history is probably an adaptation to take advantage of temporally and spatially available resources (Wilbur 1980). Consequently, Fowler's toads should respond quickly to amelioration efforts; particularly if population growth is density-dependent, as increasing evidence suggests (Greenburg and Green (submitted), this thesis). Should the carrying capacity of the breeding habitat remain low, however, the outlook for this population does not look good (COSEWIC 2010).

By measuring changes in population age structure over a longer period of time than any other study, our findings highlight the natural extent of variability inherent in the population dynamics of amphibians. Although some populations of amphibian, such as *Echinotriton chinhasiensis* (Yang et al 2011), exhibit stable age structure, changes in the relative size of particular age classes in anuran species appear to be common; e.g. *Rana temporaria* (Gibbons and McCarthy 1984; Guarino and Sindaco 2008), *Hyla arborea* (Friedl and Klump 1997), *Giocrinia alba* and *G. vitellina* (Driscoll 1999), *S. couchii* (Tinsley and Tocque 1995), *Litoria spenceri* (Gillespie 2010) and *R. catesbeiana*, *R. clamitans*, and *R. septentrionalis* (Shirose and Brooks 1997). Most studies cite variation in juvenile recruitment as the cause of unstable age structures. As our study suggests, changes in age structure can also result from variation in annual survivorship combined with age-specific survivorship. This high variability in vital rates and resulting unstable age structure precludes the construction of general life-tables because there is no single year's data that are 'typical'. Therefore, accurate projections of future population sizes for many amphibian species need to be made on a "real-time" basis, from year to year, much in the way that fisheries are managed (Blaustein 1994).

Understanding the cause(s) of any particular population increase or decrease observed in the field merits detailed examination of variation in recruitment and survival. Due to the largely stochastic dynamics of Fowler's toads, age structure was variable throughout the study period.

The invasion of Phragmites into the breeding habitat appears to be reflected in the shift toward slightly older average ages. Habitat restoration efforts for Fowler's toad at Long Point should consider both the construction of artificial breeding ponds and the incorporation of dispersal corridors. Studies of age structure may be valuable in prioritizing management efforts for declining amphibian populations by identifying vulnerable life-history stage.

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Table. 1: Reconstructed age structure and estimated survival rates and recruitment rates for sequential cohorts of *A. Fowleri* from 1992-2011.

Cohort	Sample Size	Number of individuals of each age					Av. Age	Annual Survival rate
		1	2	3	4	5		
1992	59	1207	290	151	12	0	2.02	
1993								
1994	13	311	107	66	0	0	2.28	0.21
1995	39	175	28	22	10	0	2.61	0.61
1996	36	0	39	17	5	5	2.38	0.35
1997	28	0	42	13	2	0	2.22	0.59
1998	90	183	312	25	0	0	2.04	0.25
1999	31	213	31	79	0	0	2.29	1.00
2000	24	818	64	56	24	8	2.46	
2001								
2002	45	372					2.09	
2003	84	273	185	66	0	0	2.22	0.50
2004	98	1112	236	92	19	0	2.37	0.20
2005	92	148	106	48	16	0	2.47	0.49
2006	28	152	30	52	15	7	2.73	0.75
2007	50	38	47	23	24	2	2.80	0.71
2008	45	235	41	34	16	0	2.69	0.22
2009	43	68	47	9	6	2	2.37	0.36
2010	40	13	29	17	0	0	2.37	0.39
2011	24	127	10	11	4	0	2.70	
Average of 1992-2011	48	340	97	46	10	1	2.40	0.47
Average of 1992-2001	40	484	114	54	8	2	2.29	0.50
Average of 2002-2011	55	254	81	39	11	1	2.48	0.45

Figure 1. Estimated abundances of adult and juvenile Fowler's Toads at Long Point, Ontario from 1992-2011.

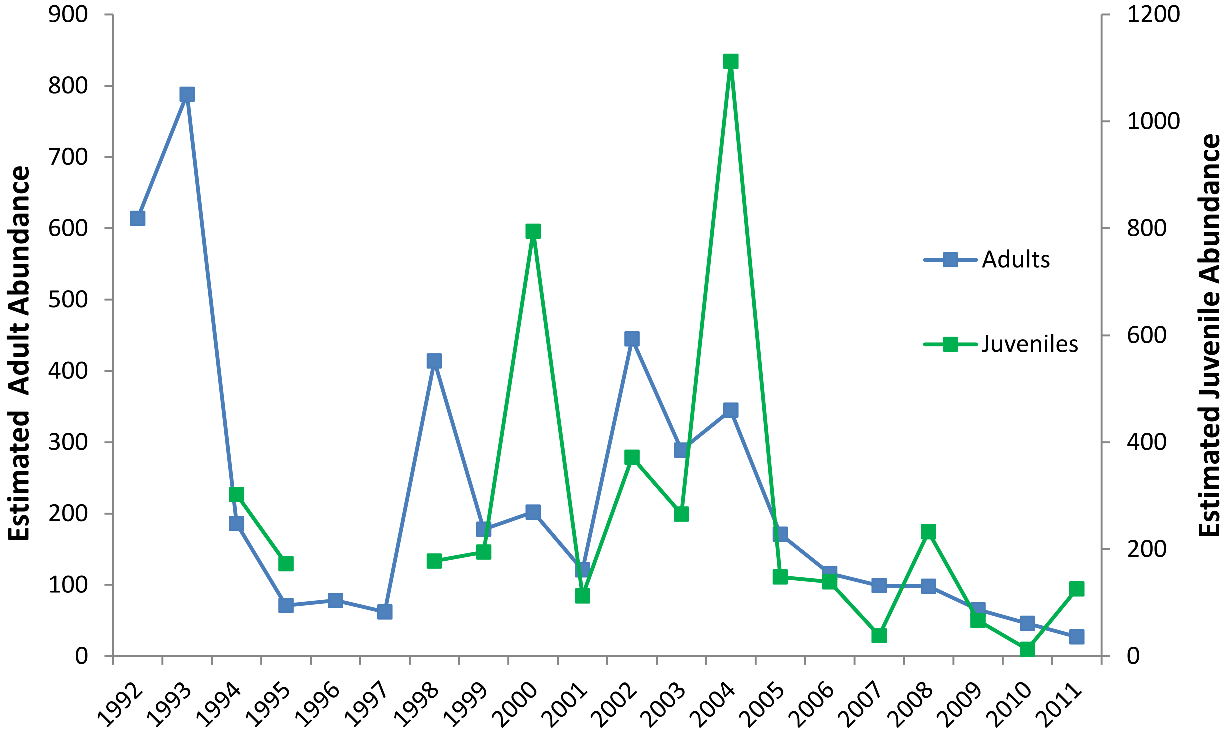


Figure 2. Hematoxylin-stained cross-sections of the phalangeal bones of Fowler's Toads of different ages. (A) one year, (B) two years, (C) three years, and (D) four years. Arrows point to lines of arrested growth (LAGs).

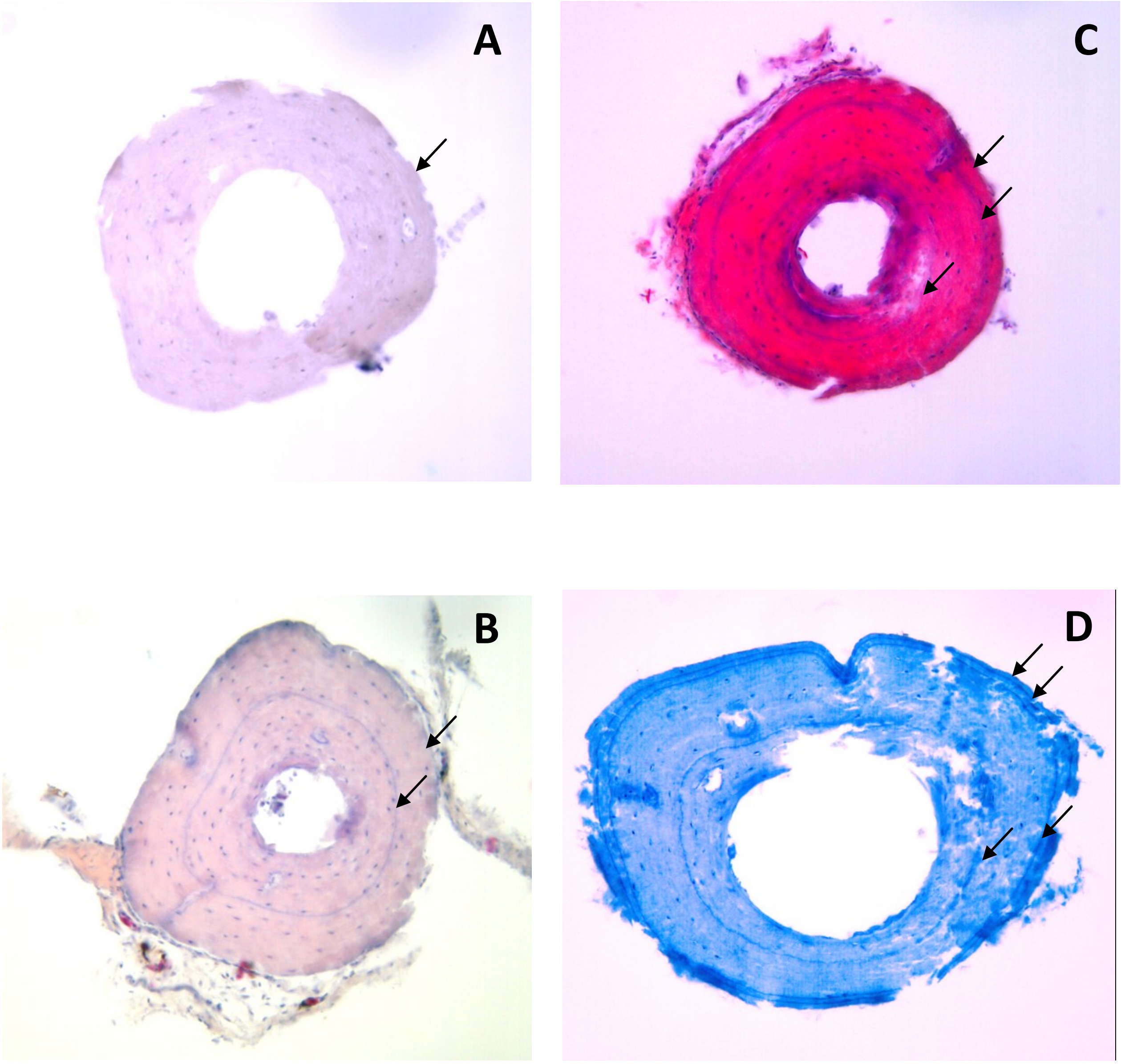


Figure 3. Age distributions of sexually mature, adult Fowler's Toads from 1992 to 2011, excluding 1993, 2001 and 2002. Distributions marked with an asterisk are statistically different (Kolmogorov-Smirinov test, $P < 0.05$).

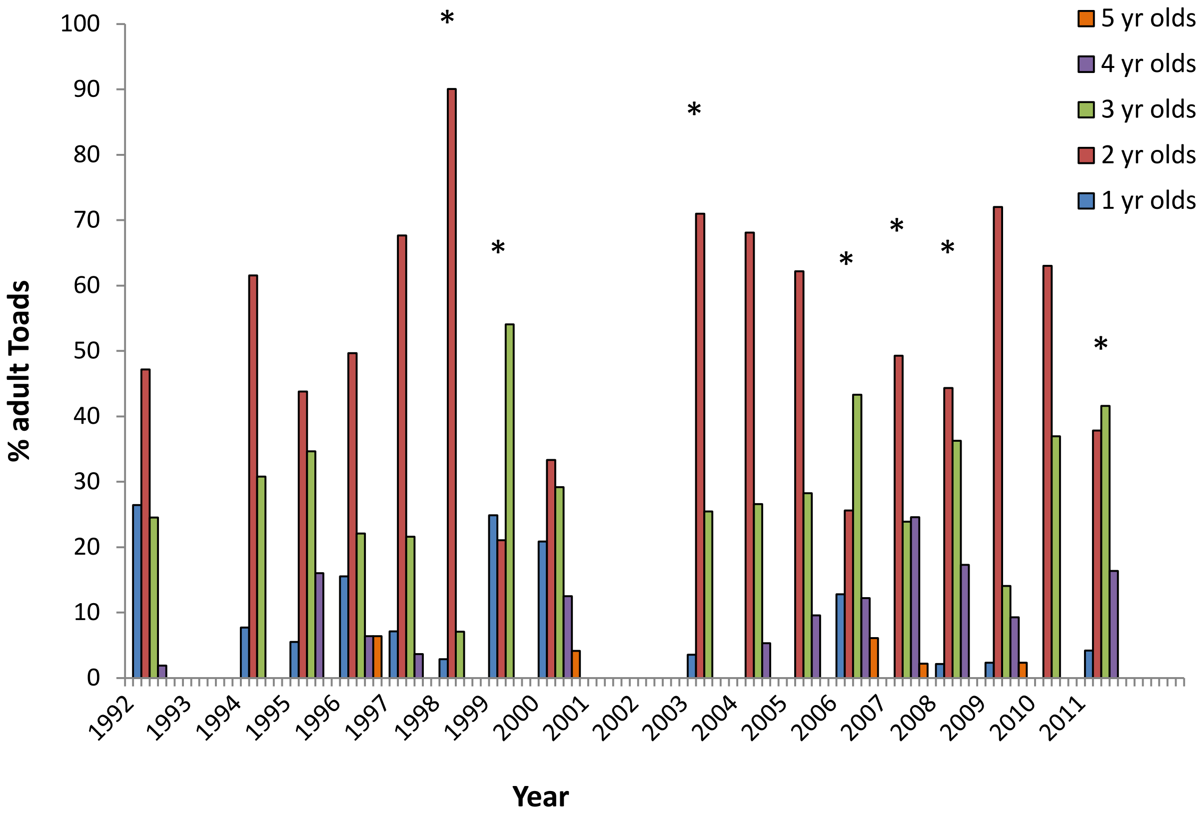


Figure 4. Average age of male and female postmetamorphic Fowler's Toads from 1992-2011.

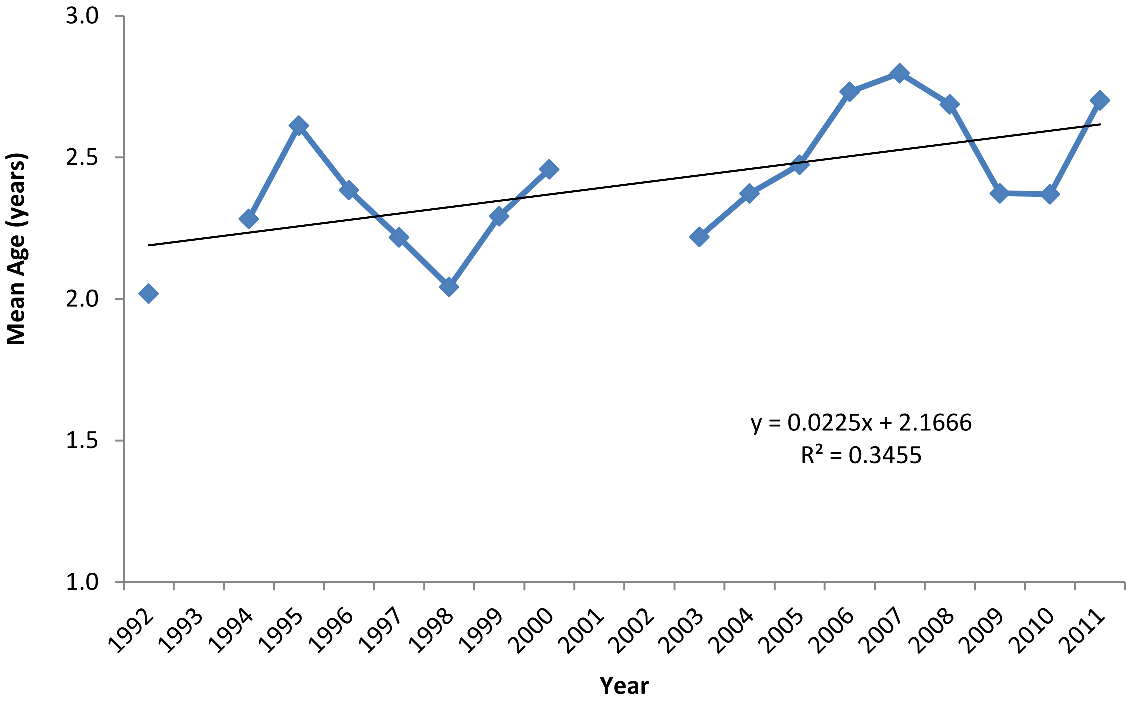


Figure 5. Annual changes in the age structure of adult Fowler's Toads from 2004-2007. The arrows follow an unusually large cohort born in 2003.

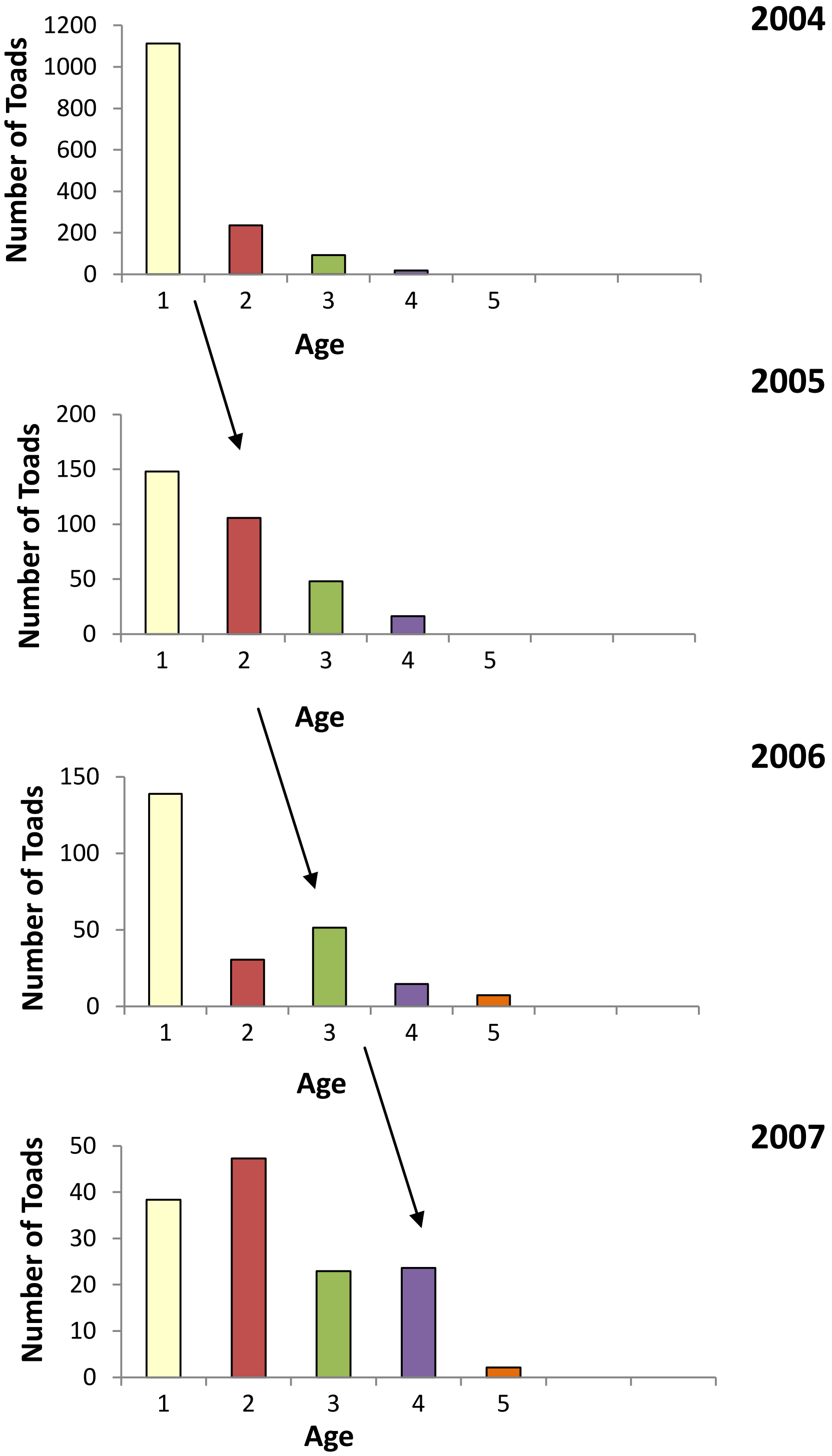


Figure 6. Average survivorship curve for Fowler's Toads at Long Point from 1992-2011, expressed as the percent of the initial number of yearlings. Curve is fitted with an exponential function.

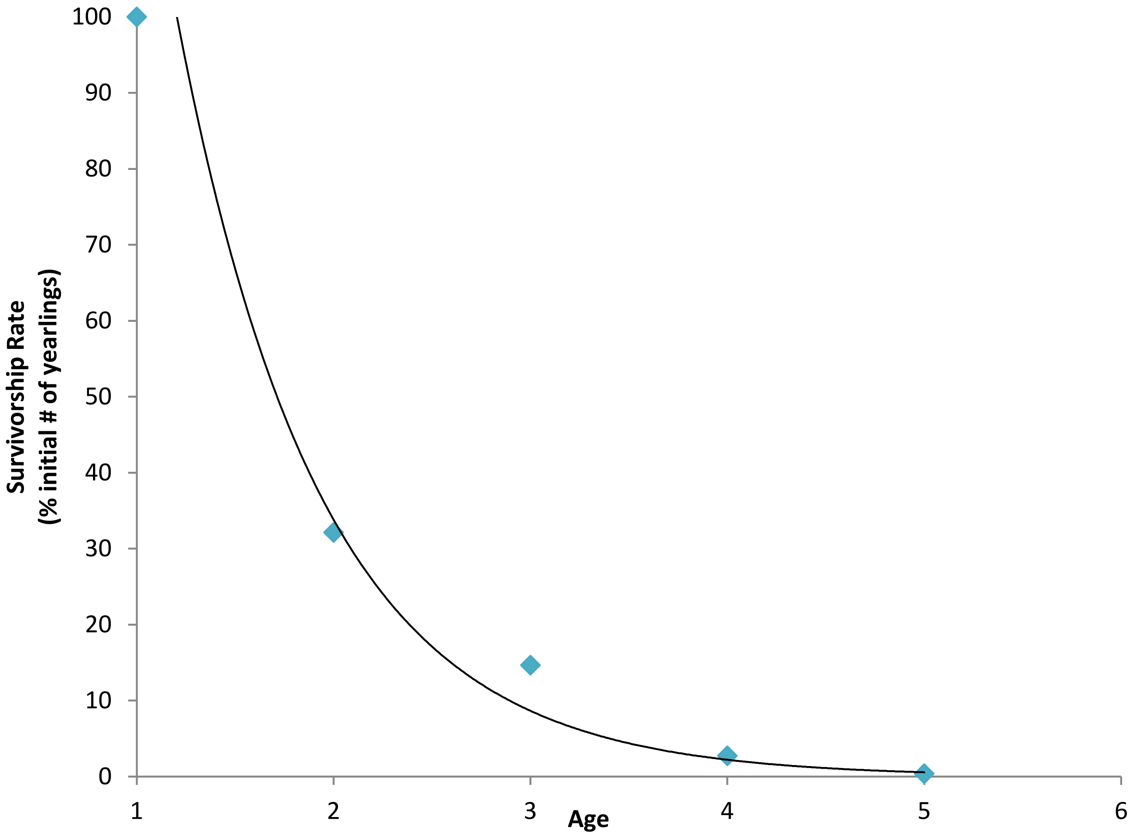
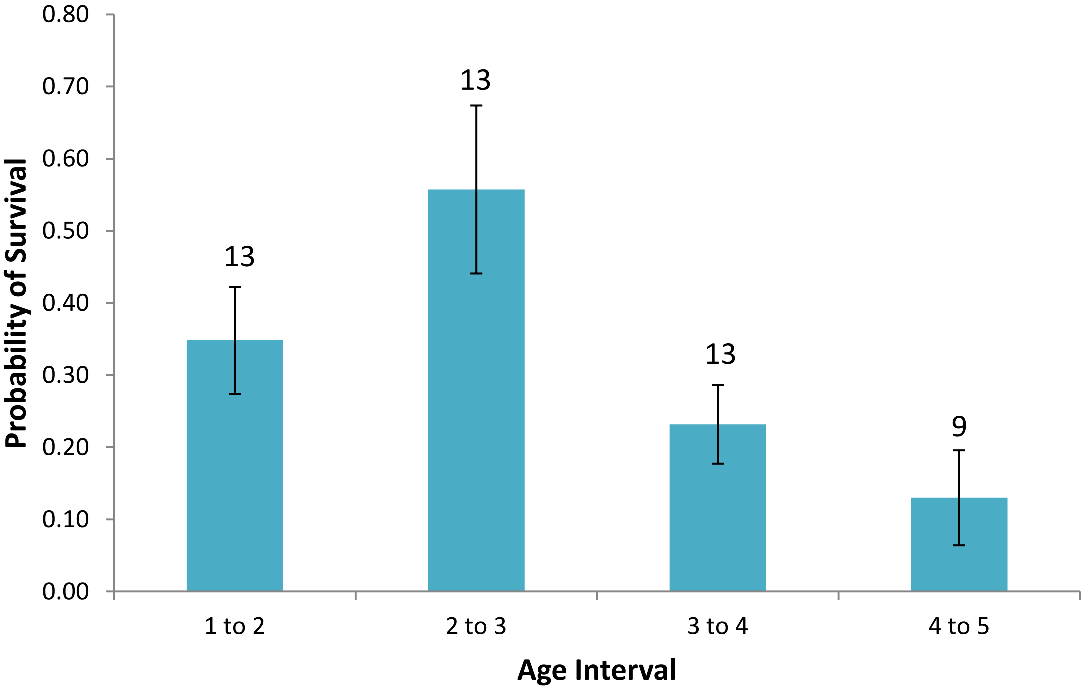


Figure 7. Average probability of a cohort surviving from age X to age X +1. Values shown above each bar and represent the number of years for which data were available.



CHAPTER 3: Density-Dependent Growth in Postmetamorphic Fowler's toads, *Anaxyrus (Bufo) fowleri*

LINKING STATEMENT TO CHAPTER THREE

Body size is also a function of growth rate. For most amphibians, the majority of growth occurs during the terrestrial life-history stage. Chapter 3 will examine changes in body size of newly metamorphosed toadlets over the summer to evaluate the potential for density-dependent somatic growth rates. Density-dependent growth rates are well documented in larval amphibians but have not yet been demonstrated in Fowler's toads.

ABSTRACT

In general among amphibians, it is advantageous to be big. Therefore, factors that influence how quickly individuals grow have important consequences for overall population dynamics. Here, we examine spatial and temporal variability in the growth rates of recently metamorphosed Fowler's toads (toadlets) in relation to population density. Snout-vent lengths were measured for samples of toadlets from study sites on regular, nightly surveys throughout for two months in the summer. Adult densities ranged from 0.01 to 0.43 toads/ meter, and toadlet densities ranged from 0.10 to 2.54 toads/ meter. Growth rates differed significantly at one site over three years, indicating that many factors likely affect growth. In 2010, the average growth rate of toadlets from the site with the lowest density of adults was twice that of toadlets from the site with the highest density of adults. Toadlet growth rates were significantly inversely related to the density of adults ($R^2 = 0.98$, $p = 0.010$), but not the density of toadlets. These results suggest that superior competition from adult Fowler's toads restricts the growth of toadlets in high density populations.

INTRODUCTION

Growth may be one of the most important traits necessary to maximize fitness in amphibians (Duellman and Trueb 1994). This is because individuals that grow faster tend to reach a larger size, which in turn provides numerous competitive, defensive and physiological advantages (Halliday and Verrel 1988). In addition, faster growth rates tend to decrease the time it takes to become sexually mature (Hemelaar 1988; Augert and Joly 1993). The majority of growth in amphibians occurs after metamorphosis (Hota 1994). In some species of anuran, for example, postmetamorphic growth in an individual accounts for at least 80% of the body size of the adult (Werner 1986). Therefore, the factors that affect growth during the terrestrial stage may be critical to the ultimate success of a population.

Numerous factors are known to influence growth of postmetamorphic amphibians. These include: genetics (Berven 1982; Bernardo 1993), carry-over effects of conditions experienced during larval development (Goater 1994; Morey and Reznick 2001), age (Breckenridge and Tester 1961, Labanick and Schlueter 1976), food quality and quantity (Scott and Fore 1995; Claussen and Layne 1983), temperature (Hadfield 1966; Lillywhite 1970), light (Richards and Lehman 1980), precipitation (Newman and Dunham 1994; Tinsley and Tocque 1995), parasites (Goater and Ward 1992; Kelehear et al. 2011) and environmental toxicants (Carey and Bryant 1995). In addition, the density of conspecifics, and possibly congeners, can negatively influence postmetamorphic growth rate (Denton and Beebee 1993; Pearson 1955; Altwegg 2003; Harper and Semlitsch 2007; Loman and Lardner 2009). The effects can be direct, such as the increased efficiency in which food is converted into growth at higher temperatures (Lilywhite 1973); or indirect, such as a decline in insect abundance (and hence food availability) in areas with heavy insecticide use (Freemark and Boutin 1995).

Density effects on the growth of postmetamorphs are primarily attributed to competition for food resources (Goater 1994, Pechmann 1994, Morey and Reznick 2001; Harper and Semlitsch 2007). The mechanism of competition likely depends on the species. For example, many salamanders are highly territorial and thus are more likely to compete for food through direct interactions (interference competition) (Jaeger 1980; Walls 1990). Anurans, on the other hand, rarely interact outside of the breeding season and so competition for food among postmetamorphic anurans is more likely to result from differences in abilities to exploit resources (exploitation competition). Population density can also affect growth indirectly through increased predator pressure in higher-density areas resulting in decreased foraging rates, as has been observed in some larval populations (Skelly and Werner 1990).

Fowler’s toads metamorphose at an extremely small size relative to ranid and hylid frogs (Werner 1986). Therefore, the growth rates experienced during the first few months after metamorphosis are particularly important for this species. The growth rates that have been reported for newly metamorphosed Fowler’s toads are rapid, but also highly variable. Labanick and Schlueter (1976) estimated an average growth rate of 0.36 mm/day for snout-vent length (SVL) and 0.15 mm/day for the tibia. Clarke (1974a) found a mean tibia growth rate of up to 0.13 mm/day, and Breden (1988) found that toadlets metamorphosing at an average size of 9 mm (SVL) were an average of 46.8 mm two months later--a growth rate we calculate as approximately 0.62 mm/day. Despite this high natural variability in growth rates, however, no one has attempted to correlate growth in Fowler’s toads with population density.

We examine the growth of Fowler’s toad toadlets--defined here as the developmental stage between metamorphosis and first hibernation--in order to assess the significance of density dependence on somatic growth in natural populations. Growth rates and population densities are compared between four study sites and within one study site over three years. Since these locations are within 140 kilometers of each other along the north shore of Lake Erie, Ontario, the habitat and weather conditions in any single year are similar. If growth rates are density dependent, then the average growth rates of toadlets should be greater in sites with a lower density of toads than in sites with a higher density.

METHODS

Description of field sites

Surveys at Long Point were conducted along the Canadian Wildlife Service Thoroughfare Beach Unit, immediately east of the traditional survey area described in Chapter 2 of this thesis. This area is located in Universal Transverse Mercator (UTM) zone 17 N, between

550700 and 553000 (m) Easting, and 4713615 and 4714200 (m) Northing (NAD 83 Datum). Similar to the rest of the Long Point shoreline, Thoroughfare beach is a continuous habitat, running almost directly east-west, without any physical barriers to animal movement.

Surveys in Niagara were conducted at Nickel Beach, located in the UTM zone 17 N between 643679 and 645233 (m) Easting and 474774 and 4748513 (m) Northing. Nickel Beach spans approximately 2125 meters and is bordered to the east and west by rocky headlands. Breeding is restricted to pools within these headlands, resulting in two distinct sources of toadlets on opposite ends of the beach. Therefore, Nickel beach can be divided into two separate study sites: Niagara East and Niagara West. The habitat at Niagara resembles that of Long Point except that there is a greater accumulation of algae along the water's edge.

Finally, surveys in Rondeau were conducted on South Beach of the Provincial Park, located in the UTM zone 17 N between 425272 and 425600 (m) Easting, and 4679191 and 4679019 (m) Northing. The portion of beach surveyed was approximately 870 meters long and was bordered on both ends by dense vegetation. The beach is at the end of a peninsula and is thus rounded in shape; the majority faces the open water of Lake Erie, but is somewhat sheltered to the west by the inner bay. Breeding along the peninsula occurs on the bay side (Dobbyn 2005).

Density estimates

The majority of density estimates were calculated from 3-day mark-recapture surveys in July, August, and early September--when all age groups are active on the beach. Toads were "marked" using either an annual web-clip or by matching the dorsal spot patterns from photographs taken upon capture, thus enabling Peterson Index estimates of abundance. Details of the specific areas and marking methods used for each site are summarized in Table 1.

Ideally, the method used to estimate population densities should have been consistent across sites. The data from Niagara, however, was collected under a larger population monitoring program by the Ontario Ministry of Natural Resources, and thus we work within the framework of their protocol in order to estimate juvenile and toadlet population densities. This involved a weekly mark-recapture survey in which adult toads were marked with unique toe-clips, providing an accurate estimate of adult population size for the entire beach (Yagi 2008). Due to time constraints for nightly surveys, toadlet counts on the west end were restricted to a length of only 300 meters of beach. To be consistent, we restricted the survey area on the east end to 300 meters as well. Because adults were relatively evenly distributed over the 2125 meters of shoreline (at least every part of Nickel Beach experienced far greater adult densities than any other study site), we used the overall adult population density to estimate the number of adults that might occur in an area of 300 meters. The abundance of juveniles and toadlets could then be roughly estimated by multiplying the number of juveniles and toadlets counted in each study area on the busiest night by the ratio of adults counted in those areas on the same night: the total estimated abundance of adults in 300 meters.

Growth rate surveys

Growth rate surveys were conducted on an approximately 10 day basis from early July until August 30. This time frame was chosen because it is when toadlet growth is at a maximum and is relatively linear (Clarke 1974*a*; Labanick and Schlueter 1976; Hota 1994). For each survey, we would walk the entire length of the study area, capturing every individual encountered. Search efforts were focused primarily within the first 5 meters of the water's edge, as this is where the vast majority of Fowler's toads can be found in the evening (Dobbyn 2005). All toads encountered were measured with dial callipers accurate to 0.1mm. Toads were held firmly in the hand and pressed flat with the thumb in order to measure them from the back of the vent to the tip of the snout in a consistent way. Adults were identified by sex, and the distinguishing male sexual characteristic(s) (darkening of the throat, vocalizations and/or vibrations) were recorded. UTM coordinates were taken for each encounter and the type of microhabitat (sand, algae or rocks) on which animals were found was noted. In 2010 and 2011, we began photographing the backs of toadlets during several surveys to allow for potential re-identification of individuals in order to test for size-specific survivorship.

Absolute growth rates

The dependent variable analysed in this study is growth rate. This is measured as the coefficient (K), the linear slope of average body size measurements against time.

Statistical analysis

The slopes representing the growth rate of toadlets at Long Point in 2009, 2010 and 2011, along with their standard errors, were compared by one-way analysis of variance (ANOVA). In order to test the effect of density on somatic growth rates, we performed a regression analysis of the growth rate and estimated densities of adults, toadlets, and the overall population densities from all sites in 2010. Across-site comparisons are limited to this year because this is the only year in which all sites were surveyed intensively. Tests were performed in SPSS version 20.0.0, with $\alpha = 0.05$.

RESULTS

Population densities

(N.B.: As the season progressed, juvenile toads increasingly resembled adults; therefore, we refer to juveniles and adults collectively under the designation of “adults”.)

The abundance of toads at Long Point varied from year to year. 2010 had the lowest number of adults but the highest number of toadlets, with estimated abundances of 25±0 and 399±125 toads over 958 meters of beach, respectively. In 2011, the estimated abundance of adults and toadlets along that same stretch of beach was 95±19 and 333±104, respectively. The year 2009 had approximately 82±11 adults along 1000 meters of beach. Toadlets were not present at the time the population census was conducted that year; however, with only 7 individuals observed on the busiest night, we can safely assume that 2009 had the lowest density of toadlets of all study sites.

Toadlets at Rondeau were found along the entire South Beach peninsula, but were slightly less concentrated to the east, facing the open lake. The three day mark-recapture survey at Rondeau yielded population estimates of 18±2 adults and 520±53 toadlets over 780 meters of shoreline.

The total estimated adult population size for the entire beach in Niagara, 2010, was 508±41 toads (Yagi, unpublished data). The busiest night of the season was July 21. Toadlets were not counted on the east side that night, so we use the second busiest night, July 26, to estimate abundance in that study area. The ratio of adults to toadlets on these nights was 32:265 in the west survey area and 13:52 in the east. This provides total abundance estimates of 92 adults and 763 toadlets in the west, and 128 adults and 416 toadlets in the east. Algae dominated the habitat on the shore of the west side and were widely used by the toadlets while foraging.

Actual density estimates for each year and site are presented in Table 1.

Distribution and dispersal

At the time that population densities were estimated, toadlets at each site were generally found throughout the study area. This was not always true at the beginning of the season, when the toadlets at some sites were concentrated in smaller sections of beach that were presumably closer to breeding ponds. At Long Point in 2010, the first toadlets were observed near the east side of the study area, but were located further and further west with each successive survey. Because Fowler's toads congregate to breed, it is likely that these toadlets were all emerging from the same pond. Thirty-one days after toadlets were first observed on the beach, the distance between the furthest traveling toadlets and the location where toadlets were first observed was approximately 1100 meters.

Growth rates

Body size measurements from each survey are presented in Table 2. Although the sizes of individuals during some surveys ranged widely, frequency distributions of body size measurements for each survey were normally distributed. This supports the assumption that toadlets at each site were born at approximately the same time. Unfortunately, there were too few within-season recaptures identified to test for size-specific survivorship. If smaller individuals were dying disproportionately, this would result in larger calculated growth rates as compared to the actual growth rates.

The changes in average snout-vent length over time appear to be linear for each survey period ($R^2 > 0.97$ for all sites) (Fig. 1). The weakest correlation was for Niagara West, where the increase in average body size was much smaller between the last two surveys (Fig. 1(*e*)). These measurements were taken 62 days after the first observation of toadlets at Niagara, making this the longest time frame over which any population was surveyed.

The growth coefficient (K) for toadlet populations ranged from 0.23 mm/day to 0.49 mm/day (Fig. 1). The toadlets at Long Point experienced some of the fastest growth rates. On an extra survey at Long Point on September 20, 2011, a 50.5 mm toadlet (stage confirmed though photo-matching from earlier that season) produced vocalizations upon capture. This may be the youngest age recorded for a Fowler's toad to exhibit a secondary sexual characteristic, as other studies referring to the age at sexual maturity cite at least one full active season post-metamorphosis.

Somatic growth rates at Long Point were statistically different across years (One-way ANOVA, $F = 64.406$, $df = 2$, $P < 0.000$) (Fig. 2). Because climate and environmental conditions also vary each year (Chapter 4, this thesis), we cannot differentiate the effects of density on growth rates at the temporal scale.

Temperature and precipitation experienced between sites in 2010 were perceived to be relatively similar. Within this year, toadlets grew fastest at Long Point and Rondeau and considerably more slowly at Niagara West and Niagara East. This produced a negative trend between overall population size and toadlet growth rates. The correlation between population density and toadlet growth rates was significant for adult density ($p = 0.010$, $R^2 = 0.98$), but not for toadlet density (Fig. 3).

DISCUSSION

As our study illustrates, growth rates of Fowler's toads can differ--not only between populations--but also within them, from one year to the next. Population densities at Long Point were relatively low for all three years, yet the growth rates of toadlets each year differed significantly. This indicates additional influences on growth rates, likely related to temporal changes in climatic and environmental factors. Measuring body size across neighbouring populations within the same year allowed us to control for some of these factors and test for effects of population density on somatic growth rates.

The main finding of this study is that the growth of Fowler's toad toadlets is density-dependent. Between the populations with the highest and lowest density of adults, the average growth rate of toadlets differed by a factor of two. This resulted in toadlets that were on average 16% larger at Long Point than Niagara East by the end of August, even though we suspect the toads at Niagara had been growing over a longer period of time.

The fact that growth rates in our populations were not also dependent on toadlet density is somewhat surprising, given that toadlet density varied to a much greater degree than adult density. However, this is reasonable if we consider the possibility that adults are far superior competitors. Indeed, the prey taxa consumed by Fowler's toads are the same regardless of differences between size classes (Clarke 1974*b*). The lack of a significant relationship could also be due to low statistical power, given the high variability in toadlet growth rates relative to the number of sample sites. Additionally, it could be that population density was not always an accurate reflection of per-capita resource availability.

A limitation to the interpretation of our results is that we did not quantify resource availability. Because the habitat between the study sites is generally very similar, we assumed that the density of resources was also relatively equal. A likely exception is Niagara in 2010, where algae was allowed to accumulate on the shoreline and became a dominant part of the habitat in the western study area. This algae, which as it dried formed large, crusty mats, is believed to have been beneficial to the toadlets. During the day, toadlets were found hiding under elevated portions of the mats, presumably using them as refuge sites from the sun and predators (Yagi, personal communication). The algae also seemed to attract an abundance of insects which likely became a fortuitous food supply for the growing toadlets. As a result, it could be that Niagara West supported a higher density of toadlets without a proportionally negative effect on individual's growth rates.

Our results agree with other studies of postmetamorphic growth in amphibians. Reading (1988, 1990) showed that common toads (*Bufo bufo*) from two populations in southern England exhibited both spatial and temporal variation in growth. In a study similar to ours, Denton and Beebee (1993) found that high density populations of *B. calimata* were composed of individuals that grew relatively slowly from metamorphosis onwards. Experimental manipulations by Pearson (1955), Altwegg (2003), Harper and Semlitsch (2007), and Loman and Lardner (2009) show density effects on postmetamorphic growth rates while controlling for resource availability. Finally, by comparing density effects of manipulations and effects of actual density in field enclosures simultaneously, Loman and Lardner (2009) confirm that this phenomenon is likely to operate in natural populations as well.

The observation that the distribution of toadlets at Long Point in 2010 expanded over time brings into question the spatial scale of density effects on natural populations of amphibians. If certain individuals are consistently at the edge of an expansion, and thus experiencing lower densities, do they grow at a faster rate than their conspecifics at the center of the distribution? And is dispersal itself density-dependent? In order to maximize fitness, individuals should prefer to be in high-quality habitat; but as more animals occupy that habitat the quality is degraded, and there comes a point at which some animals should choose to leave (Fretwell and Lucas 1969; Morris 1987). Unlike Niagara and Rondeau, where study areas were restricted by physical boundaries, the toadlets at Long Point have essentially unlimited beach along which to disperse. Thus, Long Point provides an opportune system in which to conduct large scale studies addressing dispersal (e.g. Smith and Green 2006). A better understanding of the effects of density on dispersal is necessary, because the persistence of amphibian populations in patchy environments depends on recolonization following local extinctions (Marsh 2001; Semlitsch 2010; Altwegg et al. 2012).

Whether or not density-dependent somatic growth translates into population regulation depends on the ultimate effect of growth on survival and/or reproduction. Because the probability of surviving another year in Fowler’s toads is so low (Chapter 2, this thesis), becoming sexually mature at a younger age is extremely important for ultimate population growth (Ricklefs, 1979). Fowler’s toads usually reach reproductive maturity at 2 years of age, but particularly rapid-growing individuals may reach maturity within 1 year of metamorphosis (Breden, 1987; Labanick and Schleuter 1976; Clarke 1974*a*; Kellner and Green, 1995). In our study, the finding of a large toadlet expressing secondary sexual characteristics may be preliminary evidence of density-dependent effects on reproduction. This relationship has already been shown in experimental populations of *Rana sylvatica* and *Bufo americanus* (Harper and Semlitsch 2007).

Another important factor contributing to population growth rate is adult body size (as reviewed in Chapter 1 of this thesis). Although most of the growth in Fowler’s toads occurs within the first few months of terrestrial existence, a considerable proportion remains to take place during the juvenile stage (Clarke 1974a; Breden 1988). Halliday and Verrell (1988), who review the relationship between size and age in a range of amphibian species, suggest that pre- and post-maturity growth are usually related. It remains to be seen whether size differences in Fowler’s toad toadlets are maintained into adulthood. It does, however, seem likely--assuming the same density-dependent processes that affected growth in the toadlet stage continue to operate in the juvenile stage as well.

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Table 1: Details of census methods and density estimates for each site.

SITE	Area of Survey	Date of Census	Method Used to Estimating Abundance	Density (toads/ meter)	
				Adult	**Toadlet**
Long Point 2009	1000 meters	Jun. 7-9 (adults)	Adults: Mark-recapture using annual web-clip Toadlets: count from busiest night (Aug. 24)	0.009	0.082
Long Point 2010	958 meters	Aug. 26-28	All: Mark-recap. using an annual web-clip	0.026	0.417
Long Point 2011	958 meters	Sept. 6-8	All: Mark-recapture by photo-matching of dorsal spot patterns	0.099	0.351
Rondeau 2010	870 meters	Aug. 14-16	Adults: Mark- recapture by photo-matching of dorsal spot patterns Toadlets: Mark-recap. using a web-clip	0.021	0.600
Niagara West 2010	300 meters	All season (adults)	Adults: Mark-recap. using unique toe-clips Toadlets and juveniles: adult to juvenile to toadlet ratio on busiest night (July 21)	0.307	2.543
Niagara East 2010	300 meters	All season (adults)	Adults: Mark-recap. using unique toe-clips Toadlets and juveniles: adult to juvenile to toadlet ratio on busiest night (July 27)	0.427	1.387

Table 2: Sample size, mean, standard deviation and range of snout-vent lengths for Fowler's toad for each collection site and date.

			Snout-vent length	
Site	**Date**	**Sample size**	**Mean ± SD**	**Range**
Long Point 2009	August 17	2	25.1 ± 2.90	27.0-31.1
	August 24	7	31.9 ± 1.89	30.2-34.6
	August 30	4	35.1 ± 1.53	33.5-37.2
	September 12	5	41.6 ± 3.20	38.1-45.2
Long Point 2010	July 28	5	25.0 ± 2.11	21.7-27.6
	August 5	21	28.1 ± 2.10	24.3-33.1
	August 17	48	31.8 ± 2.69	24.2-36.9
	August 26	47	36.0 ± 3.30	27.4-42.3
	August 28	38	37.6 ± 4.10	30.2-49.5
Long Point 2011	July 26	16	26.2 ± 3.22	19.9-32.9
	August 12	55	33.7 ± 2.37	29.3-37.7
	August 23	17	36.7 ± 4.01	26.7-41.6
	September 9	27	43.1 ± 2.97	37.2-49.3
Rondeau 2010	August 7	9	32.4 ± 2.88	31.0-37.0
	August 14	42	36.3 ± 2.69	30.2-43.4
	August 25	42	39.6 ± 7.1	28.7-48.6
Niagara West 2010	June 30	10	15.7 ± 1.14	13.9-16.9
	July 6	72	17.8 ± 1.86	12.6-22.6
	July 15	21	21.2 ± 2.79	15.9-25.8
	July 21	268	22.8 ± 2.46	16.4-33.7
	July 27	201	24.8 ± 2.87	16.2-32.7
	August 11	227	30.2 ± 3.44	19.5-38.9
	August 18	46	32.6 ± 4.72	22.6-43.8
	August 30	189	32.7 ± 3.18	24.9-39.7
Niagara East 2010	July 6	12	18.8 ± 1.56	16.1-22.1
	July 15	10	20.9 ± 3.33	16.6-26.7
	July 27	59	25.1 ± 4.15	15.4-39.4
	August 30	39	31.6 ± 4.38	21.0-39.3

Figure 1: Somatic growth rates of Fowler's Toad toadlets at (*a*) Long Point 2009, (*b*) Long Point 2010, (*c*) Long Point 2011, (*d*) Rondeau 2010, (*e*) Niagara West 2010, (*f*) Niagara East 2010, with equations for least-squares regression lines and R^2 values.

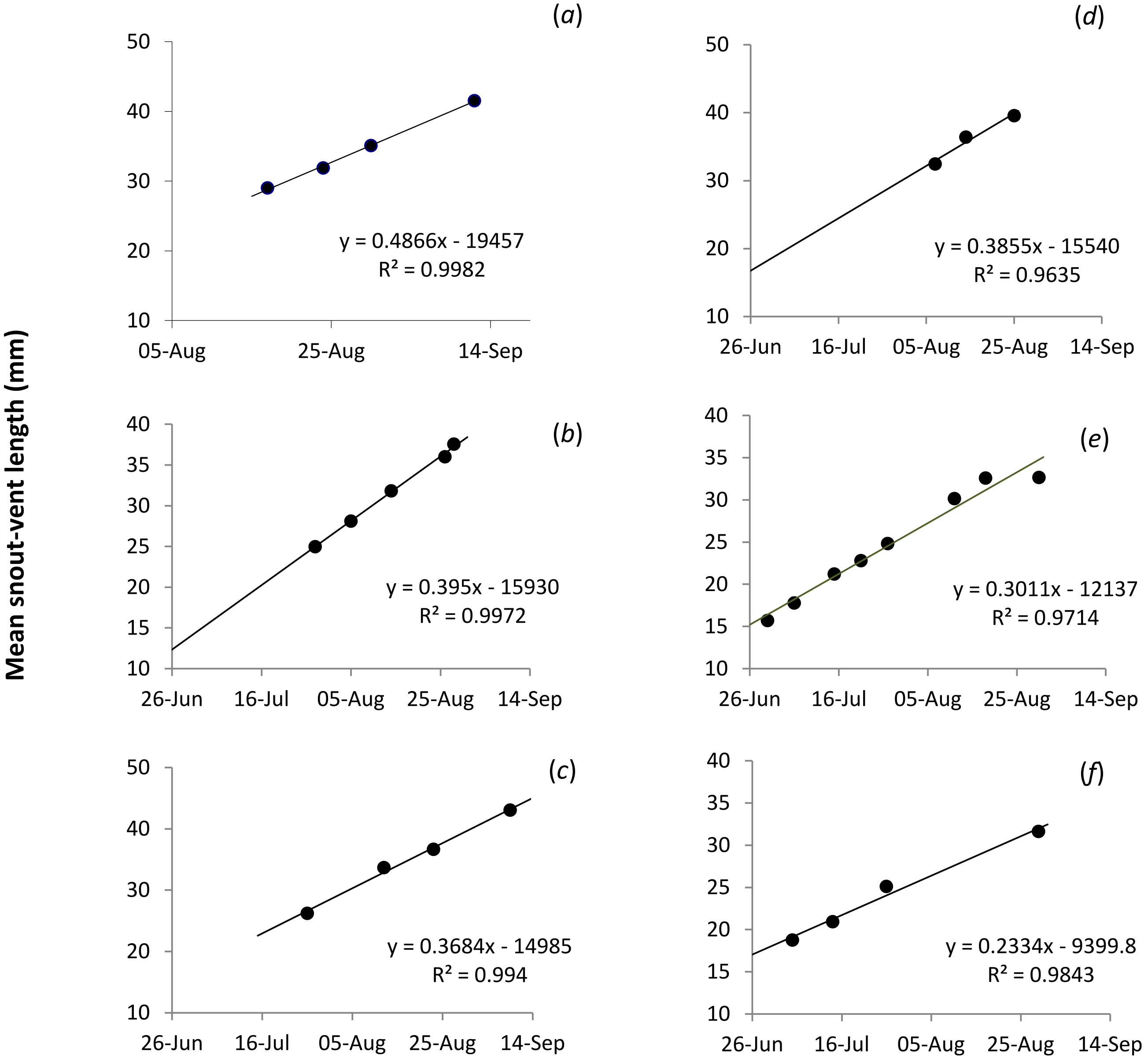


Figure 2: Regression lines with 95% confidence interval bands for the growth of Fowler's Toad toadlets at Long Point in 2009, 2010 and 2011. Body size measurements are standardized against the estimated number of days after emergence (assuming all toads metamorphose at 10mm) to benefit visual comparison. The slopes of the regression lines are statistically different across years (One-way ANOVA, F = 64.406, df = 2, P < 0.000). Growth rates for 2010 and 2011 are only marginally statistically different (t-test, P = 0.039).

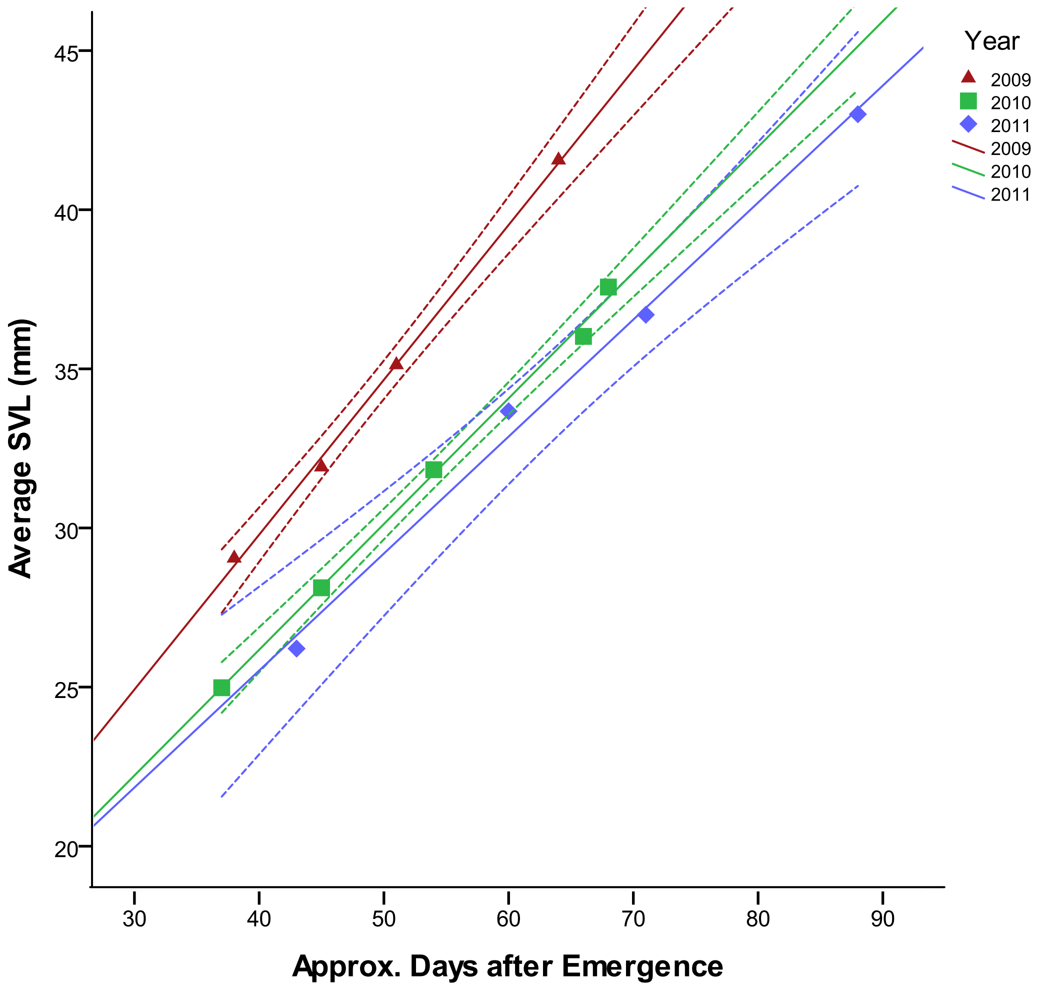
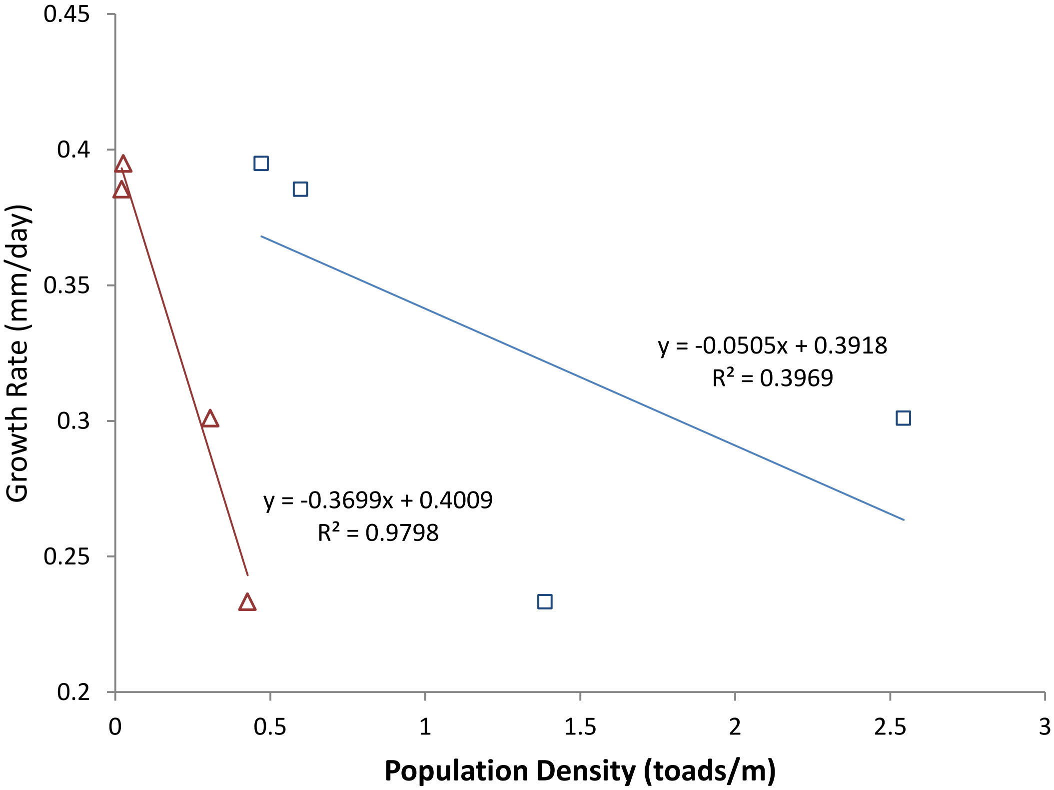


Figure 3: Regression lines of toadlet population growth coefficients (K) versus density of toadlets (□) and adults (△) at four study sites in 2010. The relationship is statistically significant between growth rates and the density of adults ($R^2 = 0.98$, $p = 0.010$), but not the density of toadlets.



CHAPTER 4: Body Size Varies with abundance, not age or climate, in an Amphibian Population

LINKING STATEMENT TO CHAPTER FOUR

Variation in the average body size of our Fowler's toad population could result from annual changes in age structure (CHAPTER 2) and/or somatic growth rates. Somatic growth rates, in turn, are affected by population density (CHAPTER 3) and climatic variation. In chapter 4, I examine the long-term variability in average body size of the Fowler's Toad population at Long Point in relation to changes in age structure, abundance, temperature, precipitation and Lake Erie water levels. In doing so, I identify the single most important factor influencing the body size-abundance relationship.

ABSTRACT

Body size variation among animals has many possible correlates, though rarely has it been studied over ecologically relevant timeframes. Large temporal body-size fluctuations within populations, if unaccounted for, could significantly affect our understanding of body size variation over large temporal and geographic scales. Adult body size was analysed over the course of 23 years (1989 to 2011) in a population of Fowler's Toads at Long Point, Ontario. We used an information theoretic approach to identify the most likely models to explain body length variation in relation to abundance, age and environmental variables, including temperature. Male toads overall averaged 53.3 mm (n = 1,962 individuals), but average body length from year to year varied from 50.9 ± 3.0 to 59.7 ± 3.3 mm (n = 23 years). The annual abundance of the toads over the 23 years was the only significant variable correlated with body size variation ($R^2 = 0.713$, $p = <0.001$). Over the last 10 years of the study, a significant trend in increased body size ($R^2 = 0.874$, $p = <0.001$) was coincident with previously detected negative trend in abundance. Body size variation in these toads is likely due to density-dependent somatic growth in the terrestrial stage. Temporal body size variation within populations may be a significant component of body size variation at larger temporal and geographic scales.

INTRODUCTION

An animal's body size affects every aspect of its biology and is, in turn, affected by multiple components of its environment and heredity (Peters 1983; White et al. 2007). At large scales of the biological hierarchy, animal body size is typically treated as a species or population characteristic (Chown and Gaston 2010) and set as the independent variable in relation to large-scale patterns of abundance, climate, or resource availability (Angilletta et al. 2004; Walters and Hassall 2006; Lewis et al. 2008). Implicitly, the average body size of a species or population is assumed to be, if not precisely constant, then at least consistent (Gaston et. al. 2008; White et al. 2007). Thus Bergmann's Rule (Bergmann 1847) and James's Rule (James 1970) concerning animal adaptation to climate at the species or population level, respectively, or the "Abundance-Size Rule" (Damuth 1981; Lewis et al. 2008), for example, are all observations cast in terms of some estimated standard species- or population-specific body size, often with data sets consisting of single samples as exemplars (e.g. Damuth 1987; Atkinson 1994; Olalla-Tárraga and Rodríguez 2007).

But what, actually, is the average body size of a species or a population? At the level of the individual, it is abundantly evident that adult body size is both phenotypically plastic and evolutionary malleable (Ozgul et al. 2010; Millien et al. 2006; Yom-Tov and Geffen 2011). An individual's eventual adult body size is influenced by many contingent factors during its growth and development, including density, temperature, age and food availability (Teder et al. 2008; McNab 2010; Huston and Wolverton 2011). Thus significant variability in body size within and between populations should be expected. Macroecological correlates to the body size are unlikely to find explanations unless they consider the microecological processes that must operate at the scale of individuals in populations (Hayward et al. 2009; Chown and Gaston 2010; Liao and Lu 2012).

Variation in body size is both spatial and temporal (Tchernov 1979). Geographic variation in body size among populations within species is common (Yom-Tov and Geffen 2006; Gaston et al. 2008). Temporal variation in body size within populations may occur both over long temporal scales, during which evolutionary change may become evident (Millien at al. 2006), and over short time frames characterized largely by phenotypic plasticity (Yom-Tom and Geffen 2011). Significant changes in average body size over periods of many years have been found within populations of animals as diverse as beetles (Braun et al. 2004), mustelids (Yom-Tov et al. 2009) and fishes (Bjørnstad et al. 1999; Martinson et al. 2008; Rogers et al. 2010). In some cases, body size within populations may change fairly rapidly. Smith et al. (1998) found a significant decrease in body mass in wood rats (*Neotoma* spp.) in less than eight years. Wikelski and Thom (2000) showed that individual Gálapagos marine iguanas, *Amblyrhynchus cristatus*, decreased body length by as much 20% within two years in response to El Niño events. Many of the recent observations of body size changes in populations (e.g. Post et al. 1997; Smith et al. 1998; Yom-Tov 2001; Reading 2007) have been attributed to accelerated climate change (Yom-Tov and Greffen 2011).

Amphibians, being small, widespread and prone to congregate in discrete breeding assemblages, should be amenable subjects for the study of body size variation. Geographic variation in body size has been identified within numerous amphibian species (Olalla-Tárraga and Rodríguez 2007) though there is no consistent correlation to any particular environmental factor. For example, patterns in body size appear to conform to Bergmann's (or, rather, James's) Rule in many amphibian species (Ashton 2002), oppose it in others (Cvetković et al. 2009; Sinsch et al. 2010) and disregard it still in others (Adams and Church 2007). Variable rates of growth within populations are known (Galatti 1992) and density-dependent growth in amphibians, particularly larvae and recently metamorphosed juveniles, is well established (Altwegg and Reyer 2003; Cohen and Alford 1993; Petranka and Sih 1993; Harper and Semlitsch 2007; Altwegg 2003; Boone 2005; Loman and Lardner 2009). There is, however, only modest documentation of temporal variation in body size in amphibian populations (Yom-Tov and Geffen 2011) as there are very few longitudinal studies of amphibian populations that present data on body size (except see Sullivan 1987; Elmberg 1987; Reading 2007; Neveu 2009; Plăiaşu et al. 2010). This is despite the many recent long-term studies of changes in their abundance (Houlahan et al. 2000; Green 2003; Lowe 2012).

If adult body size in amphibians may be strongly affected by density-dependent somatic growth rates (Harper and Semlitsch 2007) and the abundances of amphibians may be highly changeable (Green 2003), then temporal variation in body size within populations may be considerable. It might even be enough to confound, or be confused with, evidence of variation in relation to geography, temperature, resources or climate. We therefore addressed temporal variation in body size in the wild within a single amphibian population in relationship to abundance, age and environmental factors. Adult male Fowler's Toads are reported to range from 51 to 63.5 mm in body length (Dickerson 1907; Wright and Wright 1949; Conant 1958; Klemens 1993; Hulse et al. 2001). The population at Long Point, Ontario, is known to vary from year to year in abundance and population age structure to a considerable degree (Green 1997 2005; Greenberg and Green submitted). As we shall demonstrate with 23 years of continuous data, they also vary considerably in body size.

In this population of toads, if somatic growth rate of individuals is negatively correlated with their abundance, then average body size should vary in concert with abundance over time as an inverse power or lognormal relationship. Alternatively, if average body size is simply a function of age in animals with indeterminate growth (Hemelaar 1988; Liao and Lu 2012), there should be a significant correlation between age and body size. If there exists an energetic relationship between average body size and abundance (Walters and Hassall 2006), both should be correlated in such ectothermic animals with mean environmental temperature during their active growing season (Sinsch et al. 2010).

METHODS

Study Area and sampling

This study uses data on male Fowler's toads from the annual mark-recapture survey of Fowler's Toad at Long Point, Ontario, from 1989 through 2011, inclusive. See Chapter 2 of this thesis for a detailed description of the traditional study area and methods for estimating the abundance of adults. All procedures with the animals were conducted under the auspices of research permits and letters of authorization issued by the Ontario Ministry of Natural Resources and Environment Canada in accordance with the Ontario Endangered Species Act and Canadian Species at Risk Act, as well McGill University Animal Use Protocol No. 4569.

Body size determination

The snout-vent length (SVL) of every toad encountered was measured in the standard way, as described in Chapter 3 of this thesis. We limited this study to adult males because they are readily identifiable by their dark throats and by the release calls they make when handled. Adult females have no obvious morphological characteristics to distinguish them from juveniles, which all have white throats and are not vocal.

Age of adult toads

From 1992 onwards, chronological ages of adult toads were determined using skeletochronology as described in Chapter 2 of this thesis. Skeletochronological results were not available for the years 1993 and 2001.

Temperature and lake level records

Daily air temperature and rainfall records from 1988 – 2011 were obtained from the National Climate Data and Information Archive of Environment Canada (http://climate.weatheroffice.gc.ca/ climateData/canada_e.html) for the Port Colborne weather station, located near the north shore of Lake Erie approximately 100 km ENE of Long Point. Port Colborne and Long Point are both in the Eastern Lake Erie Basin climatic regime (Sly 1976) and weather patterns at the two stations can be considered comparable. Port Colborne is the closest weather station to Long Point with continuous records from 1989 to the present.

Based on observations of spring emergence of adult toads over the years of study and the minimum temperature, about 14ºC, at which the toads are active, we defined the active season for the toads as commencing 41 days after the vernal equinox, i.e. about May 1, and continuing for 154 days to about October 1. We used maximum daily air temperature in our analyses as it tends to vary over a greater range than mean or minimum temperature.

We compiled records of Lake Erie water levels, in meters, for May through August from 1988 – 2011 using data available from the Canadian Hydrographic Service (http://www.waterlevels.gc.ca/). Lake levels were measured relative to the International Great Lakes Datum 1985 for Lake Erie, set at 173.5 meters above mean sea level.

Data analysis

We used Akaike's (1974) Information Criterion adjusted for small sample sizes, AIC_C, and Akaike weights to evaluate models related to annual variation in body size among male Fowler's Toads in this population. The dependent variable was **SVL** (snout-vent length)**,** the average body size of males in each year. Since 2-year olds are the most abundant age class among the toads (Kellner and Green 1995; Middleton and Green, unpublished, we also used the average body length of the 2-year olds only as a dependent variable ($\mathbf{SVL_{age2}}$) to control for age in relation to the environmental variables.

We considered the following independent variables for analysis in our models:

Year: years from 1989-2011, inclusive, to investigate evidence of a temporal trend. Because of the change in population dynamics in this population from a regulated state during the period 1989 – 2001 incl. to a declining trend during the period 2002 – 2011 incl. (Greenberg and Green, submitted), we also considered these time periods separately.

Age: the average age, per year, of known-aged adult individuals.

***N*:** the log-transformed estimate of abundance, i.e. $\ln(\hat{N})$, each year. We used either estimated abundance of the year ($\boldsymbol{N}_{\text{yr}}$), the previous year ($\boldsymbol{N}_{\mathbf{yr-1}}$) or the year before that ($\boldsymbol{N}_{\mathbf{yr-2}}$). For 2-year old individuals, we considered either $\boldsymbol{N}_\mathbf{0}$, $\boldsymbol{N}_\mathbf{1}$, or $\boldsymbol{N}_\mathbf{2}$, which were $\ln(\hat{N})$ of all adults in their first year and in the years when they were 1- and 2-years old, respectively.

Temp: the average daily maximum air temperature of the 154-day active season during the previous year **(**$\mathbf{Temp_{yr-1}}$) or the year before that ($\mathbf{Temp_{yr-2}}$). **TempLate**, with variants as for **Temp,** was the average daily maximum air temperature during the latter half of the active season when young-of-year juveniles would experience greatest growth following metamorphosis (Hota 1994). For 2-year olds, $\mathbf{Temp_0}$ was the average daily maximum air temperature in the year when the animals would have been tadpoles and $\mathbf{Temp_1}$ was when they were 1-year of age and may be expected to have done most of their post-metamorphic growth.

Rain: Total precipitation, in mm, during the toads' 154-day active season during the previous year (**$\mathbf{Rain_{yr-1}}$**) or the year before that (**$\mathbf{Rain_{yr-2}}$**). For 2-year olds, **$\mathbf{Rain_0}$** was the total precipitation in the animals' first year of life and **$\mathbf{Rain_1}$** when they were 1 year of age.

Lake: Water level of Lake Erie of the year (**$\mathbf{Lake_{yr}}$**), one year (**$\mathbf{Lake_{yr-1}}$**) or two years (**$\mathbf{Lake_{yr-2}}$**) previous. For 2-year olds, **$\mathbf{Lake_0}$** was lake level in the animals' first year of life whereas **$\mathbf{Lake_1}$** was lake level the year after.

The several variants of ***N***, **Temp**, **Rain** or **Lake** were obtained simply shifting the data a year at a time relative to **SVL**. They are thus not independent and we first considered all the variants of each of these four variables as alternative models for explaining variation in **SVL** or **$\mathbf{SVL_{age2}}$** to identify the variant to be used for subsequent analyses according to AICc score and AICc weight. If none of the variants had a lower value of AICc than the null model, the variable was not used further.

We considered all possible models for **SVL** or **$\mathbf{SVL_{age2}}$** vs. **Year**, **Age** and informative variants of ***N***, **Temp**, **Rain** and **Lake** and calculated AIC_C and Akaike weights to identify the most informative among them with regards to their ability to explain observed variation in body size. We retained the model with the lowest AIC_C value and other models with $\Delta AIC_C < 2$. For models incorporating more than one independent variable, we ran multiple regressions to identify the relative contribution of each variable to the overall regression. Model selection and subsequent regression analyses were performed using JMP-8 software.

RESULTS

In total, 1,741 individual adult male Fowler's toads were captured and measured over 23 years. Although the life span of Fowler's toad in the wild is short, 235 captures were captured and re-measured in consecutive years, resulting in a total of 1,976 body length measurements.

The overall average SVL (1989 – 2011, incl.) was 53.6 ± 0.1 mm (S.E.) mm for all males (*n* = 1,976). However, the average annual SVL over all 23 years was 55.1 ± 0.7 mm (S.D.) since average body lengths varied considerably from year to year (Table 1, Fig. 1). Average SVL ranged from 50.9 ± 0.2 mm (S.E.) in 1993 to 61.4 ± 1.3 mm (S.E.) in 2011, a difference of

18.7%. There were also a significant trend towards larger average SVL over the course of the study (*p* = 0.001, R^2 = 0.321, *n* = 23 years), due largely to the years 2002 – 2011 (*p* = 0.001, R^2 = 0.874, *n* = 10 years). There was no significant trend in SVL over the years 1989 – 2001 (*p* = 0.088, R^2 = 0.328, *n* = 13 years)

Adult male toads averaged 2.33 ± 0.05 (S.E.) years of age (*n* = 539). The majority of adult males (62.4%) were 2 years old. No toads were older than 5-years of age. The average annual age was 2.39 ± 0.06 (S.E.) (*n* = 17 years). The average age of the toads tended to increase over the years of study (*p* = 0.036, R^2 = 0.261, *n* = 17 years). There was no discernible relationship between **SVL** in relation to **Age** (*p* = 0.324, R^2 = 0.065, *n* = 17 years).

There were 15 years in which 5 or more known 2-year olds were sampled (Table 2). $\mathbf{SVL_{age2}}$ among these 2-year olds was highly correlated with **SVL** in the population as a whole ($p < 0.001$, R^2 = 0.915). There was likewise a significant trend over time in $\mathbf{SVL_{age2}}$ (*p* = 0.028, R^2 = 0.284, *n* = 15 years).

The average daily maximum air temperature, variable **Temp**, during the 154-day active season for the toads was 22.9 ± 1.4 (S.E.) ºC over the span of 23 years from 1988 through 2010. A trend of increasing average temperature by 0.048 ºC/year was not statistically significant (*p* = 0.151, R^2 = 0.101) owing to considerable variation from year to year. There was no significant relationship between $\mathbf{Temp_{yr-1}}$ and **SVL** in the toads (Fig. 2).

Average rainfall, variable **Rain**, over the 23 years during the 154-day active season for the toads was 419.6 ± 23.0 (S.E.) mm. Although the amount of rain varied from a high of 643 mm to a low of 235 mm, no trend in **Rain** was detectable over the 23 years of study (*p* = 0.799, R^2 = 0.003). No model of **Rain** had a AICc value lower than a null model. The variable **Rain** was therefore discarded in subsequent analyses.

Lake Erie water levels, variable **Lake**, averaged 0.86 ± 0.04 (S.E.) m above the IGL Datum 1985, i.e. 174.36 m above mean sea level. Water levels were all above this average from 1989 through 1998 and below this average from 1999 to 2010 except for 2009. Water level rose above average in 2011. This long-term fluctuation rendered a significant negative trend in lake level over the course of the study (*p* = 0.034, R^2 = 0.197). Lake Erie water level (variable **Lake**) was likewise uninformative except for $\mathbf{Lake_{yr}}$ compared to SVL over the latter 10 years of the study (AICc weight = 0.498).

The best model to explain variation in **SVL** was N_{yr}, which returned a strong, negative correlation ($p < 0.001$, $R^2 = 0.713$, $n = 23$ years). The seven next best models also incorporated N_{yr} but only N_{yr} + **Age** had a ΔAICc < 2 (Table 2). The multiple regression using N_{yr} + **Age** was also significant ($p < 0.001$, $R^2 = 0.709$, $n = 17$ years) but this was due wholly to the contribution of N_{yr} ($p = <0.001$) and not **Age** ($p = 0.187$).

Using only 2-year old animals, the best model to account for variation in $\mathbf{SVL_{age2}}$ was N_2, i.e. abundance in the same year ($p < 0.001$, $R^2 = 0.702$, $n = 15$ years). The next best was N_2 + **Year** ($\Delta AIC_C = 0.591$, $p < 0.001$, $R^2 = 0.760$, $n = 15$ years) the significance of this relationship was only to the contribution of N_2 ($p = <0.001$), not **Year** ($p = 0.115$). Neither N_0 nor N_1 returned informative models compared to N_2 ($\Delta AIC_C > 2$). $\mathbf{SVL_{age2}}$ did not correlate with any environmental variables.

DISCUSSION

The results of this study clearly demonstrate that average body size in this population of toads is a function of their changeable abundance. The relationship is virtually immediate, and much faster than seen in previously notable instances of rapid body size change in animal populations (Smith et al. 1998; Loehr et al. 2007). This may be because they are short-lived animals that only breed annually (Kellner and Green 1995). As we have found, the majority of the breeding population consists of newly mature individuals that are either one or two years old (Chapter 2, this thesis). Late season growth rate prior to sexual maturity is the most probable proximate driver of average body size in these toads since growth after sexual maturity is relatively slow, in conformity to the von Bertalanffy (1938) growth curve (Hota 1994; Iturra-Cid et al. 2010; Sarasola-Puente et al. 2011). Our data, collected in early spring, reflect the relationship between size and abundance reached prior to the animals' entering into their winter dormancy the previous fall. It is this growth that is so strongly, and negatively, correlated with their abundance.

The biphasic life-history of toads readily suggests an environmental link between abundance and density in this population that is not related to climate change. The dune and beach habitat used by postmetamorphs (juveniles and adults) has remained more or less consistently available throughout the period of study. This is not true of larval habitat. With the loss of breeding ponds due to the expansion of the invasive reed, *Phragmites australis,* consequent dwindling of adult abundance without significant loss of adult habitat resulted in lower adult density and thus likely greater per-capita resource availability. This phenomenon has not been thoroughly investigated in amphibians despite evidence of density-dependent competition and growth in populations of tadpoles (Semlitsch and Caldwell 1982; Leips and Travis 1994; Stark et al. 2012) and new metamorphs (Loman and Lardner 2009).

Although there has been a steady increase in environmental temperature over the course of our study, there is no detectable effect on body size in these toads. In fact, in contrast with Bergmann's Rule applied to climate change (Millien et al. 2006) or the Temperature-size Rule for ectotherms (Atkinson 1994; Walters and Hassall 2006; Gardner et al. 2011), the toads are actually getting bigger with increasing temperatures. This only apparently contradicts studies of amphibian populations that have found correlations between body size and climate change (Neveu 2009; Tryjanowski et al. 2006; Reading 2007) since few, if any, simultaneously have considered abundance. It is, in fact, surprising how often abundance has generally been overlooked in studies of body size variation within animal populations. Japanese sardines, *Sardinops melanostictus*, in the Sea of Japan and the East China Sea clearly show a negative correlation between body size, or growth rate, and abundance from 1953 to 2006 (Ohshimo et al. 2009). Instead of looking to abundance, complex interactions between recruitment, prey biomass and climatic oscillations were proposed by to explain the observed changes in body size in these fish. Millien (2004) and Millien and Damuth (2004) found antiparallel latitudinal patterns of body size variation in two species of Japanese field mice, *Apodemus argenteus* and *A. speciosus*, but did not consider density as a factor. Braun et al. (2004) found a decrease in body size and concomitant increase in abundance in 63 species of ground beetles over 16 years but neither they nor Lövei and Magura (2006), in their reanalysis of the same data, considered the size-abundance relationship so evident in these data.

Nevertheless, long-term correlations between abundance and adult body size have been found in a number of animals (Yom-Tov and Geffen 2011). Adult body size, at least in mammals, appears to be determined to a great extent by nutrition during early development, when an individual experiences its greatest rate of growth (Geist 1987; Henry and Ulijaszek 1996; Lindstrom 1999; Ozgul et al. 2010). Thus variation in food availability may drive systematic variation in somatic growth rate, and resulting adult body mass, in mammals as diverse as deer (Skogland 1985; Toıgo et al. 2006), bears (Zedrosser et al. 2006), lemmings (Krebs 1996) and porpoises (Read and Gaskin 1990). Among birds, growth rates have been shown to vary in Great tits in relation to territory size, and thus both density and food availability (Wilkin et al. 2006). Body size variation over time is known within populations of ectothermic vertebrates as well. Loggerhead Turtles, *Caretta caretta* (Hatase et al. 2002), Australian water pythons, *Liasis fuscus,* (Madsen and Shine 2000), *Anolis* lizards (Andrews 1976) and arctic cod, *Gadus morhua* (Jørgensen 1992) all exhibit variable growth rates attributable to food availability.

Two opposing temporal patterns of size vs. abundance have been described in animal populations. Opposite to the negative correlation found among fishes (Martinson et al. 2008; Ohshimo et al. 2009), beetles (Braun et al. 2004) or Fowler's Toads, populations of voles and lemmings characteristically undergo simultaneous, positively correlated cycles of abundance and body size (the Chitty effect) (Krebs, 1996). This effect is a carry-over result of higher somatic growth rates and survival rates during the phase of population growth such that at peak abundance, the population has a great many larger and older individuals (Oli, 1999). Looking to possible effects of climate change rather than mere abundance, Ozgul et al. (2010) attributed a similarly concurrent increase in body size and abundance in Yellow-bellied marmots, *Marmota flaviventris*, in Colorado to longer growing seasons. Teplitsky et al. (2008), though, cast doubt on climate change as the driving force behind positively correlated declines in both body mass and abundance in Red-billed Gulls, *Larus novaehollandiae*. Oli's (1990) explanation for positively correlated changes in body size and abundance relies on individuals having life spans that overlap several generations. Positive versus negative correlations between size and abundance may reflect the difference between endothermic versus ectothermic physiologies.

The rapidity of the toads' response to changing abundance also implies that it is a purely phenotypic rather than evolutionary response. The body size variation we observe in this population of toads, and in other species in inverse relation to population density, appears to be distinct from the effects of selection pressures that may drive rapid evolution in body size. Differential, size-related mortality due to harvesting can produce rapid, evolutionary changes in body size in diverse species of ectothermic vertebrates (Sasaki et al. 2008; Wolak et al. 2010, Carlson et al. 2011). Selection pressure can be strong enough to drive a population to smaller body size at the same time as hunting pressure reduces population density, as seen in the snake, *Gloydius blomhoffii*, in Japan (Sasaki et al. 2008). Such effects may be especially prevalent among harvested fishes in which the pressure of fishing leads to a population-level response towards rapid maturation at smaller size. There is no harvest for Fowler's toads and the possibility that the changes we observe in body size are due to rapid evolutionary response to strong selection appears remote.

The most basic implication of strong temporal body size variability for macroecology is that estimates of average body size for a population or species may need to be treated with caution insofar as they may depend on both where and when the measurements are taken. We found an 18.7% difference between smallest and largest average male body sizes within a single population over 23 years. To compare, data on average body size within populations have been presented in a handful of studies on Bergmann's Rule in amphibians. Among 12 populations of European Common Frog throughout Sweden, largest and smallest average male body size differed by 16.8% (Laugen et al. 2005). In six populations of the Chinese toad, *Bufo andrewsi*, ranging in elevation from 6760 m to 2,100 m, average male body length in varied from about 73 mm to 82 mm, a difference of 11.8% (Liao and Lu (2012) and among three populations of the Chinese frog, *Rana chensinensis,* from 567 m to 1700 m elevation, average male body length differed up to 11.7% (Ma et al. 2009). Average male body size in five populations of Common Toad, *Bufo bufo*, from Switzerland to Norway differed by up to 26.6% (Hemelaar 1988) and, in 14 populations from Serbia to Scandinavia, differed by up to 32.4% (Cvetković et al. 2009). We conclude, therefore, that whether amphibians do (Ashton 2002) or do not (Adams and Church, 2007) follow Bergmann's or any other ecogeographic rule, temporal changes in body size within populations may also be a significant component of variation.

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Table 1. Body size averages for male Fowler's Toads at Long Point, Ontario, 1989 – 2011.

	snout-vent length (mm)					
Year	mean (± S.E.)			min	max	*n*
1989	56.1	±	0.58	51	65	37
1990	51.2	±	0.42	44	62	58
1991	51.4	±	0.18	43	60	249
1992	52.4	±	0.18	44	60	219
1993	50.9	±	0.19	43	61	255
1994	54.2	±	0.24	49	59	83
1995	55.4	±	0.53	47	64	43
1996	55.9	±	0.38	49	60	37
1997	57.1	±	0.61	49	62	31
1998	55.3	±	0.21	48	62	180
1999	56.1	±	0.4	50	65	65
2000	54.5	±	0.58	44	64	50
2001	54.4	±	0.44	47	62	57
2002	54.7	±	0.31	43	67	133
2003	53.5	±	0.44	44	62	85
2004	52.8	±	0.3	46	65	151
2005	54.2	±	0.3	50	63	76
2006	54.7	±	0.61	47	64	33
2007	54.7	±	0.58	48	63	37
2008	58.5	±	0.67	50	66	32
2009	59.1	±	0.6	53	70	34
2010	58.2	±	0.83	53	64	16
2011	61.4	±	1.27	52	69	15

[N.B. total toad measurements = 1,976, recaptured = 235. Total toads = 1,741]

Table 2. Evaluation of alternative models for Fowler's Toad average snout-vent length (SVL) relative to log abundance (N), Year and average adult age at Long Point, Ontario, Canada.

Model	Parameters	AICc	ΔAICc	Akaike weight	R^2	*p*
1	N	66.110	0.000	0.397	0.669	<0.001
2	N, Age	67.411	1.301	0.207	0.709	<0.001
3	N, Age, Year	68.222	2.112	0.138	0.760	<0.001
4	N, Year	68.390	2.279	0.127	0.692	<0.001
5	N, $\text{Temp}_{\text{yr-1}}$	69.597	3.487	0.070	0.669	<0.001
6	N, Age, $\text{Temp}_{\text{yr-1}}$	71.482	5.372	0.027	0.710	0.001
7	N, Year, $\text{Temp}_{\text{yr-1}}$	72.461	6.351	0.017	0.692	<0.001
8	N, Age, Year, $\text{Temp}_{\text{yr-1}}$	72.670	6.560	0.015	0.767	0.001
9	Year	78.327	12.216	0.001	0.321	0.001
10	Year, Age	81.773	15.663	0.000	0.322	0.066
11	Year, $\text{Temp}_{\text{yr-1}}$	81.785	15.675	0.000	0.322	0.005
12	null	81.910	15.800	0.000	-	-

Figure 1. Variation in adult male Fowler's Toads from 1989 –2011 (A – C) and environmental variables (May through September) from 1988 –2011 (D – F) at Long Point Ontario. A. Average body size measured as snout-vent length (SVL) of toads. B. Estimated abundance of toads. C. Average age of adult toads. D. Total rainfall. E. Average daily maximum air temperature. F. Lake Erie water level relative to Lake Erie IGLD 1985 datum.

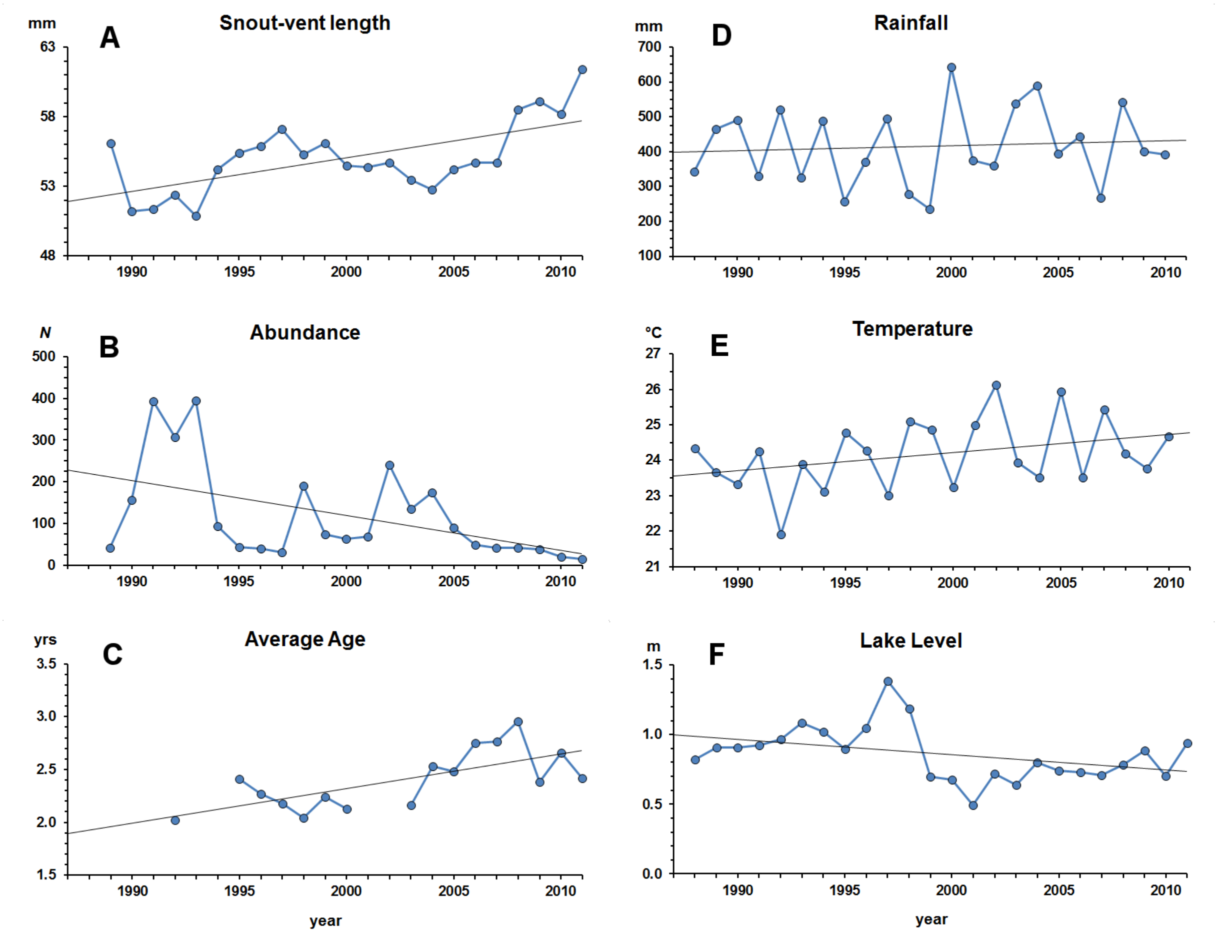
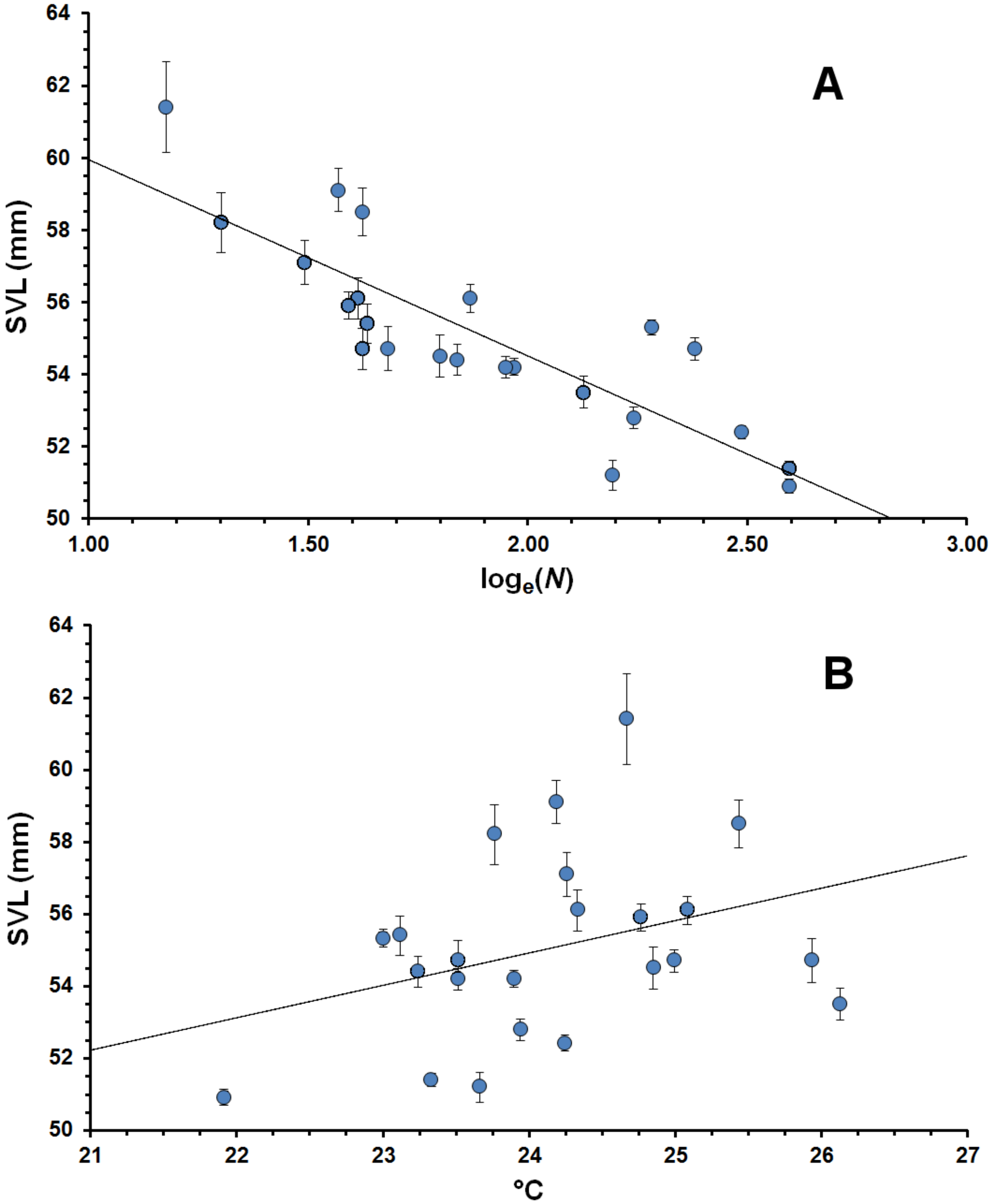


Figure 2. Relationships between average body size (snout-vent length [SVL], with standard errors) vs. (A) $\log_e$–transformed estimated annual abundance and (B) average late summer temperature for adult male Fowler's Toads from 1989 through 2011 at Long Point, Ontario. The relationship of SVL to abundance is significant (p = <0.001, R^2 = 0.713), the relationship of SVL to temperature is not (p = 0.097, R^2 = 0.125).



CHAPTER 5: CONCLUSIONS AND CONSERVATION IMPLICATIONS

The long-term persistence of populations depends on stabilizing density-dependent processes operating among individuals. It is well known that amphibian populations are regulated by density-dependent processes operating at the aquatic larval stage. As more studies are conducted on the terrestrial stage, it is becoming increasingly evident that populations may be limited by density in the terrestrial habitat as well. This study identifies a potential mechanism of population regulation in the terrestrial stage of a typical pond-breeding anuran. The primary purpose of CHAPTER 2 was to determine the age structure of the Long Point Fowler's toads for subsequent analysis on the factors influencing body size (chapter 4). An ancillary is that juvenile recruitment and postmetamorphic survivorship varied significantly from year to year, making it possible to overshoot or undershoot the carrying capacity of the terrestrial environment. Toads can respond to differences in per-capita resource availability through density-dependent somatic growth rates, as demonstrated in toadlets in CHAPTER 3. Ultimately, this results in large temporal variation in adult body size identified in CHAPTER 4.

In order to confirm that density-dependent somatic growth rates are in fact a regulatory mechanism, changes in body size must ultimately affect population growth (i.e. birth, death, immigration or emigration). The present thesis does not directly analyse these effects. However, based on the strong influence of body size on reproduction, survival, and dispersal potential, we believe our results do suggest terrestrial density may actually regulate amphibian populations-- at least in Fowler's toads.

Should the negative relationship between body size and abundance prove to be the rule among amphibian populations, there are implications for conservation in view of worldwide concerns about amphibian population declines (Houlahan 2000; Biek et al. 2002). First, because body size is relatively easy to monitor, variation in mean body size of a population may be a reasonable substitute for monitoring population size, which is difficult to estimate (Alford and Richards 1999). For example, the observation that animals are increasing in size may signify a decline in abundance requiring further investigation. Second, incorporating terrestrial density-dependence into population models may improve the accuracy with which they predict dynamics for some species (Harper and Semlitsch 2007). Population models can achieve several conservation goals, including estimating extinction risks and evaluating the potential effectiveness of multiple management options, and are increasingly used in amphibian conservation research (e.g. Biek et al. 2002, Vonesh and De la Cruz 2002, Schmidt et al. 2005, Taylor et al. 2006; Harper and Semlitsch 2007; Salice et al. 2011). Finally, the findings of this research also provide valuable demographic data for the conservation of Fowler's toads, which is currently listed as endangered in Ontario (COSSARO 2010) and Canada (COSEWIC 2010).

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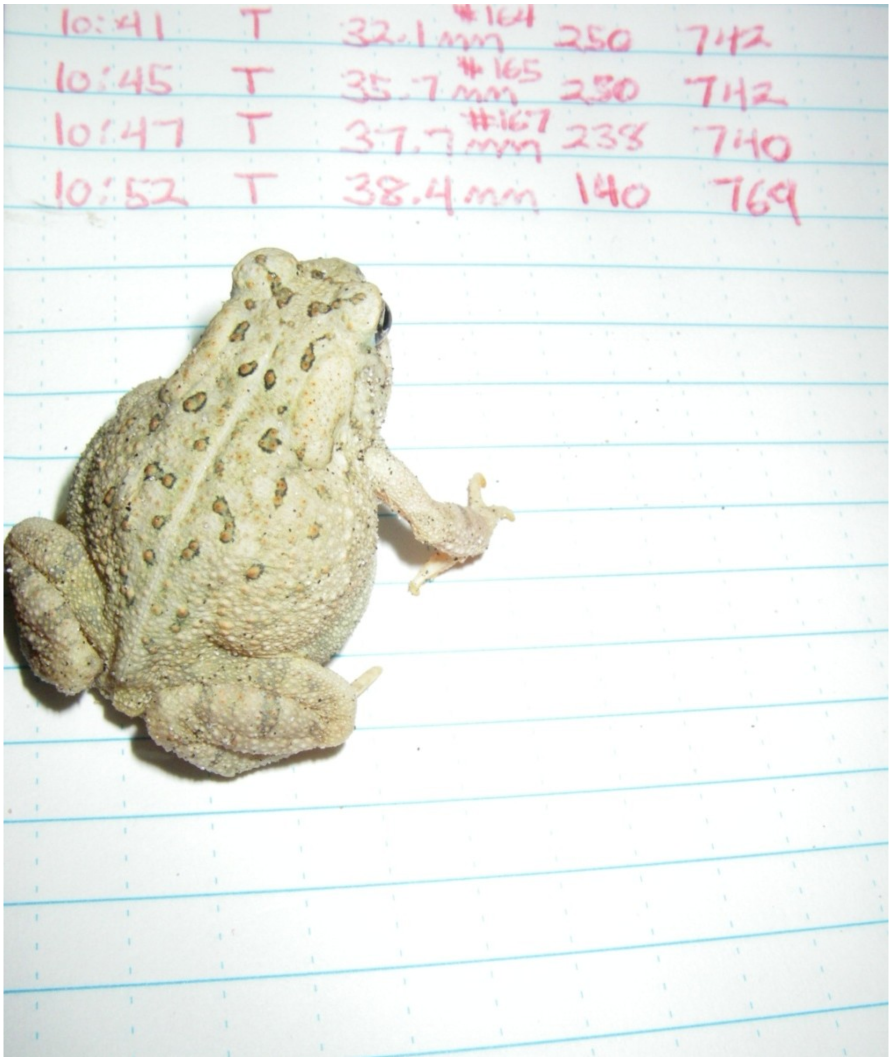
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