University of Alberta

Factors affecting larval growth and development of the boreal chorus frog *Pseudacris maculata*

by

Arthur V. Whiting

A thesis submitted to the Faculty of Graduate Studies and Research
in partial fulfillment of the requirements for the degree of

Doctor of Philosophy

in

Ecology

Department of Biological Sciences

Fall 2010
Edmonton, Alberta

Examining Committee

Cynthia Paszkowski, Biological Sciences

Lee Foote, Renewable Resources

Rolf Vinebrooke, Biological Sciences

Ross Chapman, Elk Island National Park, Parks Canada

Keith Tierney, Biological Sciences

Joseph Rasmussen, Biological Science, University of Lethbridge

Abstract

The boreal chorus frog (*Pseudacris maculata*) is a widespread species but we know little of its ecology. I examined the nature and existence of competitive mechanisms operating between larvae of the boreal chorus frog and wood frog (*Lithobates sylvaticus*) from field, mesocosm and laboratory venues spanning nutrient concentrations. I assessed larval performance and diet of tadpoles at natural ponds by measuring tadpole growth and size at metamorphosis, and stable isotope ratios for carbon and nitrogen in tadpole tissue to examine if patterns were consistent with the operation of interspecific competition. In mesocosms I measured chorus frog performance in relation to wood frog presence and nutrient enrichment to confirm the occurrence of competition and examine whether nutrient conditions typical of agriculture ponds impact tadpole performance. In the field I compared larval performance and relative abundance between agricultural ponds and those in Elk Island National Park, to examine whether habitat features surrounding ponds in farmlands reduces the abundance of tadpoles and whether tadpole performance results in reduced abundances. Lastly, I examined whether chemical interference by wood frogs occurs by raising chorus frog tadpoles with caged wood frog tadpoles and/or their feces in the laboratory.

Chorus frog performance was reduced by presence and abundance of wood frog tadpoles. Resource partitioning in natural ponds and overlap in mesocosms, based on stable isotopic analysis, suggest that resource competition occurs. In mesocosms chorus frog performance was reduced by wood frog tadpoles in fertilized treatments and nutrient conditions at agricultural sites are not in themselves detrimental to these anurans. Performance of chorus frog tadpoles in

agricultural ponds was unaffected, whereas wood frogs were larger at metamorphosis in crop ponds. Reduced tadpole abundances of both species at these ponds may be related to habitat features or conditions in croplands. The existence of chemical interference in the absence of physical interaction was confirmed, as chorus frogs exposed to wood frog tadpoles and/or feces had reduced growth and were smaller at metamorphosis which could reduce terrestrial survival and future reproduction. My research contributes to our knowledge on boreal chorus frog ecology and our general understanding of competition between larval anurans.

Acknowledgements

I never would have been able to complete this research without the advice and assistance from a number of individuals. I would first like to thank Cindy Paszkowski for taking me on as a graduate student and providing support as the writing dragged on. My doctorate experience has been a completely different one than I had during my masters, and she has restored my faith in supervision and colleagueality. I feel that I have benefitted greatly from Cindy's critical feedback throughout this project and during the writing of my thesis, her efforts to rein in my verbose prose, insightful questions and willingness to give me the feeling of free reign on the development and execution of this research. I would like to thank my field assistants who questioned my rationale and had fun playing in ponds with me: Zach Long, Curtis Van Marck and Laura Becker. I would like to thank my many lab assistants who toiled away in the dark spaces while I spent time catching frogs and having bison and moose threaten me while I was counting frogs: Jade Laramie, Kevin Liu, Vickie Lee, Eileen Tu, Courtney Spelliscy, Maia Benz, Jaskaran Singh, Melissa Tanahill, Darcie Thauvette, Samantha Jones, and Patricia Aranetta. Thanks to my supervisory committee Rolf Vinebrooke, Lee Foote and Ross Chapman for providing guidance and feedback on the research as it developed. I would like to thank Holly Wellar and Myles Stocki who analyzed the many stable isotope samples and whose advice and willingness to answer questions helped with my understanding of the results. Thanks to Rich Moses for helpful advice on analysis, and Charlene Neilson for holding my hand during my GIS analysis. Candra Schank for distracting me with frog questions and other office mates that made those days at my desk entertaining. I would also like to thank the frog crew in Cindy's lab for providing advice and discussion about my research and froggie ideas: Connie

Browne, Cam Stevens, Brett Scheffers and Candra. I would also like to thank Bill Tonn, Justin Hanisch, Eva Kuczynski and the rest of the PT lab group who helped dilute the stress on Friday's and talk about research, stable isotopes and other varied topics.

Financial and in-kind support was provided by North American Waterfowl Management Plan, Duck Unlimited, the Friends of Elk Island Society, Parks Canada, Alberta Conservation Association, Circumpolar/Boreal Alberta Resereach, Alberta Sports, Recreation, Parks, and Wildlife Foundation, Natural Sciences and Engineering Research Council (Discovery to Cindy Paszkowski), the University of Alberta and landowners in Strathcona and Lamont County.

To the many little tadpoles and frogs that make this research worthwhile and enjoyable. I am sorry to the many that met with my plastic bags, but I will ensure that your death was not in vain. I look forward to hearing and seeing you again soon.

To my family who have suffered the most during this process, I look forward to the adventures we will have. I will no longer remain in the basement wrapped in a blanket staring at the computer. To Caius, my little palaeontologist who has already written his thesis on snakes, oh how I wish I was as fast as you; I love your curiosity. To Amber, my whatcha doin', we'll make you a frog catcher yet. Thanks to my parents for you support and desire to see me finish. Finally, to Amanda for supporting and tolerating me throughout the research and raising my spirits when I was down, and dealing with me when I was wasn't. Thank you for every little thing you do and for raising two wonderful little frog catchers.

Table of contents

List of Tables

List of Figures

Chapter 1: Competition in anuran larvae

Introduction

Lotka (1925) and Volterra (1926) were among the first to quantify the effect of competition on the abundances of species. Based on their models, coexistence between competing species results when each species affects itself more than it does the other species, such that interspecific competition is weaker than intraspecific competition. However, in experiments, competition tended to drive one of the species to extinction and led to Gause's (1934) competitive exclusion principle. Connell (1980) argued that evolution prevents or reduces interspecific competition either by eliminating inferior competitors or permitting coexistence through resource partitioning. Consequently, Connell (1980) suggested that we might not observe interspecific competition, or that it might be weak, as a result of evolution of features or adaptations to reduce the effects of interspecific competition. Gause and Connell's ideas led to the belief that interspecific competition really ought not to occur, or at best be rare in nature. However, Schoener (1983) and Connell (1983) reviewed published experiments on competition and demonstrated that competition does occur between species in nature and is commonly found by studies testing for it. A meta-analysis by Gurevitch et al. (1992) on a wide variety of organisms and systems confirmed that competition generally has negative effects on a population's biomass and other performance metrics such as survival, per capita growth rates, and reproductive potential. The mechanisms behind the coexistence of species and the existence and importance of interspecific competition remain central to the study of ecology.

Interspecific competition is the process responsible for changes in an individual's fitness associated with the presence or absence of various community members. Competition may occur through resources required for growth and survival such as food, nutrients and light, or resources such as territories. The actual mechanisms behind competition are sometimes unclear or ignored, but competition is traditionally believed to act through indirect or direct mechanisms. Individuals in exploitation or scramble competition deplete shared resources, indirectly depriving others of their use. Interference competition is direct, whereby individuals harm one another, in combat or by release of toxins, or by preventing access to critical resources. Below I review both mechanisms and how coexistence between competing species occurs.

Theory on exploitative competition, such as Tilman's R* rule (Tilman 1980, 1982), suggests that the species with the capacity to subsist at the lowest concentration of the limiting resource will out-compete all other species. For a single limiting resource, the R* rule predicts that eventually only the species with the lowest R* will remain. Coexistence occurs as a result of the inability of one species to exclude another species completely; coexistence is therefore common. Complete exclusion may require a long time and many generations, but disturbances (physical disturbance and mortality or biomass loss due to grazing or predation) and temporal or spatial changes in environmental conditions provide opportunities for the "inferior" competitor to survive and persist. The intermediate disturbance hypothesis proposes that coexistence of species is a product of disturbances and changes in environmental conditions (Grime 1973, Connell 1978). Competition for multiple resources also favours coexistence as various species will be better competitors for different limiting resources, and partitioning of multiple resources

results in stable coexistence (Tilman 1982). The supply dynamics of biotic resources, like seeds, plants or other living resources, can also stabilize species interactions, as each species' ability to monopolize that resource depends on the abundance of that resource which varies seasonally or annually (Armstrong and Mcgehee 1980). Alternatively, non-equilibrium dynamics, caused by the interactions of multiple species, may create competitive chaos and allow the coexistence of many species feeding on a few limiting resources (Huisman et al. 2001). Coexistence at the local or regional scales may also occur as a result of trade-offs. Trade-offs between a species' competitive ability and its physiological tolerance to environmental gradients (also known as the competitive hierarchy hypothesis Keddy 1989, 1990), its colonization rate (e.g. Tilman 1994, Cadotte et al. 2006), or its susceptibility to predation (Paine 1994, Relyea 2000) are areas of active interest in the study of competition and mechanisms permitting coexistence of species.

Unlike exploitation, there is a paucity of general theory concerning interference competition (Case and Gilpin 1974, Vance 1984, Amarasekare 2002); most deals solely with exploitation. It is unclear why interference has not received greater attention given its commonness. For example, interspecific territoriality and other aggressive behaviours, allelopathy, overgrowth, and predation of eggs and/or young of interspecific competitors occur in many taxa including plants, invertebrates and vertebrates. The competitive mechanisms of exploitation and interference occur equally as often in the studies reviewed by Schoener (1983). Further, Amarasekare (2002) suggested that interference competition is ubiquitous in nature, which might reflect the evolution of competitive interactions as they move from pure exploitation to a situation where one (or many) species employ some form of interference (Case and Gilpin 1974). In general, interference comes at a cost,

but species and individuals employing this tactic profit as a greater share of limited resources become available (Case and Gilpin 1974). The benefits must outweigh the costs of interference for it to evolve and persist. Amarasekare (2002) modelled the outcome of competition considering both exploitation and interference competition for a biotic resource. Amarasekare found that species coexistence required a trade-off between exploitation and interference competition whereby the superior exploitative competitor suffered a net loss in per capita growth rate due to interference whereas the weaker competitor enjoyed a net benefit through interference. If the weaker competitor incurs only costs as a result of interference, then dominance by the superior competitor prevails. However, when the effects of interference are sufficiently great and the abundance of the inferior competitor is sufficiently high, then priority effects determine whether the superior exploitative competitor invades or persists within a system (Amarasekare 2002).

An individual's ability to allocate resources toward growth, survival, or reproduction is affected by interspecific interactions. The organism's energy intake is then allocated to different competing components of its life-history (Figure 1-1). Allocation of energy is governed by trade-offs as there is a finite amount of energy to be distributed among competing processes (Arendt 1997), Figure 1-1). Interactions, such as those that reduce the amount of nutrients available in the environment (exploitation) or increase foraging time (exploitation or interference), will alter an individual's growth rate, survival or future reproduction. Allocation of energy is also likely to be affected by the type and intensity of interspecific interactions.

The effects of competition can be demonstrated if we consider growth or survival rates of individuals of two or more species across sites, where there are

differences in presence or density of competitors (Wilson and Tilman 1991). A species' performance can be used as a metric to determine the relative impacts of one or more potential competitors. Petren and Case (1996) suggested that exploitation is more difficult to identify than interference competition as direct interactions can be observed between species. Petren and Case outlined five steps needed to demonstrate conclusively the operation of interspecific exploitative competition. I have adjusted their steps to accommodate short-term experiments as examined in my research on interactions between larval amphibians (e.g., over a single growing season). The first four steps are: 1) Two or more species must share one or more resources. 2) The availability of the shared resources must limit growth, survival or future reproduction of the competing species. 3) The acquisition of the resource by one species must be negatively affected by the presence of the other species. 4) Reduced acquisition must translate into reduced growth or survival which may ultimately reduce fecundity of the subordinate species. Petren and Case (1996) suggested that reduced survival and fecundity alter the species' distribution and abundance, and may trigger long-term evolutionary change. If the four steps are satisfied then, 5) interference mechanisms and other processes must be ruled out to demonstrate conclusively that interspecific exploitative competition occurs. Documentation of how species respond to interspecific interactions and the availability of resources should reveal if competition occurs and the competitive mechanism (exploitation or interference) involved.

Throughout my dissertation I use the term "interspecific competition" to describe the day-to-day effects of interactions between two species which increase the metabolic costs of acquiring resources for each species (Keddy 2001). For most organisms, growth rate is important as it ultimately determines adult body size, age

at first reproduction, reproductive potential, predation risk, and an individual's ability to compete for resources (Arendt 1997). An individual's daily growth rate integrates the effects of neighbours and of its environment. Thus, the effect of neighbours on an individual can be estimated from the difference between the growth rates of individuals growing in the same, or similar, environments with and without neighbours. Due to competition, the absolute amount of energy available to an individual is decreased and so affects the allocation of energy to growth, maintenance, reproduction or storage through trade-offs. Available energy may be further diminished if competition involves interference or alters foraging behaviours (i.e. greater activity, change in food selection). Animals with complex-life cycles such as anuran amphibians, with aquatic larvae and terrestrial adults, provide an opportunity to study competition and its influence on growth in the face of allocation trade-offs.

Competition in anuran larvae

Competition is believed to be important in structuring temperate pond-breeding anuran communities (Alford 1999). Competitive effects may be most important during the larval stage when high larval densities combined with low apparent food abundance result in resource exploitation and limit the co-occurrence of anuran species at a location (Wilbur 1997). Intra- and interspecific competition is hypothesized to be strong from the observation that only a small percentage of eggs laid give rise to larvae that survive to metamorphosis and the fact that anuran species often display non-overlapping distributions on a regional basis. Competition between species can reduce larval growth and prolong the larval period which can reduce the size at metamorphosis and survival to metamorphosis. Other factors

such as predation and hydroperiod also impact survival to metamorphosis and thus may impact local distribution of anuran species. Both predation (Relyea 2002, Resetarits et al. 2004) and hydroperiod (Wilbur 1987, Rogers and Chalcraft 2008) can interact with competition to alter the dynamics and outcome of competition. However, by accounting for their effects by observing species interactions across a range of conditions or in their absence may allow the effects of competition to be observed.

Whether competition occurs within anuran larval communities is a common research focus. A search using the Web of Science® (ISI Web of Knowledge – Thomson Reuters) with the search term" = (amphibian OR anuran) AND (larvae OR tadpole*)" uncovered 1206 studies on amphibian larvae between 2005 and 2009. Refining the search with the keyword "competition" resulted in 76 (6.3%) papers that considered some aspect of competition. Fifty-three of those examined the effect of competition on some measure of tadpole performance either through field (n = 12), mesocosm (n = 27) or laboratory experiments (n =17, of which 2 also included a field venue and another 3 also included a mesocosm venue). Five of the 54 studies considered interspecific competition with non-amphibian species (fish, mosquito larvae, snails and chironomids). Twenty-nine of the remaining 49 studies considered interspecific competition between amphibian larvae. Alford reviewed competition between amphibian larvae in 1999 and since then over 200 additional articles have considered some aspect of competition in amphibian larvae based on a search using competition AND (amphibian OR anuran).

Competition and its intensity amongst pond-breeding amphibians have been inferred from relative differences among populations and among species using

measures such as larval growth rates, duration of larval period, survival to metamorphosis, and size at metamorphosis. For northern temperate amphibians, metamorphosis provides a useful endpoint for assessing the effects of competition as reproductive fitness of individuals and survival are strongly correlated with size and date at metamorphosis (Smith 1987, Berven 1990). Monitoring larval growth rates is also convenient as larvae are generally confined to a pond and can be sampled during daylight hours in contrast to many terrestrial anurans. Growth rates provide an instantaneous measure of the effects of the rearing environment (density, food quality etc.), whereas measures at metamorphosis reflects an individual's environment over longer periods and are affected by changes in the environment throughout development. A combination of larval and metamorph measures may provide the best approach for examining competition between anurans.

Although competition is common, few studies explore direct interactions between anuran species. A number of studies suggest that competition occurs for limited food resources (e.g. periphyton). Early assessments of the abundance of foods available to tadpoles suggested that food was limited or seasonally rare (DeBenedictis 1974, Wilbur 1977), whereas subsequent studies have shown that, regardless of food abundance (quantity), it is protein or the nitrogen content (quality), which limits tadpole growth and development (Kupferberg 1997, Richter-Boix et al. 2007). The existence of energy or nutrient limitations and our uncertainty of tadpole diets (Altig et al. 2007) have led researchers to expect interspecific competition among larval anurans (Alford 1999). Exploitative competition alone is often assumed or solely its existence tested, without consideration of other mechanisms. This bias may be due to a lack of information on a) interspecific

interactions (e.g. Faragher and Jaeger 1998), b) the difference between ingestion and assimilation in tadpole digestion (as mentioned by Altig et al. 2007), and c) chemical communication in anuran larvae (e.g. Waldman 1986, Waldman and Bishop 2004, Belanger and Corkum 2009).

Although few studies have tested for interference competition (only 9 of the 45 competition studies between 2005 and 2009), knowledge about interspecific interactions, diet, and chemical communication might reveal that interference is more common than currently reported. Seven of the studies testing for interference found evidence for intraguild predation. An intraguild predator obtains energy and nutrients by reducing the abundance of its competitors through predation (Brodman and Krouse 2007, Sours and Petranka 2007, Crossland et al. 2009). Only two of the studies looked for chemical interference (Laufer and Maneyro 2008, Richter-Boix et al. 2007). Only Richter-Boix et al. observed evidence of interference: in the presence of a caged competitor (*Bufo bufo*), *Pelodytes punctatus* tadpoles switched to consumption of high protein foods, accelerating their development and reducing their exposure to a potentially poor growing environment. Previously reported cases of interference competition in lab trials were believed to be the result of chemicals excreted into the water (e.g. Rose and Rose 1965, Runkova et al. 1974, Steinwascher 1978) and cells that inhibit tadpole growth found in tadpole feces (e.g. Richards 1958, Steinwascher 1979, Griffiths et al. 1993). The effects of this type of interference have only been demonstrated in replicated laboratory experiments (e.g. Wong et al. 2000). The action of these chemicals and cells in nature has been questioned, in part because inhibitory agents released by larvae have received little attention in field trials, and it is difficult to eliminate alternative

explanations as exploitation of resources can explain virtually all the cases of growth inhibition described in the field (Petranka 1989, Biesterfeldt et al. 1993).

It still remains to be shown conclusively that the food resources of tadpoles, whatever they may be (e.g. Altig et al. 2007), are limiting and any form of competition exists. Still there is good evidence, that in most cases, resources are limiting as greater densities produce fewer, smaller metamorphs (Berven 1990, Loman 2004, Boone 2005). There is sufficient evidence from experiments in mesocosms involving a variety of species to show that food resources are limiting and species compete for them (e.g. Morin and Johnson 1988, Brown et al. 2006), whereas in natural ponds, the direct link between resource limitation and interspecific competition among larval anurans has not been shown conclusively. Demonstrating that exploitative competition occurs in mesocosms is useful, but we are uncertain of the relevance of these results to natural systems (see Skelly and Kiesecker 2001). If mesocosms lack a diversity of resources, then species may be forced to compete for the few resources available, and thus exaggerate the similarity of tadpole diets, and therefore the occurrence and strength of competition.

A poor understanding of food resources used by tadpoles in natural ponds (Altig et al. 2007) prevents a clear understanding of how species interact. Assessment of interactions between tadpoles from the large and widespread families Ranidae and Hylidae are complicated by this lack of knowledge. Exploitative competition is often the mechanism proposed for observed cases of reduced recruitment and reduced size of metamorphic frogs. There are numerous examples of anurans from the family Ranidae out-competing Hylidae larvae (e.g. Wilbur 1987, Morin and Johnson 1988, Alford 1989a, Alford 1989b, Fauth and Resetarits 1991,

Pehek 1995, Faragher and Jaeger 1998, but see Smith et al. 2004). It is believed that Ranidae tadpoles out-compete the smaller Hylidae tadpoles by consuming greater proportions of limited resources, simply as a result of their larger size. However, this assumption contradicts work by Werner (1994) with *Lithobates catesbeianus* and *L. clamitans* that showed that smaller tadpoles have a greater per unit biomass effect as competitors than larger tadpoles and tolerate the effects of competition (both intra and interspecific) better. Further, smaller tadpoles are expected to be better competitors at low resource levels, as they have lower absolute metabolic demands than their larger competitors. However, relative metabolic demands are lower in larger organisms and filter/suspension feeding is more efficient in larger tadpoles (Seale and Wassersug 1979). Whether hylid tadpoles are actually better resource competitors than ranid tadpoles remains to be shown. Therefore it is not clear if the relative differences in metabolic demands and efficiency of food acquisition led to the competitive dominance of ranid over hylid tadpoles or if ranid dominance is realized through interference. If interference competition is assumed to not exist and is rarely tested for, exploitative competition remains as the default mechanism explaining reduced performances.

Interference mechanisms may allow an inferior exploitative competitor to gain an advantage over the superior competitor and alter the outcome of competition. Larger animals tend to use interference competition to gain a competitive advantage over smaller species or individuals (Persson 1985). Body size is important in aggression and territoriality, and ranid tadpoles may use their inherently greater sizes to gain a competitive advantage through interference. Interference may result in asymmetries in the performances of competing species whereby small individuals have a lesser effect on large individuals than large

individuals have on small individuals. Interference may occur through intraguild predation (IGP), where one species preys upon the eggs, hatchlings or larvae of another species with which they also compete; this reduces the density of the competitor and may prevent dominance by the predated species (Sours and Petranka 2007). Priority effects (Knight et al. 2009), that is the order in which species breed and tadpoles hatch, are also being recognized for how they shape competitive patterns and community membership. A re-evaluation of competitive mechanisms operating between anuran larvae is needed to assess the commonness of interference and explore the role of competition in shaping the present-day diversity of communities. Knowledge on species interactions may contribute to successful reintroduction programs as one means of counteracting the recent worldwide declines in amphibian populations (e.g. Stuart et al. 2004, Mendelson et al. 2006). General knowledge of interactions and their effects is often lacking, as is true for anuran species in the aspen parkland where I conducted my research.

Anurans in the Alberta Aspen Parkland

In the aspen parkland of Alberta , Canada, four anurans breed in wetlands, but only two, boreal chorus frog (*Pseudacris maculata*) and wood frog (*Lithobates sylvaticus* formerly *Rana sylvatica* sensu Frost et al. 2006), are likely to be found at a majority of sites. These two species co-occurred in 91% of sites surveyed (n > 300) within the Beaver Hills Region, whereas 7% had only wood frogs and 2% only chorus frogs (Whiting unpublished data), as evidenced by the presence of calling frogs, tadpoles or metamorphs. The other anuran species within the aspen parkland are the boreal toad (*Anaxyrus boreas*) and the Canadian toad (*A. hemiophrys*). Both toad species are rare within the study region with *A. boreas* occurring at only 11 of

400 sites, and *A. hemiophrys* was not encountered at all (Whiting unpublished data). The leopard frog formerly occurred in the southern portions of the region, but is now largely extirpated (Seburn and Seburn 1998, Kendell 2002).

Ponds in the prairie pothole region of Alberta, which includes the aspen parkland, are highly productive systems with abundant aquatic primary production which can serve as food for tadpoles. Many of these ponds are temporary, filling in the spring as a result of snow melt and rains and then drying during the summer. Temporary ponds are considered important for breeding and the maintenance of amphibian populations (Semlitsch and Bodie 1998). Tadpoles may become food limited at any time in temporary ponds either due to blooms of inedible algae (i.e. cyanobacteria that may dominate highly eutrophic ponds) or low primary productivity during high density conditions. Intra- and interspecific competition both reduce tadpole growth and survival through food limitation and these reductions increase with tadpole densities or lower food abundance. The impacts of intraspecific is expected to be greater than interspecific competition (e.g. Relyea 2002), but under certain conditions interspecific competition may reduce growth or survival of one species much more than intraspecific competition (e.g. Morin and Johnson 1988, Faragher and Jaeger 1998, Bardsley and Beebee 2001).

Information on the basic biology and ecology of the boreal chorus frog is limited, although it is abundant in the aspen parkland of central Alberta, and in the province as a whole. I chose to examine this species' larval ecology with an emphasis on interspecific interactions and resource use. The potential for interspecific competition exists given the high frequency of co-occurrence of boreal chorus frog and wood frog populations within the aspen parkland, assuming they share food

resources or compete for specific microhabitats. Both species deposit their eggs at the same time or within a week of each other, and at times a few centimetres apart. I summarize their general biology below and then explore the potential for competition between the two species.

Study Species: boreal chorus frog and wood frog

The boreal chorus frog, of the family Hylidae, is a small anuran (adult size is 19 to 28 mm), with a short life span (likely 1-2 years in temperate populations, Smith 1983, Whiting 2004) and rapid larval development (55-83 days, Smith 1983) that can reach sexual maturity within the first year of life. The wood frog (WF), of the family Ranidae, is larger (adult males 22 to 46 mm, adult females 33 to 47 mm), with a longer life span (about 4 years, Berven 1990). Most individuals reach sexual maturity in their second or third year, and larval development is longer (77-113 days, Berven 1990). Wood frogs are larger at metamorphosis than boreal chorus frog (snout-to-urostyle length of chorus = 13.3 ± 0.25 mm, n = 265 vs. WF = 18.9 ± 0.18 mm, n = 146; from current research). The two species have large geographic ranges. The boreal chorus frog's range (Figure 1-2) extends from Great Bear Lake in the North West Territories to northern Ontario, southward to Arizona and New Mexico to Illinois with disjunct populations in southern Ontario, southern Quebec, New York and Vermont (Conant and Collins 1991, Stebbins 2003, Lemmon et al. 2007). Wood frogs are the most northern amphibian in North America, ranging (Figure 1-2) from Alaska to Labrador (Chubbs and Phillips 1998), south to northern Georgia and northern Idaho with a spotty distribution in Colorado (Hammerson 1999).

The co-occurrence of both species at the majority of my study sites in Alberta contrasts with the breeding habitat preferences reported for the two species in the literature. Chorus frogs are generally associated with shallow temporary open wetlands (Whitaker 1971), whereas wood frogs occur across a range of pond hydroperiods and pond canopy-cover gradients (Werner and Glennemeier 1999). Wood frogs are explosive breeders, and within the Beaver Hills of Alberta deposit their eggs over about five days between mid-April and mid-May depending on pond and air temperatures (personal observation). Chorus frogs in the region start calling around the same time as wood frogs. It is difficult to assess when boreal chorus frog start egg-laying given their tendency to deposit eggs on the underside and amongst clumps of fallen stalks of grasses and sedges (personal observation). Chorus frog breeding extends past that of wood frogs into mid-June. The differences in breeding phenology are interesting in light of potential competitive interactions between larvae. The prolonged breeding period of *P. maculata* allows multiple clutches to be laid. This behaviour may be an adaptation for breeding in ephemeral habitats, allowing chorus frogs to take advantage of ponds that refill with water after drying early in the season, however, intraguild predation by wood frog tadpoles is more likely to occur on later clutches if the pond does not dry (Sours and Petranka 2007). I hypothesize that prolonged breeding by the chorus frog may act to prevent intensive intraspecific competition amongst cohorts (e.g. Smith 1983, 1987) and it may lessen interspecific competition if diets of the two species change with ontogeny and densities of wood frogs are lower later in the season owing to ongoing larval mortality.

Tadpoles of the two species share few similarities. Wood frog tadpoles are capable of successfully completing development in closed canopy ponds where food

is perceived to be of low quality and quantity (Werner and Glennemeier 1999, Schiesari 2006), whereas boreal chorus frog tadpoles are rarely found in closed canopy ponds (Ouellet et al. 2009). Differences in the performances of the two species across habitat types may reflect differences in diet or ability to access resources. Boreal chorus frog tadpoles have a small oral feeding disc that lacks emarginated edges and contains fewer labial teeth rows than wood frog tadpoles (Conant and Collins 1991, Altig et al. 1998). Together the fewer teeth rows and lack of emargination around the oral apparatus may reduce the ability to grasp onto surfaces and rasp the feeding surface (Wassersug and Yamashita 2001, Wassersug pers. comm.). The shape and size of the oral apparatus likely dictates the nature of foods consumed (Altig et al. 1998, Bonacci et al. 2008). If competition occurs between these two species, then differences in their feeding and habitat preferences may allow for their co-existence and explain why they occur in the same wetlands in central Alberta.

Is competition expected between BCF and WF tadpoles?

The overlap in geographic ranges, and high degree of more local co-occurrence in the aspen parkland, suggests that interspecific interactions could be common between boreal chorus and wood frogs. Interactions between wood frogs and other species of Hylidae have been documented, including members of the genus *Pseudacris*. These interactions include exploitative competition in both mesocosm (Morin and Johnson 1988, Relyea 2002) and field settings (Skelly and Kiesecker 2001). Other forms of interference documented include intraguild predation (Sours and Petranka 2007) and aggression (Faragher and Jaeger 1998). Morin and Johnson (1988) showed that *L. sylvaticus* decreased the mass and

survival of *Pseudacris crucifer* tadpoles in artificial ponds. Morin and Johnson suggested that the wood frogs affected *P. crucifer* performance through exploitation because of their larger body size and associated greater consumption of periphyton. They also tested for chemical interference by growing *P. crucifer* in conditioned wood frog water, but found no inhibitory effects. On the other hand, western chorus frogs (*P. triseriata*) reduce wood frog tadpole growth and induce changes in the morphology of wood frog tadpoles (Relyea 2002) such that they had shorter tails and longer bodies. However, the decrease in growth rates caused by increased chorus frog density was weaker than decreases caused by increased wood frog density. Thus, Relyea (2002) demonstrated that wood frogs were affected by interspecific interactions with chorus frogs; however, he did not examine the converse effects of wood frogs on chorus frog performance.

Sours and Petranka (2007) found wood frogs to be intraguild predators of *Pseudacris feriarum* eggs and hatchlings less than one day old. In the same experiment, interspecific competition with wood frog larvae reduced larval growth and survival of *P. feriarum*. Competition between *Anaxyrus* (formerly *Bufo*) species and wood frogs has also been inferred as some toads primarily breed in ponds where wood frog tadpoles are absent or rare (Petranka et al. 1994). Whether the toad behaviour is in response to intraguild predation of eggs and larvae by wood frogs or is in response to other cues is uncertain. In addition to being predators on eggs, wood frog tadpoles are also suspected of being better resource competitors compared to other anuran larvae in unproductive, closed-canopy ponds where resources are limited or of low quality (Werner and Glennemeier 1999, Schiesari 2006).

Because wood frogs have negative effects on many other anurans, interspecific interactions, such as competition, probably occur between wood frog and boreal chorus frog larvae. I propose that competition between the species may occur, but that co-occurrence of the species within the aspen parkland region may result as competition may be weak due to variability in environmental conditions through space or time. Alternatively life-history adaptations, such as rapid development and phenotypic plasticity may reduce competition, or at least the duration of interactions, allowing co-existence. The strength and nature of the interaction may also be influenced by the high nutrient concentrations typical of ponds in the aspen parkland (Casey et al. 1999, Anderson et al. 2002, as cited in Wray and Bayley 2006, Eaves 2004). Nutrient conditions may act to alleviate competition through increased resource availability, or conditions may intensify competition by limiting the availability of suitable foods or by increasing metabolic demands. Most studies of interspecific interactions between wood frog and *Pseudacris* species have inferred exploitative competition for food resources, but have either ignored or found no evidence for interference competition. The goal of my doctoral research is to investigate how larval performance of the boreal chorus frog is affected by the presence of wood frogs (interspecific interactions) and pond nutrients (food limitation) and shed light on the diet of tadpoles in temporary ponds.

Previous work (Whiting 2004) with *Pseudacris triseriata* and *L. sylvaticus* at a pond near Montreal, Quebec, Canada suggests that interspecific competition was either weak or did not occur between these species. This research (Whiting 2004) revealed that larval survival of *P. triseriata* was highly dependent on wetland permanency and not interspecific competition. Recent mitochondrial evidence

suggests the population may be *P. maculata* rather than *P. triseriata* (sensu Lemmon et al. 2007). Seven anuran species and one salamander bred at the study wetland in addition to more than 300 breeding pairs of each *P. triseriata*, *P. crucifer* and *L. sylvaticus*. Based on this research in Quebec, as well as the variety of possible food resources within wetlands, I did not expect *P. maculata* and *L. sylvaticus* to compete for food resources at ponds in the aspen parkland.

As previously mentioned, ponds in the prairie pothole region of Alberta are highly productive systems, but resource limitations could occur for tadpoles due to a proliferation of inedible algae (i.e. cyanobacteria and metaphytic filamentous green algae) caused by eutrophication (via agriculture, and urban runoff) or at high tadpole densities. It is during periods of extreme resource limitation that exploitative or interference competition is likely to act or create the greatest differences in tadpole performance. Exploitative and interference competition may exist between *L. sylvaticus* and *P. maculata* when food resources become limited. The effects of competition may be altered by the larger size of wood frog tadpoles which is also beneficial in interference interactions. As other researchers have inferred competition between wood frog tadpoles and other *Pseudacris* larvae, it seems plausible that competition may be observed in parkland ponds. If the smaller tadpoles, such as *P. maculata*, are superior resource competitors (as theorized by Persson 1985, and demonstrated in anuran larvae by Werner 1994) and some food resources are limited, it follows that *L. sylvaticus* might employ interference mechanisms in addition to exploitation to gain a competitive advantage.

Description of research

The primary objective of this dissertation is to clarify the larval ecology of the boreal chorus frog with emphasis on its interactions with wood frog tadpoles. The secondary objective is to examine how tadpoles are affected by nutrient enrichment due to agriculture, as agricultural eutrophication of wetlands (Carpenter et al. 1998) is of global concern. Global amphibian declines (e.g. Stuart et al. 2004, Mendelson et al. 2006) have been linked to loss and degradation of wetland habitat (including due to agriculture) and it is unclear how much eutrophication is involved in those declines. I particularly wished to determine what competitive mechanisms operated between the two species and how interspecific interactions are affected by nutrients and human use of adjacent uplands. To assess the potential for resource competition, I examined the poorly known diets and trophic position of each tadpole species in relation to competitor presence and density using stable isotope analysis. Cases of possible competitive exclusion of introduced populations of western chorus frogs by the introduced wood frog (Maunder 1983, 1997), combined with uncertainty of tadpole diets, provides an opportunity to test whether competition between two abundant species might occur at northern latitudes, where anuran diversity is low and where pond nutrient concentrations are high (Casey et al. 1999, Anderson et al. 2002).

My specific research goals and approaches are:

Goal 1) Examine the larval ecology of boreal chorus frogs

Goal 2) Determine if there are patterns (such as reduced growth, survival, size at metamorphosis) consistent with competitive interactions between tadpoles by monitoring chorus frog and wood frog tadpoles

in ponds with different competitor densities and potential resource availability.

Goal 3) Determine if the two species share resources by exploring the diets of larvae in temporary ponds using stable isotopes. Stable isotopes allow a coarse determination of trophic position and the identity of resources that each tadpole species depends upon.

Goal 4) Determine if sharing resources with wood frog tadpoles affects the larval performance of boreal chorus frogs (larval growth, survival and size at metamorphosis).

Goal 5) If competition exists, determine if resource exploitation remains the only explanation for observed competitive patterns by examining possible forms of interference competition.

To address whether competitive patterns exist between boreal chorus frog and wood frog (Goal 2), in Chapter 2, I explore how tadpole performance is affected by intra- and interspecific density and interspecific interactions through surveys in natural temporary ponds in the aspen parkland of central Alberta. I also explore whether food resources are shared between the species and might indicate the existence of exploitative competition (Goal 3). To this end, I use stable isotopes of carbon and nitrogen to determine the food resources that are used by both species at two scales (in the field and in mesocosms). Stable isotopes have seldom been used to study anuran larvae and may reveal resource partitioning that is not evident from gut content or observational studies.

In Chapters 3 and 4, I explore the hypothesis that food abundance and water quality limit tadpole performance and affect interspecific interactions (Goal 4). In

Chapter 3, I examine the impact of nutrients, common in agricultural run-off, on the tadpole performance of both species. In Chapter 4, I compare tadpole density and performance in wetlands surrounded by forest to those surrounded by agricultural land. Eaves (2004) found that pond nutrients, nitrogen and phosphorous, were five times higher in ponds adjacent to crops, coincident with reduced adult densities for both chorus and wood frogs at agricultural ponds in comparison to protected forested ponds. I wanted to determine if this nutrient difference reduced tadpole performance or larval survival in mesocosms and contributed to Eaves' observation. Greater nutrient concentrations and inputs may be harmful to larval anurans, reducing their performance and survival, which leads to lower adult densities. However, the intensity of competition may be greater due to enrichment (e.g. paradox of enrichment, Rosenzweig 1971) which could reduce the performance of one or both species. Confirming resource overlap exists between the two species and that this overlap affects chorus frog tadpole growth and size at metamorphosis would confirm that exploitative competition can occur between the two species (Goal 4). Within natural ponds in pasture, crop and protected forested landscapes, I wanted to assess the effect of land use on tadpole performance through changes in wetland quality (Chapter 4). Few studies on the effects of land-use on anuran populations have monitored larval performance of pond-breeding species. This study aims to add to the knowledge of amphibians within the aspen parkland. Together, Chapters 3 and 4 increase our understanding of interspecific interactions between chorus and wood frog larvae across nutrient conditions and pond types.

In Chapter 5, I examine how chorus frog tadpoles perform in the absence of both shared resources and direct physical interaction with wood frog tadpoles. This chapter explores the potential for interference competition between the two

species, in particular chemical interference. Research reported in Chapters 2 through 4 revealed the existence of interspecific competition, but resource overlap observed between the two species in mesocosms was not as evident in natural ponds suggesting that although exploitative competition may occur, interference competition could also be operating. Thus, this chapter aims to determine if interference exists and creates performance patterns consistent with those observed in natural systems (Goal 5). To this end, I raised tadpoles in aquaria under controlled conditions where chorus frogs were physically separated from wood frog but shared water conditioned by wood frogs. In one experiment, chorus frogs had access to wood frog feces, and in second feces were removed. In a third experiment, I raised chorus frog tadpoles in the absence of wood frog tadpoles, but tested the effect of daily addition of wood frog feces to the tanks. These experiments test for the presence of an inhibitor that affects chorus frog growth, survival and size at metamorphosis, and also tests for the mechanism of release: deposition in feces or direct release into the water.

In the last chapter, I review the case for exploitative competition between boreal chorus frog and wood frog larvae and the potential importance of interspecific interference. Further, I discuss future directions in research to determine the source of interference by wood frog tadpoles and provide recommendations for those using stable isotopes and tadpole tissues in food web or dietary studies. Through my exploration of tadpole diet, food webs, land use, and the effects of interspecific interactions on larval performance, I shed light on the larval ecology of the boreal chorus frog (Goal 1).

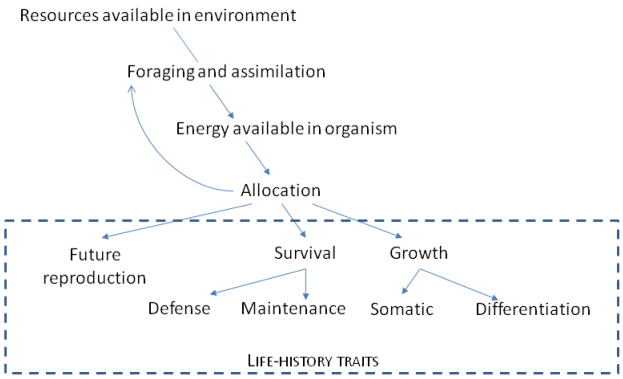


Figure 1-1: Flow of energy from resources to different life-history traits by allocation (adapted from Boggs 1992)**.**

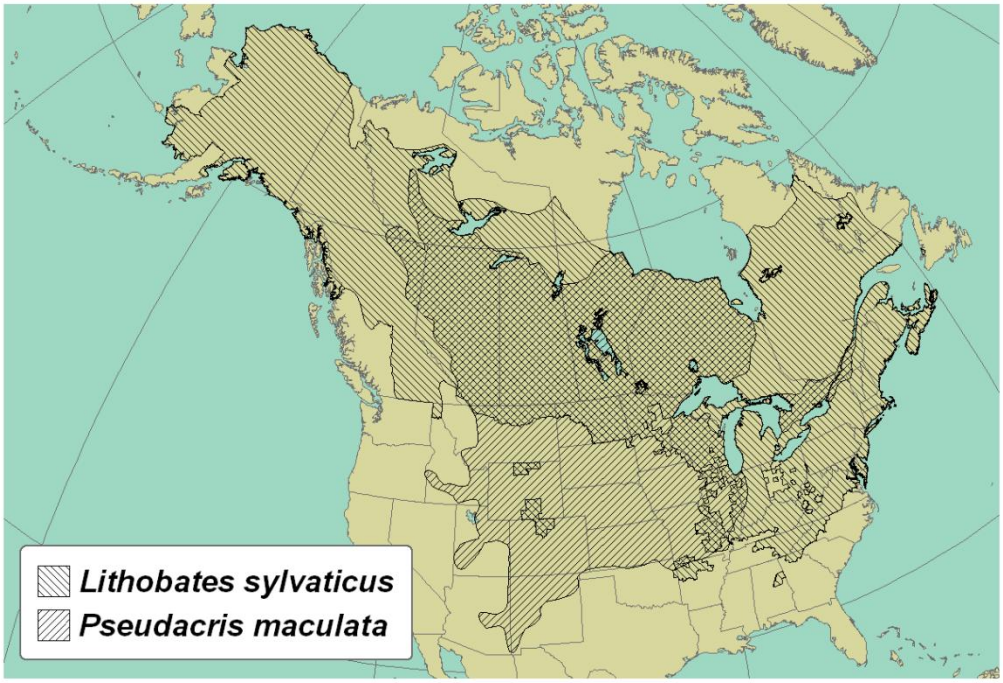


Figure 1-2: Current distribution of boreal chorus frog, *Pseudacris maculata* (sensu Lemmon et al. 2007), and wood frog, *Lithobates sylvaticus*. Data provided by IUCN (International Union for Conservation of Nature).

Literature Cited

Alford R. A. 1999. Ecology: resource use, competition, and predation. Pages 240-278 *In* R. W. McDiarmid, editor. Tadpoles: biology of anuran larvae, University of Chicago Press, Chicago.

Alford R. A. 1989a. Effects of parentage and competitor phenology on the growth of larval *Hyla chrysoscelis* . Oikos **54**:325-330.

Alford R. A. 1989b. Competition between larval *Rana palustris* and *Bufo americanus* is not affected by variation in reproductive phenology. Copeia **1989**:993-1000.

Altig R., M. R. Whiles, and C. L. Taylor. 2007. What do tadpoles really eat? assessing the trophic status of an understudied and imperiled group of consumers in freshwater habitats. Freshwater Biology **52**:386-395.

Altig R., R. W. McDiarmid, K. A. Nichols, and P. C. Ustach. 1998. A key to the anuran tadpoles of the United States and Canada. Contemporary Herpetology Information Series **2**:1-53.

Amarasekare P. 2002. Interference competition and species coexistence. Proceedings of the Royal Society of London Series B-Biological Sciences **269**:2541-2550.

Anderson A, Byrtus G, Thompson J, Humphries D, Hill B, Bilyk M. 2002. Baseline pesticide data for semi-permanent wetlands in the aspen parkland of Alberta. Edmonton: Alberta Environment; 2002.

Arendt J. D. 1997. Adaptive intrinsic growth rates: An integration across taxa. Quarterly Review of Biology **72**:149-177.

Armstrong R. A., R. Mcgehee. 1980. Competitive exclusion. American Naturalist **115**:151-170.

Bardsley L., T. J. C. Beebee. 2001. Strength and mechanisms of competition between common and endangered anurans. Ecological Applications **11**:453-463.

Belanger R. M., L. D. Corkum. 2009. A review of aquatic sex pheromones and chemical communication in anurans. Journal of Herpetology **43**:184-191.

Berven K. A. 1990. Factors affecting population fluctuations in larval and adult stages of the wood frog (*Rana sylvatica*). Ecology **71**:1599-1608.

Biesterfeldt J. M., J. W. Petranka, and S. Sherbondy. 1993. Prevalence of chemical interference competition in natural populations of wood frogs, *Rana sylvatica*. Copeia **1993**:688-695.

Boggs C. L. 1992. Resource allocation - exploring connections between foraging and life-history. Functional Ecology **6**:508-518.

Bonacci A., E. Brunelli, E. Sperone, and S. Tripepi. 2008. The oral apparatus of tadpoles of *Rana dalmatina*, *Bombina variegata*, *Bufo bufo*, and *Bufo viridis* (Anura). Zoologischer Anzeiger **247**:47-54.

Boone M. D. 2005. Juvenile frogs compensate for small metamorph size with terrestrial growth: Overcoming the effects of larval density and insecticide exposure. Journal of Herpetology **39**:416-423.

Brodman R., H. D. Krouse. 2007. How blue-spotted and small-mouthed salamander larvae coexist with their unisexual counterparts. Herpetologica **63**:135-143.

Brown C. J., B. Blossey, J. C. Maerz, and S. J. Joule. 2006. Invasive plant and experimental venue affect tadpole performance. Biological Invasions **8**:327-338.

Cadotte M. W., D. V. Mai, S. Jantz, M. D. Collins, M. Keele, and J. A. Drake. 2006. On testing the competition-colonization trade-off in a multispecies assemblage. American Naturalist **168**:704-709.

Carpenter S. R., N. F. Caraco, D. L. Correll, R. W. Howarth, A. N. Sharpley, and V. H. Smith. 1998. Nonpoint pollution of surface waters with phosphorus and nitrogen. Ecological Applications **8**:559-568.

Case T. J., M. E. Gilpin. 1974. Interference competition and niche theory. Proceedings of the National Academy of Sciences of the United States of America **71**:3073-3077.

Casey RJ, Paszkowski CA, Kendall SA, Ambrose N, Gingras B. 1999. Effects of cattle grazing intensity on water chemistry, aquatic invertebrates, waterbirds, songbirds and amphibians of pothole ponds of the Aspen Parkland, Central Alberta. Institute for Wetland and Waterfowl Research; 1999.

Chubbs T. E., F. R. Phillips. 1998. Distribution of the wood frog, *Rana sylvatica*, in Labrador: An update. Canadian Field-Naturalist **112**:329-331.

Conant R., J. T. Collins. 1991. A field guide to amphibians and reptiles. Eastern and Central North America. Houghton Mifflin Company, New York.

Connell J. H. 1983. On the prevalence and relative importance of interspecific competition - evidence from field experiments. American Naturalist **122**:661-696.

Connell J. H. 1980. Diversity and the coevolution of competitors, or the ghost of competition past. Oikos **35**:131-138.

Connell J. H. 1978. Diversity in tropical rain forests and coral reefs - high diversity of trees and corals is maintained only in a non-equilibrium state. Science **199**:1302-1310.

Crossland M. R., R. A. Alford, and R. Shine. 2009. Impact of the invasive cane toad (*Bufo marinus*) on an Australian frog (*Opisthodon ornatus*) depends on minor variation in reproductive timing. Oecologia **158**:625-632.

DeBenedictis P. A. 1974. Interspecific competition between tadpoles of *Rana pipiens* and *Rana sylvatica*: an experimental field study. Ecological Monographs **44**:129-151.

Eaves S. E. 2004. The distribution and abundance of amphibians across land-use types in Alberta's Aspen Parkland. University of Alberta. 134 p.

Faragher S. G., R. G. Jaeger. 1998. Tadpole bullies: examining mechanisms of competition in a community of larval anurans. Canadian Journal of Zoology **76**:144-153.

Fauth J. E., W. J. Resetarits. 1991. Interactions between the salamander *Siren intermedia* and the keystone predator *Notophthalmus viridescens* . Ecology **72**:827-838.

Frost D. R., T. Grant, J. Faivovich, R. H. Bain, A. Haas, C. F. B. Haddad, R. O. De Sa, A. Channing, M. Wilkinson, S. C. Donnellan, C. J. Raxworthy, J. A. Campbell, B. L. Blotto, P. Moler, R. C. Drewes, R. A. Nussbaum, J. D. Lynch, D. M. Green, and W. C. Wheeler. 2006. The amphibian tree of life. Bulletin of the American Museum of Natural History :1-370.

Gause G. F. 1934. The struggle for existence. Williams and Wilkins, Baltimore, Maryland.

Griffiths R. A., J. Denton, and A. L. C. Wong. 1993. The effect of food level on competition in tadpoles - interference mediated by Protothecan algae. Journal of Animal Ecology **62**:274-279.

Grime J. P. 1973. Competitive exclusion in herbaceous vegetation. Nature **242**:344-347.

Gurevitch J., L. L. Morrow, A. Wallace, and J. S. Walsh. 1992. A meta-analysis of competition in field experiments. American Naturalist **140**:539-572.

Hammerson G. A. 1999. Amphibians and reptiles in Colorado. University Press of Colorado, Boulder.

Huisman J., A. M. Johansson, E. O. Folmer, and F. J. Weissing. 2001. Towards a solution of the plankton paradox: the importance of physiology and life history. Ecology Letters **4**:408-411.

Keddy P. A. 2001. Competition. Kluwer, Dordrecht, The Netherlands.

Keddy P. A. 1990. Competitive hieracrchies and centrifugal organisation in plant communities. Pages 265-290 *In* J. B. Grace and D. Tilman, editors. Perspectives on plant competition, Academic Press, San Diego.

Keddy P. A. 1989. Competition. Champan and Hall, London.

Kendell K. 2002. Alberta inventory for the northern leopard frog (2000/2001). Alberta Species at Risk Report **44**:i-vii, 1-29.

Knight C. M., M. J. Parris, and W. H. N. Gutzke. 2009. Influence of priority effects and pond location on invaded larval amphibian communities. Biological Invasions **11**:1033-1044.

Kupferberg S. J. 1997. The role of larval diet in anuran metamorphosis. American Zoologist **37**:146-159.

Laufer G., R. Maneyro. 2008. Experimental test of intraspecific competition mechanisms among tadpoles of *Leptodactylus ocellatus* (Anura : Leptodactylidae). Zoological Science **25**:286-290.

Lemmon E. M., A. R. Lemmon, J. T. Collins, J. A. Lee-Yaw, and D. C. Cannatella. 2007. Phylogeny-based delimitation of species boundaries and contact zones in the trilling chorus frogs (*Pseudacris*). Molecular Phylogenetics and Evolution **44**:1068-1082.

Loman J. 2004. Density regulation in tadpoles of *Rana temporaria*: A full pond field experiment. Ecology **85**:1611-1618.

Lotka A. J. 1925. Elements of physical biology. Williams and Wilkins, Baltimore, Maryland.

Maunder J. E. 1983. Amphibians of the province of Newfoundland. Canadian Field-Naturalist **97**:33-46.

Maunder J. E. 1997. Amphibians of Newfoundland and Labrador: status changes since 1983. Herpetological Conservation **1**:93-99.

Mendelson J. R., K. R. Lips, R. W. Gagliardo, G. B. Rabb, J. P. Collins, J. E. Diffendorfer, P. Daszak, R. Ibanez, K. C. Zippel, D. P. Lawson, K. M. Wright, S. N. Stuart, C. Gascon, H. R. da Silva, P. A. Burrowes, R. L. Joglar, E. La Marca, S. Lotters, L. H. du Preez, C. Weldon, A. Hyatt, J. V. Rodriguez-Mahecha, S. Hunt, H. Robertson, B. Lock, C. J. Raxworthy, D. R. Frost, R. C. Lacy, R. A. Alford, J. A. Campbell, G. Parra-Olea, F. Bolanos, J. J. C. Domingo, T. Halliday, J. B. Murphy, M. H. Wake, L. A. Coloma, S. L. Kuzmin, M. S. Price, K. M. Howell, M. Lau, R. Pethiyagoda, M. Boone, M. J. Lannoo, A. R. Blaustein, A. Dobson, R. A. Griffiths, M. L. Crump, D. B. Wake, and E. D. Brodie. 2006. Biodiversity - confronting amphibian declines and extinctions. Science **313**:48-48.

Morin P. J., E. A. Johnson. 1988. Experimental studies of asymmetric competition among anurans. Oikos **53**:398-407.

Ouellet M., C. Fortin, and M. Grimard. 2009. Distribution and habitat use of the boreal chorus frog (*Pseudacris maculata*) at its extreme northeastern range limit. Herpetological Conservation and Biology **4**:277-284.

Paine R. T. 1994. Marine rocky shores and community ecology: an experimentalist's perspective. Excellence in Ecology **4**:1-152.

Pehek E. L. 1995. Competition, pH, and the ecology of larval *Hyla andersonii*. Ecology **76**:1786-1793.

Persson L. 1985. Asymmetrical competition - are larger animals competitively superior. American Naturalist **126**:261-266.

Petranka J. W. 1989. Chemical interference competition in tadpoles - does it occur outside laboratory aquaria? Copeia **1989**:921-930.

Petranka J. W., M. E. Hopey, B. T. Jennings, S. D. Baird, and S. J. Boone. 1994. Breeding habitat segregation of wood frogs and American toads - the role of interspecific tadpole predation and adult choice. Copeia **1994**:691-697.

Petren K., T. J. Case. 1996. An experimental demonstration of exploitation competition in an ongoing invasion. Ecology **77**:118-132.

Relyea R. A. 2002. Competitor-induced plasticity in tadpoles: consequences, cues, and connections to predator-induced plasticity. Ecological Monographs **72**:523-540.

Relyea R. A. 2000. Trait-mediated indirect effects in larval anurans: reversing competition with the threat of predation. Ecology **81**:2278-2289.

Resetarits W. J., J. F. Rieger, and C. A. Binckley. 2004. Threat of predation negates density effects in larval gray treefrogs. Oecologia **138**:532-538.

Richards C. M. 1958. The inhibition of growth in crowded *Rana pipiens* tadpoles. Physiological Zoology **31**:138-151.

Richter-Boix A., G. A. Llorente, A. Montori, and J. Garcia. 2007. Tadpole diet selection varies with the ecological context in predictable ways. Basic and Applied Ecology **8**:464-474.

Rogers T. N., D. R. Chalcraft. 2008. Pond hydroperiod alters the effect of density-dependent processes on larval anurans. Canadian Journal of Fisheries and Aquatic Sciences **65**:2761-2768.

Rose S. M., F. C. Rose. 1965. The control of growth and reproduction in freshwater organisms by specific products. Mitteilungen Internationale Vereinigung Fur Theoretische und Angewandte Limnologie **13**:21-35.

Rosenzweig M. L. 1971. Paradox of enrichment: destabilization of exploitation ecosystems in ecological time. Science **171**: 385-387.

Runkova G. G., Z. L. Stepanova, and L. A. Koval'chuk. 1974. Organ specificity in the action of metabolites of amphibian larvae on their endogenous metabolism under conditions of increased population density. Doklady Akademii Nauk SSSR **217**:328-329.

Schiesari L. 2006. Pond canopy cover: a resource gradient for anuran larvae. Freshwater Biology **51**:412-423.

Schoener T. W. 1983. Field experiments on interspecific competition. American Naturalist **122**:240-285.

Seale D. B., R. J. Wassersug. 1979. Suspension feeding dynamics of anuran larvae related to their functional morphology. Oecologia **39**:259-272.

Seburn CNL, Seburn DC. 1998. COSEWIC status report on the northern leopard frog *Rana pipiens* (southern mountain and western boreal/prairie populations) in Canada. Ottawa: Committee on the Status of Endangered Wildlife in Canada.

Semlitsch R. D., J. R. Bodie. 1998. Are small, isolated wetlands expendable? Conservation Biology **12**:1129-1133.

Skelly D. K., J. M. Kiesecker. 2001. Venue and outcome in ecological experiments: Manipulation of larval anurans. Oikos **94**:198-208.

Smith D. C. 1987. Adult recruitment in chorus frogs: effects of size and date at metamorphosis. Ecology **68**:344-350.

Smith D. C. 1983. Factors controlling tadpole populations of the chorus frog (*Pseudacris triseriata*) on Isle Royale, Michigan. Ecology **64**:501-510.

Smith G. R., H. A. Dingfelder, and D. A. Vaala. 2004. Asymmetric competition between *Rana clamitans* and *Hyla versicolor* tadpoles. Oikos **105**:626-632.

Sours G. N., J. W. Petranka. 2007. Intraguild predation and competition mediate stage-structured interactions between wood frog (*Rana sylvatica*) and upland chorus frog (*Pseudacris feriarum*) larvae. Copeia **2007**:131-139.

Stebbins R. C. 2003. A field guide to western reptiles and amphibians. Houghton Mifflin, New York.

Steinwascher K. 1979. Host-parasite interaction as a potential population-regulating mechanism. Ecology **60**:884-890.

Steinwascher K. 1978. Interference and exploitation competition among tadpoles of *Rana utricularia*. Ecology **59**:1039-1046.

Stuart S., J. S. Chanson, N. A. Cox, B. E. Young, A. S. L. Rodrigues, D. L. Fishman, and R. W. Waller. 2004. Status and trends of amphibian declines and extinctions worldwide. Science **306**:1783-1786.

Tilman D. 1994. Competition and biodiversity in spatially structured habitats. Ecology **75**:2-16.

Tilman D. 1982. Resource competition and community structure. Princeton University Press, Princeton.

Tilman D. 1980. Resources - a graphical-mechanistic approach to competition and predation. American Naturalist **116**:362-393.

Vance R. R. 1984. Interference competition and the coexistence of two competitors on a single limiting resource. Ecology **65**:1349-1357.

Volterra V. 1926. Fluctuations in the abundance of a species considered mathematically. Nature **118**:558-560.

Waldman B. 1986. Preference for unfamiliar siblings over familiar non-siblings in American toad (*Bufo americanus*) tadpoles. Animal Behaviour **34**:48-53.

Waldman B., P. J. Bishop. 2004. Chemical communication in an archaic anuran amphibian. Behavioral Ecology **15**:88-93.

Wassersug R. J., M. Yamashita. 2001. Plasticity and constraints on feeding kinematics in anuran larvae. Comparative Biochemistry and Physiology A-Molecular and Integrative Physiology **131**:183-195.

Werner E. E. 1994. Ontogenetic scaling of competitive relations: size-dependent effects and responses in two anuran larvae. Ecology **75**:197-213.

Werner E. E., K. S. Glennemeier. 1999. Influence of forest canopy cover on the breeding pond distributions of several amphibian species. Copeia **1999**:1-12.

Whitaker J. O. 1971. A study of the western chorus frog, *Pseudacris triseriata* in Vigo County, Indiana. Journal of Herpetology **5**:127-150.

Whiting A. V. 2004. Population ecology of the western chorus frog, *Pseudacris triseriata*. Montreal, Quebec: McGill University.

Wilbur H. M. 1997. Experimental ecology of food webs: complex systems in temporary ponds. Ecology **78**:2279-2302.

Wilbur H. M. 1987. Regulation of structure in complex-systems - experimental temporary pond communities. Ecology **68**:1437-1452.

Wilbur H. M. 1977. Density-dependent aspects of growth and metamorphosis in *Bufo americanus*. Ecology **58**:196-200.

Wilson S. D., D. Tilman. 1991. Components of plant competition along an experimental gradient of nitrogen availability. Ecology **72**:1050-1065.

Wong A. L., R. A. Griffiths, and T. J. C. Beebee. 2000. The influence of tadpole size on cell-mediated competition between anuran larvae (*Bufo calamita* and *Rana temporaria*) in the laboratory. Amphibia-Reptilia **21**:431-438.

Wray H. E., Bayley S. E. 2006. A review of indicators of wetland health and function in Alberta's prairie, aspen parkland and boreal dry mixedwood regions. The Water Research Users Group, Alberta Environment; 90 p.

Chapter 2: Interspecific competition between northern temperate anurans

Introduction

Interspecific competition may result in the exclusion of a species from a habitat, but exclusion can only be inferred from differences in species' distributions and from translocation experiments. The study of competition and its effects on sympatric species provide mechanistic explanations of species' abundances and distribution patterns. Competition alters the resources available for consumption and increases the metabolic costs of individuals acquiring resources. These increased costs limit growth and survivorship of competing individuals and species, and can alter the spatial and temporal distribution of species as well as their abundance. If competition is severe, it leads to the exclusion of the inferior competitor from certain habitats, but coexistence occurs until exclusion. Species too similar in their resource use cannot coexist for long if shared resources are limiting; coexisting species must differ sufficiently in their resource use. Theory suggests that competition may be difficult to detect as niche differentiation driven by competition minimizes observed interspecific competition (Connell 1980, Keddy 1989). Interspecific competition may still occur if conditions such as environmental variability (as reviewed by Tylianakis et al. 2008), trade-offs between a species' competitive ability and its colonization ability (e.g. Tilman 1994, Cadotte et al. 2006), or susceptibility to predation (Paine 1994, Relyea 2000) permit similarity of species' traits and resource requirements. A similarity in species' resource use may also be maintained if the abundance of critical resources exceeds the needs of the competitors, either because populations are small or resource availability varies

temporally or spatially. However, conditions may change and then competition between species in natural systems may be observed.

Interactions between larvae of anuran species and other members of their aquatic food webs are commonly used as models to document the nature of competition and to examine how it structures communities. Alford (1999) suggested interspecific competition may be common in tadpole assemblages at high tadpole densities, because individuals of several species with low perceived niche differences co-occur at many sites. Similar niches combined with temporal food-limitations in aquatic environments (DeBenedictis 1974, Wilbur 1977), should intensify interspecific competition.

My goal in this chapter is to ascertain whether competition occurs between larval anuran species within temporary ponds in the aspen parkland of Alberta. Anuran species richness here is low compared to other temperate locations within North America. Because interspecific competition increases the metabolic costs of acquiring resources, I want to determine if there are interactions between the larvae of the boreal chorus frog (*Pseudacris maculata*) and wood frog (*Lithobates sylvaticus* formerly *Rana sylvatica* sensu Frost et al. 2006). These are the most abundant anuran species in Alberta (Russell and Bauer 2000). If these species compete, I would expect to find one of several patterns: 1) overlap in larval diets, 2) patterns of reduced performance, such as reduced growth rate, survivorship and size at metamorphosis, in one or both species with increased density of heterospecific larvae, and 3) differences in the spatial distribution of larvae of the two species within ponds. I elaborate on each of these patterns below.

The first and most basic evidence for interspecific competition would be the overlap of larval diets of the two species. A similarity in tadpole diets and limited food resources are necessary for competition to occur between the species. The diets of anuran larvae are not well documented (Altig et al. 2007), but tadpoles are believed to be herbivores and graze periphyton and other algal resources. This perception was supported by gut content studies where the contents closely matched the availability of algae in tadpoles' environments (e.g. Farlowe 1928, Seale and Beckvar 1980). However, observations of tadpoles feeding opportunistically on protozoans from the water column (Test and McCann 1976) and on carrion (Beiswenger 1975, Harp and Petranka 2006) are changing our understanding of tadpole diets. Such observations prompted Petranka and Kennedy (1999) to call for a re-evaluation and further study of the functional roles and trophic status of tadpoles. The problem of gut content studies and their use in determining tadpole diets is the difference between ingestion and assimilation. Gut contents provide snapshots of the diet, whereas the absorption of stable isotopes of nitrogen and carbon integrate dietary components over longer periods and show actual assimilation (Hesslein et al. 1993). The inclusion of amphibian larvae in SIA of food webs is novel (but see Finlay and Vredenburg 2007, Verburg et al. 2007, Schiesari et al. 2009).Thus, stable isotope ratios provide an opportunity to determine if assimilated diets of two or more species are similar and can provide evidence of interspecific competition.

The second pattern that I consider as evidence of interspecific competition is the reduction of tadpole performance in the presence of, or at increased density of, a competitor. Performance metrics commonly used to evaluate the effects of interspecific competition in anurans include tadpole growth, and fitness

components measured at metamorphosis, such as survival, size at metamorphosis and duration of larval period. The allocation of resources to growth and development are likely to be affected by increased metabolic costs of acquiring resources in the face of competition (Arendt 1997). The effects of increased metabolic costs due to neighbours and the environment are integrated into the day-to-day growth of an individual. When the effects of the environment are accounted for, then we can measure the effects of neighbours on an individual tadpole's growth. A convenient stage to measure the effects of competition is metamorphosis, as it integrates the effects of neighbours and of the environment that an individual experiences during its larval period. Attributes of individuals at metamorphosis are useful for evaluating the impacts of competition in anurans as the lifetime reproductive fitness and survival of an individual after emergence is related to date and size at metamorphosis (Smith 1987, Berven 1990, Loman 2004).

The final pattern that I consider, as evidence for competition between boreal chorus frog and wood frog tadpoles, is spatial distribution of larvae within ponds. Differences between species in spatial distribution within a pond may result from factors such as predation, resource availability, or other environmental preferences, such as temperature. Competition may also alter spatial distribution of tadpoles within a pond (Kiesecker and Blaustein 1997, 1998). Behavioural interference, such as tail nipping or aggressive behaviours (Kupferberg 1996, Faragher and Jaeger 1998), might cause the smaller chorus frog tadpoles to avoid locations used by larger wood frog tadpoles. Similarly, competition for food resources may cause species to alter their use of space or resources and lead to segregation or spatial avoidance of competitors.

In this research I investigate the following questions:

1) Do chorus frog and wood frog tadpoles exhibit resource partitioning, or do they overlap in their diets which might lead to competition?
2) Does the performance of boreal chorus frogs decrease with increasing wood frog density or is performance more clearly related to other environmental parameters?
3) Is the spatial distribution of chorus frog tadpoles within a pond affected by the presence of a potential competitor, the wood frog?

To address Question 1 and determine whether chorus frog and wood frog tadpoles might compete for food, I use a comparative study of the two species' feeding ecology using SIA of co-occurring tadpoles from natural ponds and stocked mesocosms. I measure the natural abundance of two stable isotopes, ^{15}N and ^{13}C from tadpole tissues from ponds and mesocosms. ^{15}N is used to determine the relative trophic position (Kling et al. 1992) and ^{13}C the source of food (Peterson and Fry 1987). I use these two stable isotopes to determine if the species utilize the same food resources and therefore compete for food. Wood frog tadpoles are able to develop in a wide range of habitats, from closed-canopy forested ponds with low productivity to highly productive open-canopy ponds (Werner and Glennemeier 1999, Schiesari 2006). In contrast, boreal chorus frogs are usually found in relatively productive open-canopy, temporary ponds (Whitaker 1971). Inherent differences in the resources used by each species' larvae may exist because they exist in different habitats, but again the general uncertainty of tadpole diets and their plasticity in response to resource availability does not exclude the possibility of exploitative competition for food when the species co-occur. If competition for food exists, I

would expect the two species to display comparable stable isotopic signatures indicating the species occupy similar trophic levels and rely on similar carbon sources. A complicating factor is differences in isotopic signatures may result either from the use of different food resources or species specific differences in assimilation. In order to interpret results of SIA on tadpoles from ponds and mesocosms, I performed lab experiments in which I fed tadpoles two different known food types. I fed tadpoles one of two formulated diets, differing in protein content, in aquaria stocked with conspecifics. Results from these feeding trials were used to verify whether potential differences observed in natural ponds and in mesocosms reflected real interspecific differences in resource use or differences in species specific rates of isotope assimilation. If the two species assimilated the isotopes at the same rate regardless of food source then I felt confident that overlap in stable isotopes signatures observed in ponds or mesocosms reflect the feeding ecology of tadpoles and support interspecific competition for food.

I approach Question 2 (on page 36) through a comparative study of the performance of chorus frog larvae in naturally occurring populations. Work by others (Wilbur 1987, Morin and Johnson 1988, Alford 1989ab, Fauth and Resetarits 1991, Pehek 1995, Skelly 1995, Faragher and Jaeger 1998) suggests that ranid tadpoles (such as wood frog) are superior competitors to hylid tadpoles (such as boreal chorus frog) because of inherent differences in tadpole size, differences in their ability to deplete shared resources, or differences in the efficiency of converting shared foods into energy. In addition, ranid tadpoles may also employ behavioural and/or chemical interference (Faragher and Jaeger 1998) thereby reducing the performance of hylid tadpoles. The preceding observations and evidence in my literature review (Chapter 1) suggest that if competition occurs

between larvae of these two species, the performance of boreal chorus frog would be reduced. To verify this prediction, I investigated whether performance, measured in terms of growth, larval period, and size at metamorphosis, of chorus frogs was related to the density of wood frog tadpoles across ponds differing in tadpole densities. In addition, I examine these three metrics in light of other variables related to water chemistry and intraspecific density of chorus frogs.

To address Question 3 (on page 36), I surveyed natural populations over three years at ponds in the Beaver Hills region of Alberta. To determine whether chorus frog tadpole distribution within a pond was affected by wood frog tadpole presence, I compared the abundance of tadpoles at different locations within ponds to evaluate whether competition or some other factor alters the spatial distribution of tadpole species within a pond. There are a number of possible distribution patterns depending on what factors most strongly affect tadpoles; such factors include predation, resource distribution, thermal preferences, competition and temporal difference in foraging habits of tadpoles. I predict that if competition is for food or space then the inferior competitor, assumed to be the boreal chorus frog, would avoid sites with wood frogs. Avoidance may occur due to direct behavioural interference by wood frogs or because chorus frogs are unable to acquire sufficient resources in patches with wood frog tadpoles.

Methods

Study area

I conducted this study over three years (2005-2007) within the aspen parkland ecoregion of central Alberta, Canada. Study ponds were located within Elk Island National Park (EINP, 53°35’34”N, 112°52’15”W). Additional ponds in the

surrounding agricultural areas were added in 2006 and 2007 (Appendix Table 1-1). Because only two ponds with chorus frog (CF) alone were found during breeding call surveys in 2005 at ponds within EINP (n = 400, < 0.3 ha), plans to compare CF tadpole performance when allopatric or sympatric with wood frogs (WF) were revised. I instead examined CF performance relative to WF density. I selected 15 of the ponds surveyed in 2005 within EINP (hereafter referred to as park ponds) based on ease of access and density of WF eggs found. All park ponds (Appendix Table 1-1) were temporary ponds, or Class II based on Stewart and Kantrud's (1971) prairie wetland classification. All ponds had sedge (Cyperaceae – *Carex* spp.) as emergent vegetation and only one park pond had cattails (*Typha latifolia*). Most ponds were bordered by tall grasses (Poaceae – *Poa* spp. and *Glyceria* spp.). Upland vegetation consisted mainly of trembling aspen (*Populus tremuloides*) and balsam poplar (*P. balsamifera*) with some willow (*Salix* spp.) within or near the water's edge. In 2006 and 2007, I re-surveyed six park ponds and 10 ponds in the nearby agricultural lands (referred to as agricultural ponds; Appendix Table 1-1). Agricultural ponds were temporary to semi-permanent ponds (Class II to IV, Stewart and Kantrud 1971): three were accessed by cattle and one by sheep, six were within or immediately adjacent to crops (canola, barley, clover or corn). Agricultural ponds differed in extent of riparian vegetation, but all contained some emergent shoreline vegetation of sedges, tall grasses (*Poa* and *Glyceria* spp.), and/or cattails (for a more detailed list of species found within ponds see Appendix Table 2-2).

Competitive pattern – Diet overlap

Feeding experiment – do different species assimilate isotopes at different rates?

To determine if CF and WF tadpoles assimilated carbon and nitrogen isotopes similarly, I measured the fractionation rates of ^{15}N and ^{13}C isotopes in

tadpoles relative to diets consisting of constant known food sources. Eggs of CF and WF were collected from a pond in EINP and hatched in separate plastic containers with de-chlorinated water. I used 12 aquaria (50 × 25 × 27 cm) filled with 28 L of de-chlorinated city water (22.5 cm deep) and placed an airstone at a low bubble in each aquarium. Six aquaria received 40 randomly selected CF hatchlings and the other six aquaria 40 WF hatchlings. Species tanks were randomly assigned to one of two shelves and then randomly assigned to one of two food treatments consisting either exclusively of rabbit pellets (Hagen ART.# H-1153, 16% protein) or of spirulina pellets (Ocean Star International #1235 medium floating/sinking pellets, 42% protein). All pellets were ground to a powder using a coffee grinder. Once a day, tadpoles were fed at 12.5% of the average mass of tadpoles among aquaria within a species and food treatment. I chose this rate of feeding, as prior experience with feeding tadpoles at 15% of average mass resulted in water fouling (see Chapter 5). Aquaria were cleaned every other day and water replaced weekly. I used dry-ice to euthanize eggs, hatchlings and tadpoles of both species prior to the experiment. To monitor how each tadpole species assimilated isotopes from foods, I analyzed both foods and samples from up to five developing tadpoles from tanks on day 14 and 28. Additional samples were taken on day 20 and 35 and then all remaining tadpoles were euthanized on day 42.

Diet overlap in natural ponds

In order to determine if CF and WF tadpoles overlap in assimilated resources and therefore potentially compete for food, I collected animals for SIA from the field. Animals were collected using dipnets at random locations throughout each pond. I used isotopic signatures of carbon and nitrogen to determine the degree of diet similarity between CF and WF. In 2005, I sampled three to five

tadpoles from park ponds weekly starting 19 May, park and agricultural ponds once between 5 and 29 June 2006, and six agricultural ponds on 11 June 2007. Monthly I collected snails and other macro-invertebrates (Appendix Table 2-1), as well as several potential tadpole food sources: epiphytic and epipelic periphyton, floating algal mats, macrophytes, particulate organic matter from the water column, sediment, and plant detritus. In 2005, I kept tadpoles in plastic containers with de-chlorinated water to void guts for 24 hours before euthanization. Tadpoles were euthanized in plastic bags placed within dry ice. In 2006 and 2007, I kept tadpoles in plastic bags in a cooler with local pond water for up to 8 hours and then euthanized the tadpoles at day's end. I am confident there was little material in tadpole guts prior to analysis as tadpoles are capable of voiding their guts in 2 hours (Schiesari 2004). All animals were euthanized by rapid freezing in dry ice and samples were kept frozen at -20°C prior to sample preparation and analysis, which unlike other preservation methods does not alter isotopic ratios (Hobson et al. 1997).

Diet overlap in outdoor mesocosms

An experiment (described in detail in Chapter 3) to test the influence of fertilization and the presence of WF tadpoles on CF tadpole performance provided an opportunity to examine tadpole diet overlap in a simplified natural system. Sixteen fibreglass cattle troughs (tanks) were placed in an empty dugout at the Meanook Biological Research Station. Tanks were placed in a 4 × 4 grid, treatments consisted of 99 CF held with 0 or 50 WF with or without fertilizer addition (both nitrogen and phosphorous). Tanks had a sandy bottom ~ 5 cm deep to which I added 20 L of dried detritus taken from a nearby pond. Tanks were filled with approximately 1300 L of water from the same pond on May 7, 2007. The intake hose of the water pump had a nylon mesh bag (mesh size ≈1 cm) which excluded large

macro-invertebrates but allowed small zooplankton and snails (*Physa* sp.) to colonize the tanks. After allowing periphyton and other microflora to establish for a week following the addition of water and detritus, tadpoles were introduced. At this point, CF hatchings were six days old with a mean body length of 2.23 mm (range 1.6 – 2.9 mm, n = 40), whereas WF tadpoles were nine days post hatching with a mean body length of 3.71 mm (range 2.9 – 4.8 mm, n = 30). For further details on animal collection, selection and treatments see Chapter 3, "Methods". SIA was performed on two to five tadpoles collected on June 19 and 26 from two replicates of each treatment to examine the overlap in isotopic diet between CF and WF in tanks. I also wanted to compare isotopic signatures of tadpoles to those of snails, which depend upon attached algae resources that tadpoles may also eat; thus at the end of the experiment when tadpoles were absent I collected two to three snails on July 17 from each tank.

Stable isotope analysis

Whole-body samples were freeze-dried at -80°C for 24 h and ground to a fine powder, in glass scintillation vials using surgical scissors and a stainless steel scoop to avoid loss due to static electricity. Between 0.8 and 1.2 mg of the dried powder was added to a 3 mm × 5.5 mm tin capsule, folded and placed in an individually labelled sample tray. Stable isotope results are calculated relative to a reference standard. The global standard for $\delta^{13}C$ is PeeDee belemnite and for $\delta^{15}N$ is atmospheric nitrogen. Sample analyses were completed at one of two labs (Table 2-1).

Competitive pattern – performance versus competitor density

To determine how the presence and density of WF tadpoles affect CF tadpole performance, I measured both CF and WF tadpole growth, duration of larval period and size at metamorphosis from natural ponds. I determined growth rates by measuring body length to the nearest 0.1±0.05mm using callipers for the first 10-15 tadpoles of each species captured on a given survey date. Body length was defined as the distance from the snout to the point where the tail meets the body and hind limbs emerge. Body length was preferred over total length as tail breakage was common. I used dipnets to capture tadpoles and continued until I had collected 10 tadpoles or had spent more than 20 minutes searching. If more than 10 individuals were captured, I measured all individuals to prevent bias in tadpoles selected for measurement. Measurements were made weekly at seven park ponds in 2005, starting on May 19. Subsequently, measurements were taken every other week, starting May 19 in 2006 and May 15 in 2007, at six of the park ponds and seven agricultural ponds (six of the agriculture ponds were used in each year, Appendix Table 1-1). The duration of egg and larval periods were measured from the date of peak calling for each species to the date when the first metamorph (Gosner stage 43-45, Gosner 1960) was found. I used peak calling as CF eggs are inconspicuous and easily missed, whereas peak calling in WF coincided with peak egg-laying (personal observation). Due to difficulty in finding metamorphs amongst dense vegetation, not all ponds were included in larval period analysis (n = 10 of 15 in 2005, n = 7 of 14 in 2006 and 13 of 14 in 2007). Larval periods are likely overestimates due to the biweekly sampling schedule.

The last performance response measured was size at metamorphosis. At each location I measured the snout-to-urostyle length (SUL) of emerging CF to the

nearest 0.1±0.05mm. Only those individuals still showing signs of a tail were included in measures as individuals with no tail may have been living in the terrestrial environment for some time and grown since emergence. I confirmed that CF SUL does not change during tail resorption by holding recently emerged individuals at stage 42 (Gosner 1960) until full tail resorption (Gosner stage 45). My goal had been to measure at least 10 metamorphs at each site, but captures at some locations were low and those sites with only one metamorph were removed from the analysis. Size at metamorphosis was recorded at 12 of 15 ponds in 2005, five of 14 in 2006 and 12 of 14 ponds in 2007 ($\bar{x}$ number of metamorphs measured per site = 11, range 3-24). I could not determine survival to metamorphosis due to differences in detectability associated with vegetation at the perimeter of ponds and uncertainty in how many eggs or tadpoles were initially present at each location.

Determining tadpole density

To determine if WF has an effect on CF growth, larval period, and size at metamorphosis, I compared each CF metric against a gradient of WF densities which was determined from a single, more intensive, sampling of each pond in mid-June of each year. Sampling was completed across all ponds within one week (14 –23 June 2005, 21–28 June 2006, and 13–14 June 2007). An assistant and I sampled eight to 15 randomly selected locations based on a 1 m^2 grid. Sampling points were rejected if they were within 5 m of another sampled point, if they were dry, or an obstruction prevented efficient sweeping with the net. Net sweep paths were 1 m long by 37 cm wide, and repeated three times to ensure adequate sampling at each location. Sweep contents were placed into marked plastic buckets and identified at the end of all sweeps. All tadpoles were identified and staged using a field classification. Water depth was measured from markings on the side of the sampling net. Animal density

for each sample was determined by dividing the number of individuals by the volume of water sampled; volume sampled was calculated using the following equation:

$$\frac{r^2}{2}\left[2 \times \arccos\left(\frac{r-d}{r}\right) - \sin\left(2 \times \arccos\left(\frac{r-d}{r}\right)\right)\right] \times 1000 \ \frac{\mathrm{L}}{\mathrm{m}^3} \times length$$

Where r is the radius of the net (0.185 m) and d is the depth of water sampled and *length* is the sweep length (1 m). Where water was higher than the net, a volume of 107.5 L was used and the sweep was taken along the pond bottom.

Spatial distribution of tadpoles

I used tadpole densities from intensive tadpole surveys from each year (described above) to determine the average distribution of tadpoles within ponds. For each pond I determined the mean percent of CF tadpoles collected at each sample location. I used the pond means to determine the relationship between CF and WF density across ponds. I also determined the proportion of locations within a pond that were occupied by only CF, only WF, and by both species. If CF tadpoles avoided WF due to behavioural interference, I expected few locations would contain both species.

Pond environment measurements

To determine if pond environment might contribute to differences in CF performance metrics, I measured pond chemistry, depth and temperature each year. In 2006 and 2007, I measured total nitrogen (TN), total phosphorous (TP), ammonia (NH_4), all collected from a single water sample from an open water area of the pond (all measured in µg/L). Pond pH was measured with a handheld meter. Pond depth measured around June 15 of each year. For pond hydroperiod, I counted the number

of days from the date of the first frog calling to pond drying in each year. I measured pond temperatures continuously using temperature loggers (Thermochron® iButton DS-1921G – Maxim Integrated Products) at 30 minute intervals from 6 June until pond drying in each year and then took the average of daily averages for use in correlations.

Data analysis

Due to the small sample sizes and investigative nature of this study (not explanatory), I considered effects significant with P-values less than 0.10 instead of the more widely used 0.05. All analyses were done using SYSTAT 12.02 (SYSTAT Software INC. 2007).

Tadpole diet

For the feeding trial, I selected two aquaria from each species/diet combination and measured mean tadpole isotopic signatures on day 14 and 28. Isotopic fractionation of tadpole tissues for each species and food was determined by the formula: $[\delta^{15}N_{tadpole}$ (or $^{13}C)] - [\delta^{15}N_{food}$ (or $^{13}C)]$ (as per Schiesari 2006, Schiesari et al. 2009). I compared the fractionation rate between foods within species for each sampling date using ANOVA, treating aquaria as replicates and tadpoles as subsamples.

For natural ponds and mesocosms, I calculated pond (or tank) mean isotopic values for each species from a given sampling date. I corrected $\delta^{13}C$ values ($\delta^{13}C_{corr}$) for variation in pond carbon signature using the mean snail $\delta^{13}C$ signal across ponds in each year and then adding the difference $[\delta^{13}C\ (snail_{pond}) - \delta^{13}C\ (snail_{mean\ ponds})]$ to the tadpole $\delta^{13}C$ value (Post 2002). I calculated tadpole trophic level as: $1+[\delta^{15}N_{tadpole} - \delta^{15}N_{snail}]/\delta^{15}N_{fractionation\ rate}$ (Post 2002). Snails are a useful baseline reference as

they have similar generation times to tadpoles, are consumers of benthic primary production and are specialized for grazing periphyton and detritus (Post 2002, Schiesari et al. 2009).

To determine if mean CF and WF diets ($\delta^{13}C_{corr}$, and trophic level) differed, I used a paired *t*-test with ponds as replicates. I also compared uncorrected $\delta^{13}C$ and trophic level between CF and snails (*Physa* sp.), as snails commonly consume periphyton and if it is limited may compete with tadpoles. To determine if tadpole densities or pond environmental variables influenced the similarity of tadpole diets, I constructed a Pearson's correlation matrix using the difference in $\delta^{13}C_{corr}$, CF – WF (which more often gave positive values), and relative trophic level. I chose a univariate approach as I was interested in examining the response of tadpole diet metrics to individual variables.

In mesocosms, to determine if WF presence and fertilization affected CF carbon signature and trophic level, I used a two-way ANOVA with sampling dates (s = 2) as repeated measures. Two separate fixed effects split-plot analyses were performed to establish whether WF and CF tadpoles shared the same trophic level and carbon source. Fertilizer was used as the between subjects effect and species as within subjects effect with tanks as the split plot. To compare diets between *Physa* sp. with CF tadpoles, I also used a fixed effects split-plot analysis with fertilizer and WF presence as between subjects' effect and species as within subjects' effect with tanks as the split plot.

Tadpole performance in natural ponds

Mean tadpole growth rates were determined using linear regression on the linear portion of the growth trajectory for each pond-year, generally until the two-

leg stage (equivalent to Gosner stage 39). I chose arithmetic growth as opposed to the specific growth rate, as I was mainly interested in CF growth and its response to other factors; direct comparisons of patterns between CF and WF was of secondary interest. I used the standard error in the slope estimate to estimate uncertainty in the growth rate for each species. Larval period, as previously mentioned, was the length of time required for the first tadpole to emerge from each pond, producing a single value for each pond. Mean SUL of metamorphs for each pond was compared across ponds. Tadpole densities were calculated as the average density from intensive sampling in June of each year. Tadpole densities were transformed by $x^{1/4}$ to improve normality. I used Pearson's correlation followed by simple linear regression to determine if there were any relationships between tadpole densities and CF growth rates, larval period and mean metamorph length to assess effects of competition. As ponds differed in tadpole densities, pond size, and predator densities from year to year (see Results), which all are likely to affect tadpole performance, I chose to use each pond-year as individual independent measures (ignoring pond identity) within the analyses. This increased the sample size from 14 ponds to up to 26 ponds in the regressions. Negative relationships would be expected if competition, either intra or interspecific, occurred for a limited food resource in ponds. In addition, I compared CF performance metrics to TN, TP, NH_4, depth, hydroperiod and daily average pond-water temperature. Pond environmental variables were compared using Pearson's correlations within years across sites (Appendix Table 4-3). Water chemistry parameters were natural log-transformed and depth was square root transformed to improve normality.

Spatial distribution of tadpoles

Within each pond, I calculated the proportion of locations with only CF tadpoles as: CF only locations divided by all locations with tadpoles. Sample points without any tadpoles were thus excluded (about 23% of locations within any given pond were excluded). The mean proportion of locations with only CF tadpoles across ponds within a year was compared to 1 (CF avoid WF completely) and 0 (CF and WF co-occur at all sample locations) using two separate one-sample *t*-tests. For this analysis I excluded those ponds where all samples had either only CF or only WF tadpoles. Data were arcsine square root transformed prior to analysis.

Results

Evidence for resource overlap between CF and WF tadpoles

Calibration of isotopic fractionation

Tadpole isotopic signatures changed from hatching through development and reflected signatures of the provided food. Isotopic signatures of both tadpole species were enriched in both $\delta^{13}C$ and $\delta^{15}N$ relative to their food (Table 2-2). Due to a number of WF deaths, followed by replacement, isotopic values for WF had not equilibrated with their foods as evidenced by enrichment from day 14 to 28 (especially in animals fed spirulina). CF isotopic values, on the other hand, were stable between these dates. Due to WF mortality and replacement, I only consider values from day 28 in species comparisons. I found no evidence that fractionation of either isotope in tadpole tissue differed between species or food types (Table 2-2). There was no interaction between species or food for either isotope ($p \geq 0.29$). The resulting mean isotopic fractionation of larval anuran tissues was 1.70 ± 0.09‰ for $\delta^{13}C$ and 2.54 ± 0.12‰ for $\delta^{15}N$. I use the $\delta^{15}N$ fractionation factor for all further

calculations of trophic level in this study. The absence of differences in isotopic fractionation between species and food types suggests that differences observed in ponds and mesocosms should reflect niche differentiation with respect to assimilated foods.

Evidence for overlap in tadpole diets from natural ponds

To determine if WF and CF tadpoles shared food resources, I compared corrected $\delta^{13}C$ and $\delta^{15}N$ isotopic signatures between the species across sites. WF tadpoles were generally more depleted in $\delta^{13}C$ compared to CF tadpoles within a pond (mean difference of paired comparisons 1.02‰), this difference was significant (Figure 2-1, paired $t_{24} = 3.63$, $p = 0.001$; or $t_{15} = 3.71$, $p = 0.002$ if tadpole values are averaged for a pond across years). The trophic level of CF tended to be higher than WF (more enriched in $\delta^{15}N$ by 0.24‰), and this difference was statistically significant (Figure 2-2, mean difference = 0.096, paired $t_{24} = 1.92$, $p = 0.067$). Repeated sampling within ponds in 2005 (between May 19 and July 27) revealed seasonal shifts in carbon source in both species, but interspecific differences were generally consistent (see appendix #3 and Appendix Figure 3-1).

I have included six representative food webs to show the relative position of tadpoles within these temporary ponds (Figure 2-3). In general, tadpoles within these pond food webs had equivalent trophic levels to known aquatic predators such as anisopteran larvae and predatory dytiscids, based on *Physa* sp. as the reference for calculating trophic level.

Comparisons of each tadpole species with snails for both $\delta^{13}C$ and trophic level (based on $\delta^{15}N$) indicated divergence in diets. Compared to snails, tadpoles were significantly more depleted in $\delta^{13}C$ (*paired t*-test, snail – CF = 3.03 ‰ - t_{24} =

6.53, $p < 0.001$; snail – WF = 4.05 ‰ - $t_{24} = 8.10$, $p < 0.001$) and occupied significantly higher trophic levels (CF = 1.47, one sample $t_{25} = 6.58$, $p < 0.001$; and WF = 1.40, $t_{25} = 5.17$, $p < 0.001$; see Figure 2-2, snail trophic position = 1.0).

Evidence for overlap in tadpole diets from mesocosms

The source of carbon in CF tadpole diets in mesocosm was affected by an interaction between WF presence and fertilization (Table 2-3). In fertilized tanks, CF tadpoles assimilated foods with similar $\delta^{13}C_{corrected}$ regardless of WF presence; however, in the absence of fertilization, CF tadpoles assimilated foods significantly more enriched in $\delta^{13}C$ in tanks with WF (Figure 2-4). In comparisons between CF and WF tadpoles, the two species shared similar carbon resources based on tadpole $\delta^{13}C$ regardless of fertilization (Table 2-4). WF tadpoles, however, occupied a higher trophic level than co-occurring CF tadpoles in both fertilized and unfertilized tanks (Table 2-4), though differences were not statistically significant.

The comparisons between CF tadpoles and *Physa* snails revealed that CF tadpoles and snails fed at similar trophic levels, but CF tadpoles assimilated carbon sources that were significantly more depleted than snails regardless of WF presence or fertilization (Table 2-5).

Are trophic level and $\delta^{13}C$ affected by the pond environment?

To examine whether tadpole isotopes were influenced by pond environments, I examined the relationship of tadpole diet metrics and tadpole densities, and also diet metrics and pond environmental variables. CF and WF carbon and nitrogen ratios were significantly related within a pond ($\delta^{13}C$: $r = 0.84$, $\delta^{15}N$: $r = 0.77$, n = 25, $p < 0.001$ for both) reflecting a pond specific isotopic signature for tadpoles. I found no significant relationship between $\delta^{13}C$ and tadpole densities

for either species ($0.08 \leq r \leq 0.27$, $p > 0.20$). WF trophic level, on the other hand, increased with both CF and WF density such that it was significantly related to total tadpole density ($r = 0.36$, n = 23, $p = 0.10$). The difference (CF-WF) in tadpole trophic levels decreased with tadpole densities, and was significantly related to total tadpole density and to CF density ($r = -0.52$, $p = 0.01$ and $r = -0.58$, $p < 0.01$, respectively, n = 22). With respect to diet and pond environment, CF $\delta^{13}C$ increased with average daily pond temperature, whereas WF $\delta^{13}C$ showed little to no increase with temperature resulting in a significant relationship between the difference in $\delta^{13}C$ (CF – WF) and mean pond temperature (Table 2-6). There was also a weak significant positive relationship ($p = 0.08$) between CF $\delta^{13}C$ and pond hydroperiod (Table 2-6); a similar but non-significant relationship also existed for WF $\delta^{13}C$. There were no other significant relationships between pond variables (depth, TN, TP, and NH_4) and tadpole diet metrics (Table 2-6).

Evidence for competitors decreasing CF performance

Tadpole densities varied across years and patterns differed for the two species (Figure 2-5). CF tadpole density was significantly different across years ($F_{2,35} = 8.43$, $p = 0.001$) and was lowest in 2006 (Tukey's $p < 0.001$). Reduced CF tadpole densities in 2006 reflected lower CF calling ranks recorded at ponds than in 2005 (unpublished data). WF tadpole density also differed across years ($F_{2,35} = 17.12$, $p < 0.001$), increasing an order of magnitude over the three year study (0.009 to 0.123 tadpole/L). Increased WF density occurred despite greater number of egg masses at ponds in 2006 compared to 2007 (60.6 vs. 49.0 egg masses from five park ponds, *paired* $t_4 = 2.49$, $p = 0.07$; egg mass counts were not made in 2005). Some of the changes in tadpole density might reflect the reduction in pond area over the study period. Pond area was greatest in 2005 among park ponds compared to the

following years (of those measured each year, RM ANOVA $F_{2,10}$=13.49, p = 0.001, Tukey's p = 0.003 for both). Daily pond temperature, though tending to be lower in 2005 (mean across ponds within year, 2005: 14.6°C; 2006: 16.0°C; 2007: 16.3°C), did not differ significantly within ponds across the years (RM ANOVA $F_{2,14}$ = 1.77, p = 0.21). Pond hydroperiods were significantly longer in 2005 compared to both 2006 and 2007 (mean across ponds within year, 124 days in 2005 versus 91 days in both 2006 and 2007, RM ANOVA – $F_{2,16}$ = 22.23, p < 0.001, Tukey's p < 0.001 for both). The remaining environmental variables (Appendix Table 4-1) did not differ over the study period.

Growth rate

Mean tadpole growth rates (Figure 2-5) across study sites were generally similar across years for CF ($F_{2,29}$ = 0.04, p = 0.97) and WF ($F_{2,29}$ = 0.99, p = 0.39). Tadpole growth rates were significantly higher in WF compared to CF (Figure 2-5, t_{14} = 4.71, p < 0.001 when growth rates across years were pooled by pond). From the environmental variables measured and density estimates, only WF density affected CF growth significantly (Table 2-7). CF growth was negatively related to WF density and interspecific density had a stronger effect than intraspecific density (Table 2-7, Figure 2-6). WF growth rate did not show a reciprocal relationship, but rather was only significantly positively related to ammonia concentration (r = 0.42, p = 0.027, n = 27) and weakly to all other variables ($r \leq 0.17$).

Larval period

Mean CF larval periods (Figure 2-5) were shortest in 2007 (62.9 ±2.3 days), longer in 2006 (70.9 ±2.0 days) and significantly longer in 2005 than in 2007 (72.9 ± 3.4 days, $F_{2,27}$ = 4.15, p = 0.027, Tukey's p = 0.03). Pond environmental variables had

stronger effects than tadpole densities, as larval periods increased significantly with pond hydroperiod (Table 2-8 and Figure 2-7), and decreased significantly in warmer ponds (Table 2-8 and Figure 2-7). Temperature and hydroperiod were significantly related over the years (r = –0.45, p = 0.006, n = 36 pond-years). Relationships of other environmental variables and CF larval period were not significant. In addition to the effect of shorter hydroperiods and warmer temperature, greater tadpole densities also decreased larval periods, although tadpole densities increased with pond hydroperiod (r = -0.41, df = 36, p = 0.01; including all ponds across years). Of the density measures only the negative relationship between CF larval period and total tadpole density was significant (Table 2-8 and Figure 2-7).

Metamorph size

SUL of CF metamorphs differed significantly across years (Figure 2-5, $F_{2,21}$ = 3.39, p = 0.053) with greater sizes in 2005 and the smallest metamorphs in 2006. A reduction in size by 10.9% in 2006 relative to 2005 may be ecologically relevant as small size at metamorphosis is negatively related to the survival of metamorphs post emergence (Smith 1987, Boone 2005) and lifetime reproductive fitness (Smith 1987, Berven 1990). Increases in WF density had a significant, negative effect on CF metamorph size (Table 2-9, Figure 2-8). The effect of CF density on CF size at metamorphosis was in the opposite direction as WF density, although the former relationship was not significant (p > 0.50). The effects of total tadpole density were in the same direction as WF density, but were not significant (p > 0.4, Table 2-9, Figure 2-8). There were no significant relationships between pond environmental variables and CF size at metamorphosis (Table 2-9).

Does tadpole diet affect tadpole performance (growth, larval period or metamorph size)?

Again searching for patterns using correlations, I tested whether diets influenced growth and development. Firstly, CF growth rate was significantly (+) related to size at metamorphosis within a pond (r = 0.50, p = 0.02, from 22 pond-years), whereas CF larval period was not significantly related to either growth rate of larvae or size at metamorphosis though trends were negative for both ($p \geq 0.38$). With respect to correlations of tadpole performance and stable isotopes, there was a significant reduction in CF growth rate in ponds where CF or WF occupied higher trophic levels (r = -0.45, n = 25, p = 0.03 for both; Figure 2-9), suggesting that greater consumption of animal material coincided with lower CF growth. However, there were no comparable relationships for WF. With respect to carbon source, there were no significant correlations with tadpole growth. Larval period for CF increased with the difference in trophic levels between CF and WF tadpoles (r = 0.49, n = 19, p = 0.035), though larval period was no significantly related to either CF(+) or WF (–) trophic level on their own ($p \geq 0.50$). Size of CF at metamorphosis was significantly negatively related to CF trophic level (r = -0.52, n = 18, p = 0.027) and also with WF $\delta^{13}C$ (r = -0.56, n = 17, p = 0.021).

Evidence for spatial avoidance of competitors

To determine if CF tadpoles tended to avoid WF tadpoles or locations with higher densities of WF tadpoles, I examined the proportion of locations within natural ponds with only CF tadpoles present. About 23% of sites within any given pond were excluded due to the absence of tadpoles in sweeps (Figure 2-10, range 0-75). CF were absent from 36% of sample locations within a pond (range 0-87%). In ponds with WF, they were absent from 41% of sample locations (0-90%). I found no

evidence that CF tadpoles strictly avoided locations with WF tadpoles, as the proportion of sites with only CF differed significantly from complete avoidance in any year (Table 2-10). The proportion of sites within a pond with only chorus frogs decreased significantly with the density of wood frogs at ponds (based on pond-years Spearman r = - 0.86, $p < 0.001$, df = 34; Figure 2-10), such that chorus frog were rarely alone in 2007 when WF densities were highest (Figure 2-5). The proportion of total tadpoles in any given sample that were CF was 81.6% in 2005 but decreased in 2006 and 2007 to less than 50% (Table 2-10). This decrease and the lower proportion of locations within a pond with only CF present coincided with increases in WF density across the years (Figure 2-10), even though CF density in 2007 was similar to that in 2005 (Figure 2-5).

Discussion

Previous investigations of interactions between tadpoles of anuran species have inferred competition from changes in larval performance associated with the presence of another species (e.g. Wilbur and Alford 1985, Morin and Johnson 1988, Alford 1989b, Werner 1994, Skelly 1995). The reduction in performance was hypothesized to result from competition for food resources. High overlap in consumed resources may intensify competition and competitive hierarchies (Keddy 1990) may emerge based on tadpole size (Wilbur 1984). Greater sizes are related to greater resource acquisition rates (Seale and Wassersug 1979, Wassersug and Hoff 1979, Seale 1982), and may lead to interference behaviours (Persson 1985). My use of SIA to examine whether anuran tadpoles share foods in natural ponds and mesocosms is novel and complements my evaluation of performance. The results of my study are consistent with interspecific competition as tadpole species overlap in

trophic level and potentially in carbon source (food eaten), such that the two species may partition resources or consume the same resources while feeding from the same locations within a pond. Interspecific competition is also inferred from the reduced larval performance of CF in ponds in the presence of WF tadpoles and with greater densities of the second species.

Tadpoles exhibit diet overlap

The absence of significant species differences in fractionation for both $\delta^{13}C$ and $\delta^{15}N$, from feeding trials, suggests that overlap in isotopic signatures measured from ponds and mesocosm reflects real dietary overlap. Overlap in diet is necessary for resource competition to affect the performance of one or both species. Resource competition may result in a change in feeding niche in the presence of a competitor, when one species is more efficient or prevents access to a shared resource (Keddy 2001). In comparing CF isotopic values from mesocosms across the four treatments, I found CF tadpoles in unfertilized treatments were more enriched in $\delta^{13}C$ when WF were present, but WF $\delta^{13}C$ were similar to CF values, and suggests that food signatures changed as a result of WF presence. In comparing CF and WF values in mesocosms, there was a small difference in trophic level as WF fed at a higher trophic level in fertilized treatments. This may reflect greater availability and consumption of heterotrophic microorganisms which might result in $\delta^{15}N$ enrichment and a small increase in $\delta^{13}C$. Despite differences in trophic level, the two species utilized the same carbon resources in both treatments, and in unfertilized treatments also fed at a similar trophic level. Together the SIA evidence from mesocosms combined with reduced growth of CF in the presence of WF in

mesocosms (Chapter 3) suggests that the two tadpole species shared common foods in this experimental setting and is consistent with resource competition.

Though dietary overlap of tadpoles from ponds was weaker than seen in mesocosms, it was still suggestive of resource competition. Schiesari et al. (2009) observed similar patterns of $\delta^{13}C$ similarity across experimental venues. Schiesari et al. examined isotope overlap in two pairs of ranid tadpoles (*Lithobates sylvaticus* = WF with *L. pipiens* and *L. catesbeianus* with *L. clamitans*) and found that tadpoles from pond enclosures were more similar to each other than were those free-ranging tadpoles in the same ponds (species differences: 1.23‰ versus 3.18‰, and 0.12‰ versus 1.04‰ respectively). Carbon similarity of CF and WF tadpoles from my pond surveys (CF enriched by 1.02‰ relative to WF; mean absolute difference was 1.41‰ but ranged from 0.02-4.01‰, n = 25 pond-years) were similar to those observed by Schiesari et al. between *L. catesbeianus* and *L. clamitans*. Both species pairs used in Schiesari's trials have previously been demonstrated to be interspecific resource competitors based on reduced performance (growth and size at metamorphosis) of larvae from natural ponds (Werner 1994, Werner and Glennemeier 1999, Schiesari 2006). I believe that as CF and WF occupied similar trophic level values, $\delta^{15}N$, and $\delta^{13}C$ differences overlap the ranges observed by Schiesari et al., suggesting that the two species display resource partitioning, and that share some common foods. If the assumption that food limitation is correct, the two tadpoles likely compete. Further, as both CF and WF $\delta^{13}C$ and trophic level values showed strong correlations from natural ponds, it appears that the tadpole feeding niche is conserved, and that $\delta^{13}C$ differences among ponds reflect isotopic identity of each pond. Therefore, it seems that if CF and WF compete for resources, that they do so across a range of pond conditions and at varying intensities.

Why was dietary overlap greater in mesocosms than from natural ponds? The intensity of interspecific competition in mesocosms is generally greater than in replicated field enclosures (Skelly and Kiesecker 2001, Skelly 2002). Greater local densities and reduced spatial heterogeneity in mesocosms increase the potential for resource competition. Other factors that might create differences amongst venues are differences in resources availability and diversity. For example, the abundance of attached algal resources was likely lower in mesocosms because surface area available for algal colonization was lower in mesocosms without physical structure compared to most natural ponds. In natural ponds I found that the difference in $\delta^{13}C$ was on average 1‰ (0 to 4‰), whereas in mesocosms the difference was within the error of replicated samples resulting in no difference between the species. Further, I found that even after correcting $\delta^{13}C$ across venues, tadpoles from ponds were significantly more depleted in $\delta^{13}C$. The similarity of tadpole $\delta^{13}C$ and a significant enrichment in ^{13}C in mesocosms may reflect the lack of diversity (Schiesari et al. 2009) or availability of food resources in these artificial systems and may have contributed to the degree of overlap in tadpole SIA diets.

Resource partitioning with respect to carbon source, $\delta^{13}C$, may reflect species differences in their ability to obtain, ingest and assimilate resources. If the source of competition is periphyton (such as epiphyton, epipelon, epilithon) species may differ in their ability to scrape particles from these surfaces (Wassersug and Yamashita 2001) or to filter particles dislodged by grazing or that become suspended (e.g. Seale and Wassersug 1979). In this fashion, the two species may feed from the same types of sites, but collect different proportions of the multi-species community. Grazing also depletes resources used by the other species which contributes to interspecific competition. Another mechanism that may contribute to

species differences in $\delta^{13}C$, is that WF tadpoles respond to competition by elongating their guts, prolonging food passage time (Relyea and Auld 2004), which allows them to extract more nutrition and may result in greater fractionation or absorption of resources with depleted carbon. I am unaware of reports of comparable increases in gut length for CF tadpoles. However, reduced CF growth and size at metamorphosis in the presence of WF, as discussed below, and the absence of a high degree of resource overlap (based on $\delta^{13}C$) in natural ponds may indicate that some mechanism other than exploitative resource competition is responsible for the reduced performance.

WF reduce CF tadpole performance

Of the three performance metrics measured, growth rate and size at metamorphosis showed clear negative relationships with WF density. Duration of larval period, however, showed a much stronger relationship with aspects of the pond environment than with tadpole densities. I will first discuss larval period, then size at metamorphosis and finally growth rate.

CF larval period was affected by pond temperature (–), hydroperiod (+) and, to a lesser extent by total (CF+WF) tadpole density (–); these factors increase the risk of death and decrease gains associated with remaining in a deteriorating, drying environment (Wilbur and Collins 1973). Greater temperatures and shorter hydroperiods in 2007 resulted from ponds being significantly smaller and shallower at snow melt than in 2005. Weak effects of CF and WF tadpole densities separately translated into a significant effect of total tadpole density. Morin and Johnson (1988) in contrast found that the presence and density of WF increased the larval period of *Pseudacris crucifer* in mesocosms, however, they used constant water volumes.

Tadpoles in ponds, however, generally respond to the threat of pond drying (e.g. Rowe and Dunson 1995), which may increase competition or reduce food quality, which tadpoles may respond to by increasing their development rate (Wilbur and Collins 1973). If similarity in tadpole trophic level is an indication of increased resource overlap, then in my study ponds competition may explain reduced CF larval periods as differences in trophic levels between CF and WF decreased with tadpole densities. CF larvae responded to changes in pond environments in 2007 by increasing development rates and left ponds 8-13 days sooner than in previous years (*paired* $t_5 = 2.33$, $p = 0.067$ vs. 2005 and $t_6 = 6.01$, $p < 0.001$ vs. 2006) likely owing to increased tadpole density, pond temperature, and reduced hydroperiods.

I found that CF size at metamorphosis was significantly affected by WF tadpole density. Morin and Johnson (1988) also found significant reductions of 67% in mass at metamorphosis (which is correlated with SUL) for *Pseudacris crucifer* in WF treatments. In my field study, SUL in *P. maculata* was reduced by 11 and 13% at the two WF densities used by Morin and Johnson; percent reduced was calculated by regressing chorus frog SUL on measured WF density from my survey ponds. Direct comparisons between my study and those involving *P. crucifer* are difficult as the target species differs and the other studies used mass at metamorphosis, whereas I used length. The absence of an effect of intraspecific density on metamorph size for CF in my study was surprising as Smith (1983, 1990) has demonstrated that size at metamorphosis is significantly affected by chorus frog density (*P. maculata*, formerly *P. triseriata* sensu Lemmon et al. 2007); though maximum density in Smith (1983, 1990) was an order of magnitude higher than the highest chorus frog density observed in my ponds. There were no significant effects of pond environmental factors on size at metamorphosis, even though hydroperiod has previously been

shown to affect size at metamorphosis (Wilbur and Collins 1973). However, size at metamorphosis was significantly positively related to CF larval growth rates which decreased with WF densities. As size at metamorphosis is strongly correlated to adult fitness in anurans (Smith 1987, Berven 1990), reduced growth rates and smaller metamorphs due to competition with WF tadpoles could reduce survival of terrestrial CF frogs, delay the onset of sexual maturity and reduce total reproductive output of both individuals and ultimately the pond population. These reductions could lead to smaller CF population sizes, and if combined with other factors like predation and poor recruitment due to pond drying or disease, competition with WF could lead to the extirpation of small populations such as seen for *P. triseriata* populations introduced to Newfoundland (Maunder 1983, 1997).

CF growth rates responded more strongly to tadpole densities than to environmental variables, with WF densities decreasing CF growth more than intraspecific densities. Intraspecific density did decrease CF growth rates, but relationships were not significant within years, and in 2005 CF growth increased with CF density (also when WF densities were lowest). Sours and Petranka (2007) similarly found no intraspecific effect of *Pseudacris feriarum* density on its own growth rate across the density range found within my study ponds. This is in contrast to work by Smith (1983, 1990) who showed that *P. triseriata* growth decreased with intraspecific density (0.01-5.25 tadpoles/L). The absence of a reciprocal relationship in my study where WF growth was affected by CF density suggests some form of asymmetric interaction between the two species as tadpoles. WF tadpoles are larger and size advantages (Seale and Wassersug 1979, Wassersug and Hoff 1979, Seale 1982) may account for the greater effect of interspecific over intraspecific density on CF growth. Despite the observed relationships between

growth and density, growth rates of the two species in each pond were positively correlated, suggesting a linkage to resource quality or availability or that a good environment for CF is also good for WF. If trophic level is an indication of tadpole food availability or quality, higher trophic levels appear to signal lower abundance of foods available as CF tadpole growth rate decreased with tadpole trophic level. Starvation, carnivory and necrophagy may be associated with higher tadpole trophic levels and may explain the relationships between CF tadpole growth (–), trophic level (+), and densities. Tissue $\delta^{15}N$ content may increase, raising trophic level, as a result of starving tadpoles metabolizing their own tissues or tadpoles resorting to greater levels of carnivory or in cases of high mortality, necrophagy. The patterns of reduced growth at higher competitor densities and indications that food was limiting, evidenced by shifts in trophic level, are consistent with the operation of interspecific competition.

Studies of competition between hylids and ranids generally find hylid growth rates decrease with ranid density (but see Smith et al. 2004). For example, Sours and Pertranka (2007) found that *Pseudacris feriarum* growth (final mass) decreased by 15% and 37% in high and low food treatments when WF was present versus absent (at a WF density of 0.283 tadpole/L). Based on the linear regression of CF growth on WF density, I observed a 30% decrease, on average, in CF growth rates (change in length) in the presence of WF. Using data on change in length and change in mass from mesocosms (described here and in Chapter 3) this translates to about a 21% reduction in change in mass per unit time which is within the range observed by Sours and Petranka (however, regression between change in mass on change in length was marginally significant $p = 0.08$, $R^2 = 0.43$). Morin and Johnson (1988), also found a significant effect of interspecific competition at WF densities within the

range observed in my study, but in contrast *P. crucifer* growth rates (change in mass) were reduced by 75-80% in the presence of WF within mesocosms. Skelly (1995), on the other hand, found little evidence of interspecific competition between *P. crucifer* and *Rana clamitans,* as *P. crucifer* growth rate (measured as mm/day) appeared unaffected by increasing ranid density within field enclosures. Faragher and Jaeger (1998), in a laboratory setup, observed up to an 80% decrease in *Hyla versicolor* tadpole growth (final mass) when this species directly interacted with *R. utricularia* tadpoles. However, when *R. utricularia* tadpoles were separated from *H. versicolor* tadpoles by a screen, *H. versicolor* growth was only reduced by 25%. Faragher and Jaeger (1998) explained the reduced performance of *H. versicolor* as a product of chemical interference in the second example and a combination of chemical and agonistic interference in the full interaction treatment. The intensity of competition within mesocosms may be greater, relative to that in natural ponds, due to lower diversity and/or abundance of resources available, the choice of species, and the stages at which competitors are introduced (Skelly and Kiesecker 2001, Skelly 2002). The reduced growth of CF at higher WF densities is similar to reductions seen in other studies that have inferred interspecific competition between hylids (and specifically *Pseudacris* species) with WF and other ranids.

CF tadpoles do not avoid WF tadpoles

I found that CF and WF occupied the same sites within a pond, with no evidence that agonistic behaviours caused one species to avoid the other. However, sweeping for tadpoles using a dipnet prevents recognition of species choosing different microhabitats, depths or temperatures within a sample location. If behavioural interference is responsible for the reduction in CF growth rates with increasing tadpole density, it does not appear to alter the spatial distribution of CF

tadpoles relative to WF tadpoles within a pond. Faragher and Jaeger (1998) reported that ranid tadpoles were aggressive toward *H. versicolor*, but I found no evidence of aggression in mesocosms or aquaria (personal observation). Sampling to determine spatial distribution was only done once per pond in any given year, and would not have captured daily and seasonal changes in tadpole distributions. Regardless, it may be that both CF and WF prefer the same locations because food conditions or environmental conditions are optimal for growth, and the cost of abandoning these areas for suboptimal ones may outweigh the costs of directly interacting with the other species. This is evident as tadpoles were not present at all locations within a pond, suggesting that they may have been clumped where resources availability was greatest (Figure 2-10). Of the 324 total locations sampled, 25% lacked tadpoles of either species. Such locations might also have been avoided because of abiotic conditions, such as water depth or temperature. The overlapping distribution of larvae of both species within ponds might have heightened resource competition and does not preclude the existence of chemical interference, either of which could affect the performance of CF.

Does competition exist between CF and WF?

I recorded several patterns consistent with the operation of interspecific competition between CF and WF. The outcome of competition appeared to be asymmetric with CF tadpoles experiencing reduced growth rates and smaller size at metamorphosis, whereas WF growth rates were unaffected by CF tadpoles. The reductions in CF growth rate and size at metamorphosis are comparable to responses reported by other studies inferring competition between anuran larvae. SIA of tadpole tissues suggests the operation of resource partitioning, but if both species rely upon similar resources, such as biofilms, then competition for the

undisturbed resource may occur such that disturbed surfaces may no longer provide adequate nutrition until they recover. If the differences in $\delta^{13}C$ are a result of differences in foods consumed, due to differences in oral morphology, and not due to differences in assimilation of foods, then the mechanism that best explains the competitive patterns and reduced CF tadpole performance metrics is interference competition. However, the absence of spatial segregation of tadpoles of the two species suggests either that agonistic behaviours by the larger WF tadpole may not occur or are rare, or that other interference mechanisms operate in ponds. One mechanism that might result in reduced growth and size at metamorphosis of CF, but no change in tadpole spatial distribution, is chemical interference. Faragher and Jaeger (1998) found similar reduction in tadpole growth occurred not as a result of resource exploitation but due to chemical interference. The evidence for chemical interference by anuran larvae in natural ponds is equivocal (Petranka 1995), but the patterns observed in my study are consistent with effects of chemical inhibitors released by WF tadpoles. In order to determine if WF affect CF performance in the absence of resource limitation or direct interactions, and whether evidence exists for chemical interference under these conditions, I performed the laboratory experiments described in Chapter 5.

Table 2-1: Comparisons of the two labs where stable isotope analysis was completed.

	University of Alberta BGCA	University of Saskatchewan SIF
Mass Spectrometer	Continuous flow GV Instruments IsoPrime	Tracer /20; Delta V[b]
Elemental Analyzer	Eurovector Euro EA 3000	ANCA G/S/L; Costech ECS4010[b]
Standard for Carbon	IAEA-CH-3 IAEA-CH-6 IAEA-CH-7	IAEA-CH-6
Standard for Nitrogen	IAEA-N-2 IAEA-NO-3 USGS 34	IAEA-N-1 IAEA-N-2 IAEA-NO-3 USGS 24
Reference material		
Animal	Chicken egg albumin	Chicken egg albumin
Plant	Peach leaves	Yellow field pea seed
Sediment	High organic sediment[a]	Yellow field pea seed
95% Confidence of $\delta^{13}C$		
Animal	0.20‰	0.29‰
Plant	0.27‰	0.14‰
Sediment	0.30‰	0.14‰
95% Confidence of $\delta^{15}N$		
Animal	0.35‰	0.33‰
Plant	0.43‰	0.17‰
Sediment	0.38‰	0.17‰

Note that confidence intervals from University of Alberta Biogeochemical Analytical Laboratory (BGCA) are reported based on several years of analysis, whereas those for the University of Saskatchewan Stable Isotope Facility Laboratory (SIF) are based on submitted reference material[c].

[a] Cat. No. B2151, batch No. 2824 Elemental Microanalysis Ltd.

[b] For analysis conducted on animal and plant/sediment tissues respectively

[c] Reference samples for animal tissue n = 64, and plant and sediment n = 43

Table 2-2: Summary of tadpole fractionation of $\delta^{13}C$ and $\delta^{15}N$ for both chorus frog and wood frog fed rabbit chow and spirulina pellets. Mean isotopic and fractionation values are given ± 1 SE.

		Species $\delta^{13}C$		**Species $\delta^{15}N$**	
Diet		Chorus Frog	Wood Frog	Chorus Frog	Wood Frog
Rabbit Chow	Tadpole	-25.55 ± 0.08	-25.11 ± 0.15*	4.31 ± 0.03	4.36 ± 0.14*
	Food	-27.05		1.71	
	Fractionation	1.50 ± 0.08	1.94 ± 0.15*	2.60 ± 0.03	2.66 ± 0.14*
Spirulina	Tadpole	-21.17 ± 0.25	-21.24 ± 0.22	9.83 ± 0.07	10.42 ± 0.32
	Food	-22.94		7.65	
	Fractionation	1.77 ± 0.25	1.70 ± 0.22	2.18 ± 0.07	2.77 ± 0.32

Source of variation	Significance test	Significance test
Species	$F_{1,3} = 0.73$, $p = 0.46$	$F_{1,3} = 2.35$, $p = 0.22$
Food	$F_{1,3} = 0.002$, $p = 0.97$	$F_{1,3} = 0.57$, $p = 0.51$
Species × Food	$F_{1,3} = 1.39$, $p = 0.32$	$F_{1,3} = 1.62$, $p = 0.29$
Mean isotopic fractionation	**1.70 ± 0.09**	**2.54 ± 0.12**

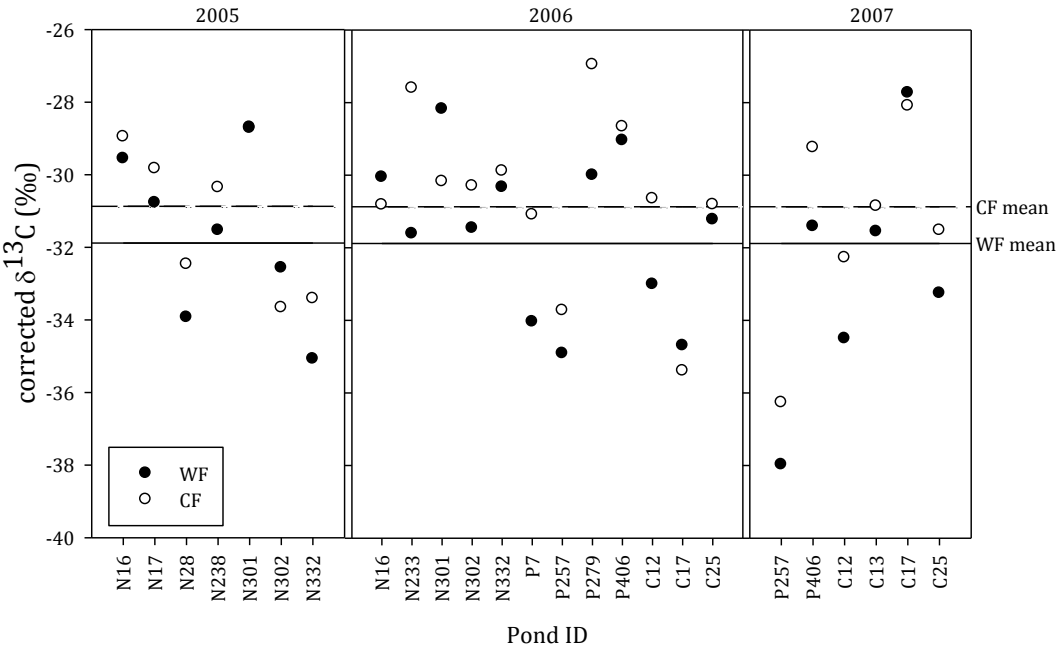


Figure 2-1: Relationship between chorus frog (CF) and wood frog (WF) $\delta^{13}C_{corrected}$ in natural ponds. Only ponds sampled from June in each year were used in comparisons.

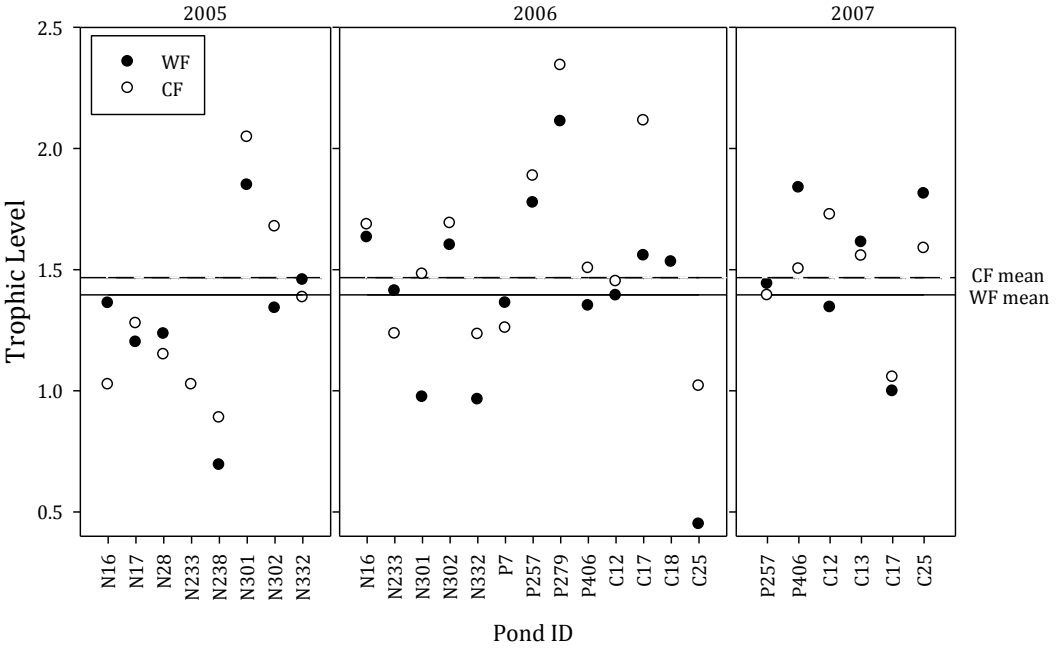


Figure 2-2: Relationship between chorus frog (CF) and wood frog (WF) trophic level across natural ponds. Only ponds sampled from June in each year were used in comparisons.

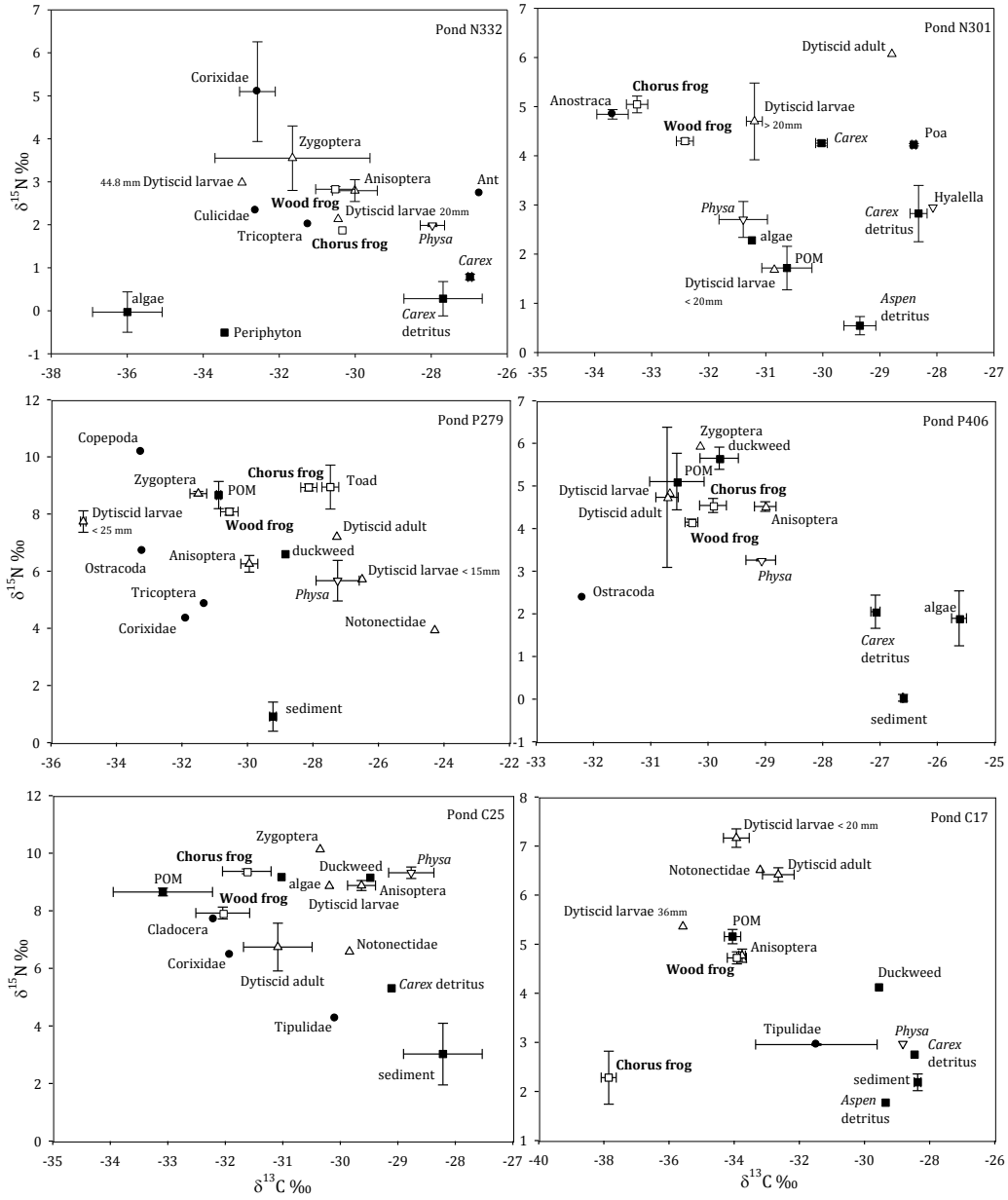


Figure 2-3: Carbon (*x*-axis) and nitrogen (*y*-axis) isotopic signatures of representative organisms and substrates in selected pond food webs. Upper plots are from park ponds (2005), middle plots from pasture ponds (2006), and bottom plots from crop ponds (2006). Open square represent tadpoles: boreal chorus frog, wood frog, western toad (Toad). Inverted triangles represent reference herbivores generally snails (*Physa*), or *Hyallela azteca*. Triangles represent different potential tadpole predators. Filled squares represent primary producers and other potential food sources such as plant based detritus and particulate organic matter (POM) collected from the water column. Algal sources were mainly filamentous green alage. Filled circles represent other animals. Points represent means ± 1 SE for samples; for animals points represent 1-5 samples of which each sample could be composed of many individuals (such as for ostracods). For macrophytes and other material such as algae and detritus multiple samples were homogenized into one or two samples.

Table 2-3: Analysis of chorus frog $\delta^{13}C$ and Trophic Position across wood frog (WF) and fertilizer (FERT) treatments from 16 mesocosms. Only the results of the repeated measures ANOVA for carbon are shown as there were significant interactions between sampling date and treatments.

Source of Variation	F	df	P - value
Chorus frog $\delta^{13}C$			
WF	3.63	1, 4	0.13
FERT	0.18	1, 4	0.69
WF × FERT	4.41	1, 4	0.10
Date	0.042	1, 4	0.85
Date × WF	6.38	1, 4	0.065
Date × FERT	5.90	1, 4	0.072
Date × WF × FERT	0.034	1, 4	0.86
Chorus frog Trophic Position			
WF	2.562	1, 4	0.185
FERT	1.297	1, 4	0.318
WF × FERT	0.307	1, 4	0.609

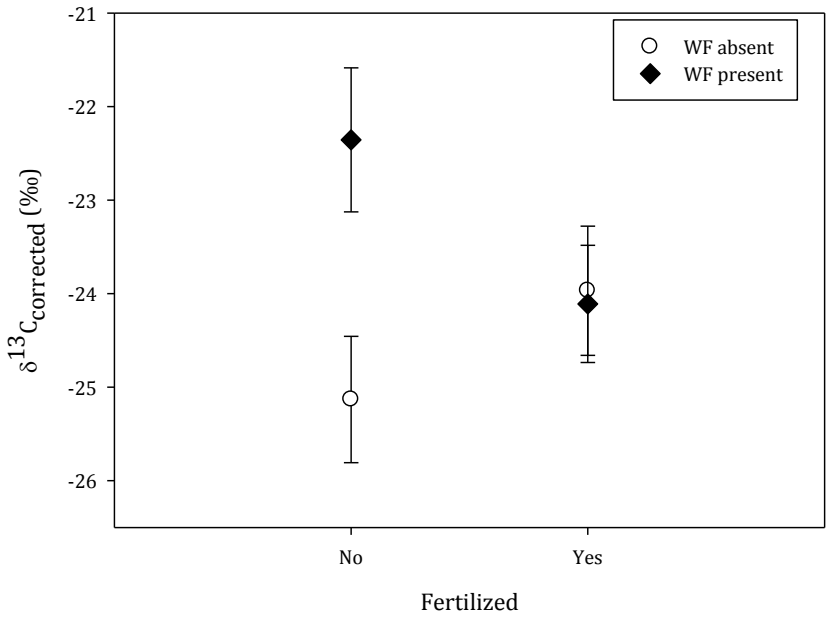


Figure 2-4: Effect of fertilization and wood frog (WF) presence on chorus frog carbon isotopic signature in mesocosms. Tadpole $\delta^{13}C$ was corrected using *Physa sp*. snails as the baseline reference.

Table 2-4: Summary of tadpole stable isotope diet comparison between chorus frog and wood frog from fertilized (Fert) and unfertilized (noFert) mesocosms using a split-plot ANOVA with species (chorus vs. wood frog) and fertilization (FERT) as fixed effects with replicate mesocosms (Tank, n = 2) as the split-plot.

Treatment	Species $\delta^{13}C_{corrected}$		Species Trophic Position	
	CF	WF	CF	WF
Fert + WF	-24.11 ± 0.63	-23.66 ± 0.63	1.07 ± 0.14	1.46 ± 0.31
noFert + WF	-22.36 ± 0.77	-22.58 ± 0.39	0.93 ± 0.08	1.16 ± 0.06

Source of Variation	df	MSE	*F*	*P* - value	df	MSE	*F*	*P* - value
Species	1	0.025	0.36	0.61	1	0.195	8.00	0.11
Species × FERT	1	0.228	3.24	0.21	1	0.014	0.56	0.53
Species × Tank(FERT)	2	0.070			2	0.024		
FERT	1	3.990	2.73	0.24	1	0.097	0.95	0.43
Tank(FERT)	2	1.460			2	0.102		

Table 2-5: Summary of stable isotope diet comparison between chorus frog tadpoles and *Physa* sp. from fertilized (Fert) and unfertilized (noFert) mesocosms with wood frogs present (+ WF) or absent (CF) using a split-plot ANOVA with species (chorus vs. *Physa*), fertilization (FERT) and wood frog presence (WF) as fixed effects and replicate mesocosms (Tank, n = 2) as the split-plot.

Treatment	Species $\delta^{13}C_{actual}$		Species Trophic Position	
	CF	*Physa* sp.	CF	*Physa* sp.
noFert CF	-25.04 ± 0.86	-22.69 ± 0.18	1.20 ± 0.28	1.09 ± 0.45
noFert + WF	-23.05 ± 1.35	-23.48 ± 0.58	1.29 ± 0.12	1.47 ± 0.09
Fert CF	-23.29 ± 0.89	-22.11 ± 0.20	1.50 ± 0.30	1.11 ± 0.39
Fert + WF	-24.19 ± 0.34	-22.86 ± 0.28	1.34 ± 0.08	1.17 ± 0.26

Source of Variation	df	MSE	*F*	*p*	df	MSE	*F*	*p*
Species	1	4.884	10.13	0.03	1	0.063	1.28	0.32
Species × FERT	1	0.086	0.18	0.70	1	0.096	1.96	0.23
Species × WF presence	1	1.742	3.61	0.13	1	0.065	1.33	0.31
Species × WF × FERT	1	2.124	4.41	0.10	1	0.002	0.03	0.87
Species × Tank(WF × FERT)	4	0.482			4	0.049		
WF	1	0.815	0.54	0.50	1	0.002	0.01	0.94
FERT	1	0.051	0.03	0.86	1	0.034	0.13	0.74
WF × FERT	1	2.024	1.35	0.31	1	0.078	0.30	0.62
Tank(WF × FERT)	4	1.500			4	0.266		

Table 2-6: Correlation and regression results of chorus frog (CF) and wood frog (WF) tadpole diet metrics as determined by SIA to pond environmental variables.

	$\delta^{13}C$			Trophic Level		
Variable	CF	WF	(CF-WF)	CF	WF	(CF-WF)
Temperature	0.31	0.05	0.50*	0.24	0.37†	-0.29
Hydroperiod	-0.36†	-0.30	-0.07	-0.08	-0.18	0.12
Depth	-0.05	-0.14	0.23	0.29	0.29	0.01
Total Nitrogen	0.22	0.28	-0.16	-0.26	-0.14	-0.15
Total Phosphorous	0.22	0.13	0.08	-0.28	-0.12	-0.20
Ammonium	0.21	-0.03	0.36	-0.07	-0.09	0.02

† $p \leq 0.10$; * $p \leq 0.05$; ** $p \leq 0.01$

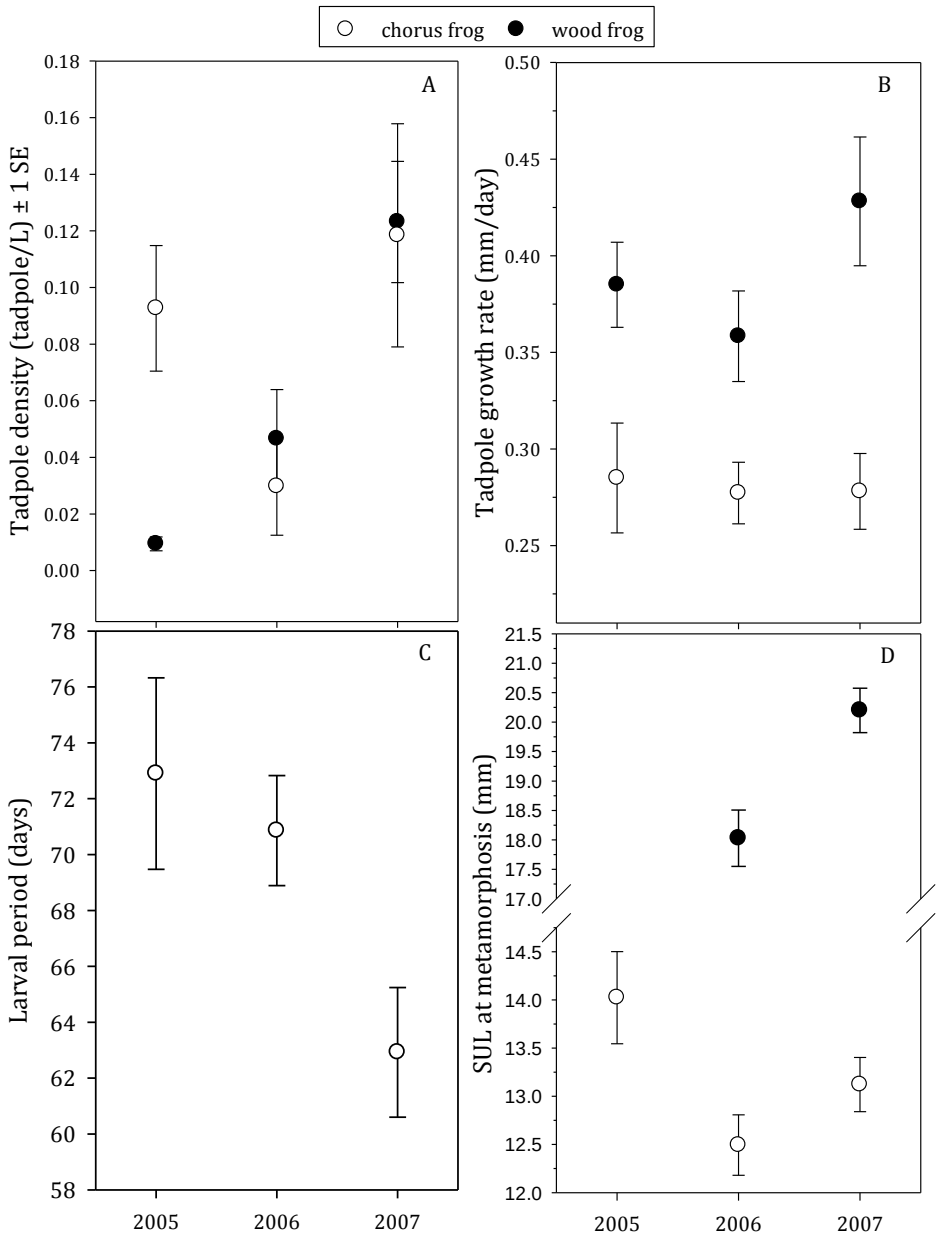


Figure 2-5: Mean annual variation ± SE for boreal chorus frog and wood frog tadpoles in density A), tadpole growth rates B), larval period C), and mean metamorph snout-to-urostyle length (SUL) D) from ponds in the Beaver Hills region of Alberta. Larval period and the 2005 metamorph SUL for wood frogs were not measured.

Table 2-7: Results of regressions analyses of boreal chorus frog tadpole growth rate in natural ponds on selected variables. Tadpole densities, chorus frog (CF) and wood frog (WF) were measured per litre. TAD = mean total tadpole density within ponds (CF+WF). Environmental variables are defined in text, with TN, TP and NH_4 measured in µg/L and depth in cm, with the appropriate transformation (trans) to satisfy normality, Pearson's correlation (*r*) and regression statistics are noted for each variable.

	Variables	*trans*	*r*	*F*-value	df	*p*	β ± se	*t*	*p*	α ± se
density	WF/L	$x^{1/4}$	-0.36	3.91	1,26	0.06	-0.128 ± 0.065	-1.97	0.06	0.340 ± 0.030
	CF/L	$x^{1/4}$	-0.26	1.89	1,26	0.18	-0.116 ± 0.082	-1.37	0.18	0.338 ± 0.041
	TAD/L	$x^{1/4}$	-0.31	2.82	1,26	0.11	-0.138 ± 0.083	-1.68	0.11	0.364 ± 0.049
Environmental variables	Temperature	x	0.06	0.15	1,27	0.70	0.003 ± 0.007	0.39	0.70	0.238 ± 0.117
	Hydroperiod	x	-0.02	0.53	1,30	0.47	0.000 ± 0.001	-0.73	0.47	0.325 ± 0.063
	Depth	√x	-0.06	0.03	1,28	0.86	-0.002 ± 0.012	-0.18	0.86	0.294 ± 0.069
	TN	ln(x)	0.21	0.05	1,23	0.83	0.003 ± 0.011	0.22	0.83	0.261 ± 0.079
	TP	ln(x)	0.08	0.82	1,23	0.37	0.021 ± 0.023	0.91	0.37	0.106 ± 0.189
	NH_4	ln(x)	0.43	1.86	1,23	0.19	0.016 ± 0.012	1.36	0.19	0.213 ± 0.049
	WF growth	x	0.37	4.65	1,29	0.04	0.236 ± 0.109	2.16	0.04	0.185 ± 0.043

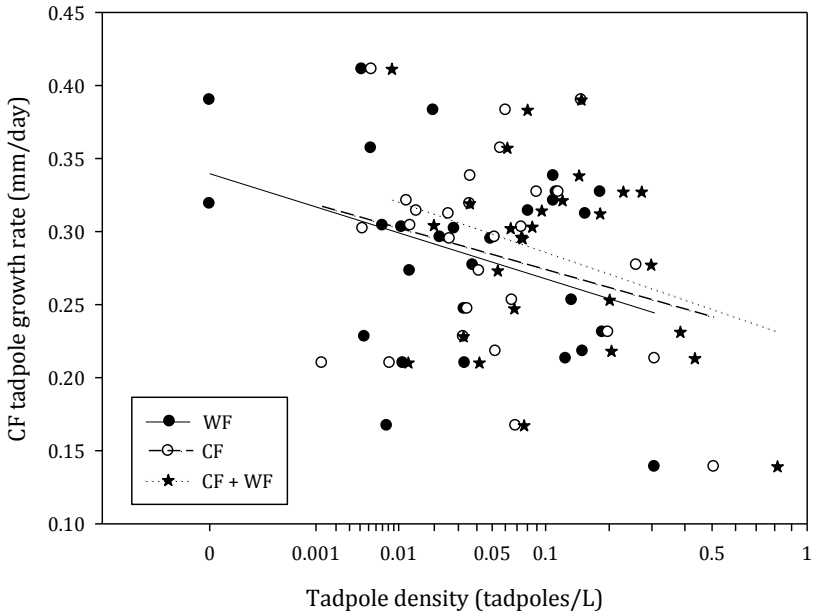


Figure 2-6: Relationships between chorus frog growth rate and mean tadpole densities of wood frogs (WF), chorus frogs (CF) and chorus frog + wood frog (CF + WF).

Table 2-8: Results of regression analyses of boreal chorus frog larval period in natural ponds on selected variables. Tadpole densities, chorus frog (CF) and wood frog (WF) were measured per litre. TAD = mean total tadpole density within ponds (CF+WF). Environmental variables are defined in text, with TN, TP and NH_4 measured in µg/L and depth in cm, with the appropriate transformation (trans) to satisfy normality, Pearson's correlation (*r*) and regression statistics are noted for each variable.

	Variables	*trans*	*r*	*F*-value	df	*p*	β ± se	*t*	*p*	α ± se
density	WF/L	$x^{1/4}$	-0.32	2.67	1,24	0.11	-15.8 ± 9.6	-1.64	0.11	74.7 ± 4.6
	CF/L	$x^{1/4}$	-0.26	1.80	1,24	0.19	-19.0 ± 14.2	-1.34	0.19	77.7 ± 7.6
	TAD/L	$x^{1/4}$	-0.36	3.59	1,24	0.07	-25.7 ± 13.6	-1.90	0.07	83.5 ± 8.4
Environmental variables	Temperature	x	-0.54	9.38	1,23	0.01	-3.0 ± 1.0	-3.06	0.006	116.1 ± 15.7
	Hydroperiod	x	0.63	17.85	1,27	0.00	0.3 ± 0.1	4.22	0.000	33.7 ± 8.3
	Depth	√x	0.01	0.01	1,25	0.94	0.2 ± 2.7	0.071	0.94	67.1 ± 15.0
	TN	ln(x)	-0.24	1.14	1,18	0.30	-3.6 ± 3.4	-1.07	0.30	95.0 ± 27.6
	TP	ln(x)	-0.36	2.66	1,18	0.12	-2.8 ± 1.7	-1.63	0.12	85.6 ± 12.3
	NH_4	ln(x)	-0.19	0.67	1,18	0.43	-1.6 ± 1.9	-0.82	0.43	72.4 ± 8.4

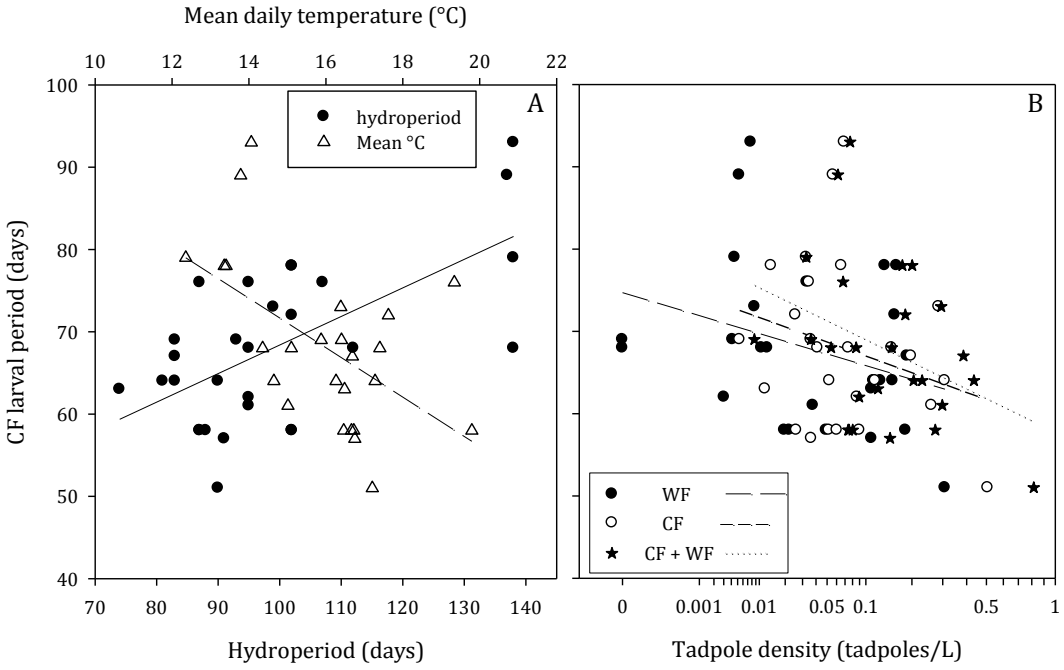


Figure 2-7: Relationship between chorus frog (CF) larval period and A) pond hydroperiod (lower axis, solid line) and mean daily pond temperature (upper axis, dashed line) from natural ponds pooled across years. B) Relationship between chorus frog larval period and wood frog, chorus frog and total tadpole density and associated regression lines.

Table 2-9: Results of regression analyses of boreal chorus frog size at metamorphosis from natural ponds on selected variables. Tadpole densities, chorus frog (CF) and wood frog (WF) were measured per litre. TAD = mean total tadpole density within ponds (CF+WF). Environmental variables are defined in text, with TN, TP and NH_4 measured in µg/L and depth in cm, with the appropriate transformation (trans) to satisfy normality, Pearson's correlation (*r*) and regression statistics are noted for each variable.

	Variables	*trans*	*r*	*F*-value	df	*p*	β ± se	*t*	*p*	α ± se
density	WF/L	$x^{1/4}$	-0.45	5.06	1,20	0.04	-2.72± 1.21	-2.25	0.04	14.5 ± 0.6
	CF/L	$x^{1/4}$	0.03	0.01	1,20	0.91	0.25 ± 2.27	0.11	0.91	13.2 ± 1.2
	TAD/L	$x^{1/4}$	-0.19	0.73	1,20	0.40	-1.75 ± 2.04	-0.85	0.40	14.4 ± 1.3
Environmental variables	Temperature	x	-0.35	2.56	1,19	0.13	-0.25 ± 0.16	-1.60	0.13	17.1 ± 2.4
	Hydroperiod	x	0.27	1.66	1,22	0.21	0.01 ± 0.01	1.29	0.21	11.9 ± 1.2
	Depth	√x	0.25	1.41	1,21	0.25	0.40 ± 0.34	1.19	0.25	11.1 ± 1.9
	TN	ln(x)	-0.18	0.45	1,13	0.51	-0.28 ± 0.42	-0.67	0.51	15.2 ± 3.4
	TP	ln(x)	-0.19	0.50	1,13	0.49	-0.17 ± 0.24	-0.71	0.49	14.1 ± 1.7
	NH_4	ln(x)	0.24	0.82	1,13	0.38	0.23 ± 0.26	0.91	0.38	12.0 ± 1.1

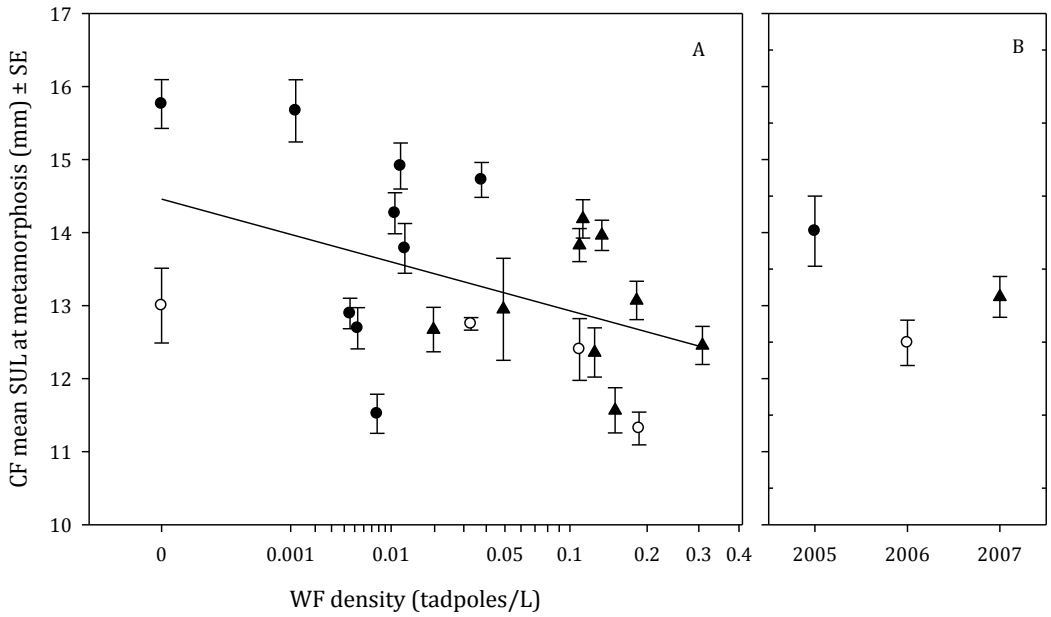


Figure 2-8: Relationship of snout-to-urostyle length (SUL) of recently metamorphosed chorus frog (CF) larvae from individual ponds to respective wood frog (WF) tadpole density A) and mean SUL across all ponds in a given year B). Symbols in A are as in B, with nine ponds in 2005, five ponds in 2006 and 10 ponds in 2007.

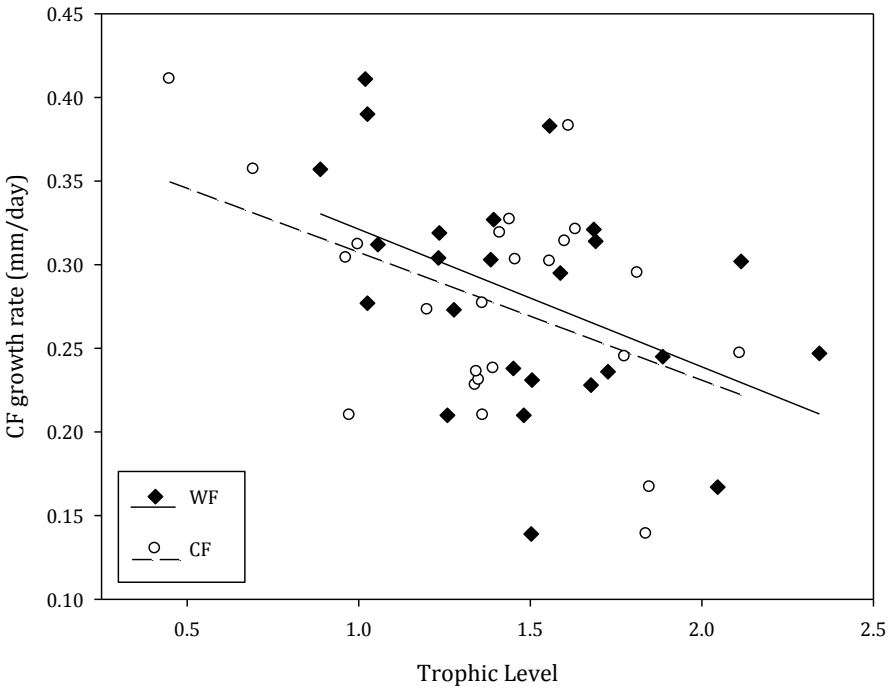


Figure 2-9: Relationship between growth rate and trophic level of chorus frog (CF) and wood frog (WF) tadpolesfrom 15 natural ponds. Trophic level is based on tadpole $\delta^{15}N$ relative to *Physa* $\delta^{15}N$ divided by the tadpole fractionation of ^{15}N.
Dashed line : CF growth = -0.076 (CF trophic level) + 0.384, $F_{1,23} = 5.77$, $p = 0.025$
Solid line : CF growth = -0.082 (WF trophic level) + 0.403, $F_{1,22} = 5.64$, $p = 0.027$

Table 2-10: Summary of spatial distribution of chorus frog (CF) tadpoles in relation to wood frog (WF) tadpoles across sites within natural ponds of the Beaver Hills region of Alberta. I excluded ponds where only one species was found during the density estimates.

Year	Mean % of samples with CF alone	Ponds sampled (# CF only)	CF avoid WF (one sample t-test μ = 1)		CF always with WF (one sample t-test μ = 0)		Mean % tadpoles that were CF in a sample
			t	*p*	*t*	*p*	
2005	53.9 ± 6.2	14 (2)	-11.26	< 0.001	12.57	< 0.001	81.6 ± 4.0
2006	22.8 ± 7.3	11 (1)	-10.94	< 0.001	4.19	0.003	34.8 ± 7.0
2007	8.8 ± 5.3	12 (0)	-16.40	< 0.001	1.81	0.098	43.9 ± 6.6

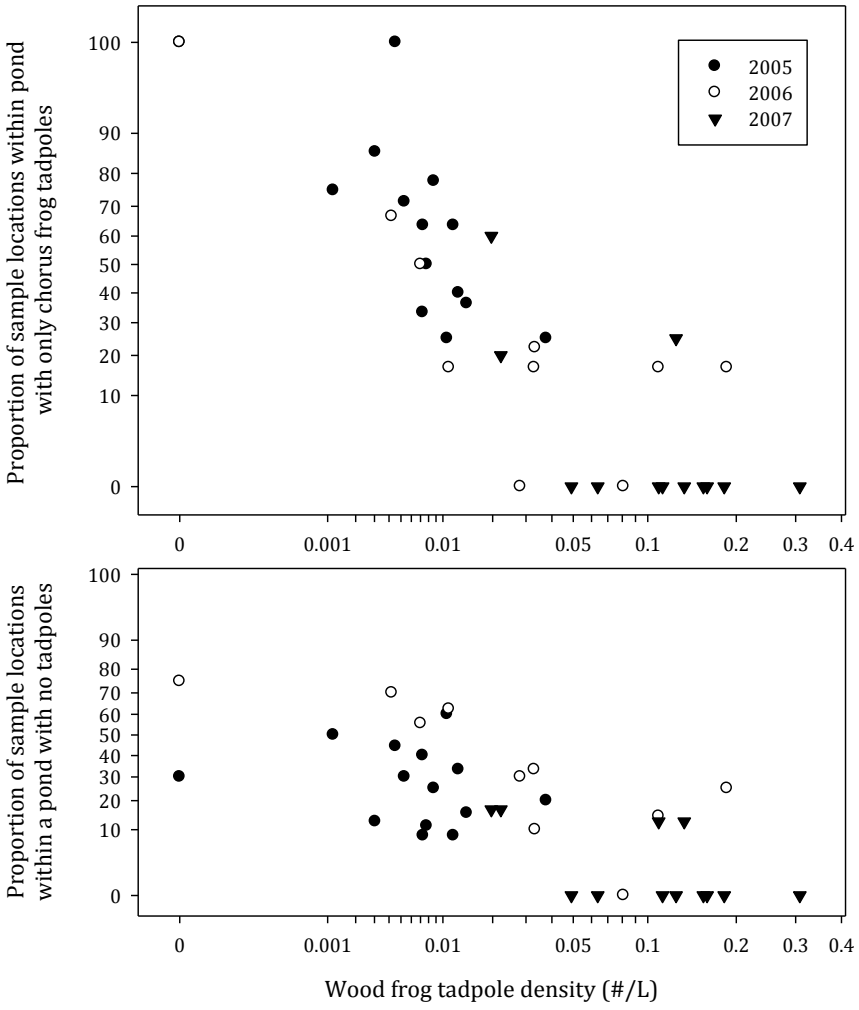


Figure 2-10: Relationship between percent of sample locations within each pond (5-16 samples per pond) that contained only chorus frog tadpoles (upper pane) compared to the mean density of wood frog tadpoles in those ponds (n = 10-14 ponds per year) and the proportion of locations with no tadpoles in sweeps (lower pane). Note locations with no tadpoles are not included in the calculations in the upper pane.

Literature Cited

Alford R. A. 1999. Ecology: resource use, competition, and predation. Pages 240-278 *In* R. W. McDiarmid, editor. Tadpoles: biology of anuran larvae, University of Chicago Press, Chicago.

Alford R. A. 1989a. Effects of parentage and competitor phenology on the growth of larval *Hyla chrysoscelis*. Oikos **54**:325-330.

Alford R. A. 1989b. Competition between larval *Rana palustris* and *Bufo americanus* is not affected by variation in reproductive phenology. Copeia **1989**:993-1000.

Altig R., M. R. Whiles, and C. L. Taylor. 2007. What do tadpoles really eat? assessing the trophic status of an understudied and imperiled group of consumers in freshwater habitats. Freshwater Biology **52**:386-395.

Arendt J. D. 1997. Adaptive intrinsic growth rates: An integration across taxa. Quarterly Review of Biology **72**:149-177.

Beiswenger R. E. 1975. Structure and Function in Aggregations of Tadpoles of the American Toad Bufo-Americanus. Herpetologica **31**:222-233.

Berven K. A. 1990. Factors affecting population fluctuations in larval and adult stages of the wood frog (*Rana sylvatica*). Ecology **71**:1599-1608.

Boone M. D. 2005. Juvenile frogs compensate for small metamorph size with terrestrial growth: Overcoming the effects of larval density and insecticide exposure. Journal of Herpetology **39**:416-423.

Cadotte M. W., D. V. Mai, S. Jantz, M. D. Collins, M. Keele, and J. A. Drake. 2006. On testing the competition-colonization trade-off in a multispecies assemblage. American Naturalist **168**:704-709.

Connell J. H. 1980. Diversity and the coevolution of competitors, or the ghost of competition past. Oikos **35**:131-138.

DeBenedictis P. A. 1974. Interspecific competition between tadpoles of *Rana pipiens* and *Rana sylvatica*: an experimental field study. Ecological Monographs **44**:129-151.

Faragher S. G., R. G. Jaeger. 1998. Tadpole bullies: examining mechanisms of competition in a community of larval anurans. Canadian Journal of Zoology **76**:144-153.

Farlowe V. 1928. Algae of ponds as determined by an examination of the intestinal contents of tadpoles. Biological Bulletin **55**:443-448.

Fauth J. E., W. J. Resetarits. 1991. Interactions between the salamander *Siren intermedia* and the keystone predator *Notophthalmus viridescens* . Ecology **72**:827-838.

Finlay J. C., V. T. Vredenburg. 2007. Introduced trout sever trophic connections in watersheds: Consequences for a declining amphibian. Ecology **88**:2187-2198.

Frost D. R., T. Grant, J. Faivovich, R. H. Bain, A. Haas, C. F. B. Haddad, R. O. De Sa, A. Channing, M. Wilkinson, S. C. Donnellan, C. J. Raxworthy, J. A. Campbell, B. L. Blotto, P. Moler, R. C. Drewes, R. A. Nussbaum, J. D. Lynch, D. M. Green, and W. C. Wheeler. 2006. The amphibian tree of life. Bulletin of the American Museum of Natural History :1-370.

Gosner K. L. 1960. A simplified table for staging anuran embryos and larvae with notes on identification. Herpetologea **16**:183-190.

Harp E. M., J. W. Petranka. 2006. Ranavirus in wood frogs (*Rana sylvatica*): Potential sources of transmission within and between ponds. Journal of Wildlife Diseases **42**:307-318.

Hesslein R. H., K. A. Hallard, and P. Ramlal. 1993. Replacement of sulfur, carbon, and nitrogen in tissue of growing broad whitefish (*Coregonus nasus*) in response to a change in diet traced by delta-S-34, delta-C-13 and delta-N-15. Canadian Journal of Fisheries and Aquatic Sciences **50**:2071-2076.

Hobson K. A., H. L. Gibbs, and M. L. Gloutney. 1997. Preservation of blood and tissue samples for stable-carbon and stable-nitrogen isotope analysis. Canadian Journal of Zoology **75**:1720-1723.

Keddy P. A. 2001. Competition. Kluwer, Dordrecht, The Netherlands.

Keddy P. A. 1990. Competitive hieracrchies and centrifugal organisation in plant communities. Pages 265-290 *In* J. B. Grace and D. Tilman, editors. Perspectives on plant competition, Academic Press, San Diego.

Keddy P. A. 1989. Competition. Champan and Hall, London.

Kiesecker J. M., A. R. Blaustein. 1998. Effects of introduced bullfrogs and smallmouth bass on microhabitat use, growth, and survival of native red-legged frogs (*Rana aurora*). Conservation Biology **12**:776-787.

Kiesecker J. M., A. R. Blaustein. 1997. Population differences in responses of red-legged frogs (*Rana aurora*) to introduced bullfrogs. Ecology **78**:1752-1760.

Kling G. W., B. Fry, and W. J. O'Brien. 1992. Stable isotopes and planktonic trophic structure in arctic lakes. Ecology **73**:561-566.

Kupferberg SJ. The ecology of native tadpoles (*Rana boylii* and *Hyla regilla*) and the impact of invading bullfrogs (*Rana catesbeiana*) in a northern California river. California, USA: University of California at Berkeley; 1996. 289 p.

Lemmon E. M., A. R. Lemmon, J. T. Collins, J. A. Lee-Yaw, and D. C. Cannatella. 2007. Phylogeny-based delimitation of species boundaries and contact zones in the trilling chorus frogs (*Pseudacris*). Molecular Phylogenetics and Evolution **44**:1068-1082.

Loman J. 2004. Density regulation in tadpoles of *Rana temporaria*: A full pond field experiment. Ecology **85**:1611-1618.

Maunder J. E. 1983. Amphibians of the province of Newfoundland. Canadian Field-Naturalist **97**:33-46.

Maunder J. E. 1997. Amphibians of Newfoundland and Labrador: status changes since 1983. Herpetological Conservation **1**:93-99.

Morin P. J., E. A. Johnson. 1988. Experimental studies of asymmetric competition among anurans. Oikos **53**:398-407.

Paine R. T. 1994. Marine rocky shores and community ecology: an experimentalist's perspective. Excellence in Ecology **4**:1-152.

Pehek E. L. 1995. Competition, pH, and the ecology of larval *Hyla andersonii*. Ecology **76**:1786-1793.

Persson L. 1985. Asymmetrical competition - are larger animals competitively superior. American Naturalist **126**:261-266.

Peterson B. J., B. Fry. 1987. Stable isotopes in ecosystem studies. Annual Review of Ecology and Systematics **18**:293-320.

Petranka J. W. 1995. Interference competition in tadpoles - are multiple agents involved? Herpetological Journal **5**:206-207.

Petranka J. W., C. A. Kennedy. 1999. Pond tadpoles with generalized morphology: is it time to reconsider their functional roles in aquatic communities? Oecologia **120**:621-631.

Post D. M. 2002. Using stable isotopes to estimate trophic position: models, methods, and assumptions. Ecology **83**:703-718.

Relyea R. A. 2000. Trait-mediated indirect effects in larval anurans: reversing competition with the threat of predation. Ecology **81**:2278-2289.

Relyea R. A., J. R. Auld. 2004. Having the guts to compete: how intestinal plasticity explains costs of inducible defences. Ecology Letters **7**:869-875.

Rowe C. L., W. A. Dunson. 1995. Impacts of hydroperiod on growth and survival of larval amphibians in temporary ponds of Central Pennsylvania, USA. Oecologia **102**:397-403.

Russell A. P., A. M. Bauer. 2000. The amphibians and reptiles of Alberta: a field guide and primer of boreal herpetology. University of Calgary Press, Calgary.

Schiesari L. 2006. Pond canopy cover: a resource gradient for anuran larvae. Freshwater Biology **51**:412-423.

Schiesari L., E. E. Werner, and G. W. Kling. 2009. Carnivory and resource-based niche differentiation in anuran larvae: implications for food web and experimental ecology. Freshwater Biology **54**:572-586.

Schiesari LC. Performance tradeoffs across resource gradients in anuran larvae. United States -- Michigan: University of Michigan; 2004.

Seale D. B. 1982. Obligate and facultative suspension feeding in anuran larvae: feeding regulation in Xenopus and Rana. Biological Bulletin **162**:214-231.

Seale D. B., N. Beckvar. 1980. The comparative ability of anuran larvae (genera, *Hyla*, *Bufo* and *Rana*) to ingest suspended blue-green-algae. Copeia **1980**:495-503.

Seale D. B., R. J. Wassersug. 1979. Suspension feeding dynamics of anuran larvae related to their functional morphology. Oecologia **39**:259-272.

Skelly D. K. 2002. Experimental venue and estimation of interaction strength. Ecology **83**:2097-2101.

Skelly D. K. 1995. Competition and the distribution of spring peeper larvae. Oecologia **103**:203-207.

Skelly D. K., J. M. Kiesecker. 2001. Venue and outcome in ecological experiments: Manipulation of larval anurans. Oikos **94**:198-208.

Smith D. C. 1990. Population-structure and competition among kin in the chorus frog (*Pseudacris triseriata*). Evolution **44**:1529-1541.

Smith D. C. 1987. Adult recruitment in chorus frogs: effects of size and date at metamorphosis. Ecology **68**:344-350.

Smith D. C. 1983. Factors controlling tadpole populations of the chorus frog (*Pseudacris triseriata*) on Isle Royale, Michigan. Ecology **64**:501-510.

Smith G. R., H. A. Dingfelder, and D. A. Vaala. 2004. Asymmetric competition between *Rana clamitans* and *Hyla versicolor* tadpoles. Oikos **105**:626-632.

Sours G. N., J. W. Petranka. 2007. Intraguild predation and competition mediate stage-structured interactions between wood frog (*Rana sylvatica*) and upland chorus frog (*Pseudacris feriarum*) larvae. Copeia **2007**:131-139.

Stewart R. E., H. A. Kantrud. 1971. Classification of natural ponds and lakes in the glaciated prairie region. U.S. Fisheries and Wildlife Service Resource Publication **92**:57.

SYSTAT Software INC. 2007. Systat. 12.02. San Jose, California.

Test F. H., R. G. McCann. 1976. Foraging behaviour of *Bufo americanus* tadpoles in response to high densities of micro-organisms. Copeia **1976**:576-578.

Tilman D. 1994. Competition and biodiversity in spatially structured habitats. Ecology **75**:2-16.

Tylianakis J. M., R. K. Didham, J. Bascompte, and D. A. Wardle. 2008. Global change and species interactions in terrestrial ecosystems. Ecology Letters **11**:1351-1363.

Verburg P., S. S. Kilham, C. M. Pringle, K. R. Lips, and D. L. Drake. 2007. A stable isotope study of a neotropical stream food web prior to the extirpation of its large amphibian community. Journal of Tropical Ecology **23**:643-651.

Wassersug R. J., M. Yamashita. 2001. Plasticity and constraints on feeding kinematics in anuran larvae. Comparative Biochemistry and Physiology A-Molecular and Integrative Physiology **131**:183-195.

Wassersug R. J., K. Hoff. 1979. A comparative study of the buccal pumping mechanism of tadpoles. Biological Journal of the Linnean Society **12**:225-259.

Werner E. E. 1994. Ontogenetic scaling of competitive relations: size-dependent effects and responses in two anuran larvae. Ecology **75**:197-213.

Werner E. E., K. S. Glennemeier. 1999. Influence of forest canopy cover on the breeding pond distributions of several amphibian species. Copeia **1999**:1-12.

Whitaker J. O. 1971. A study of the western chorus frog, *Pseudacris triseriata* in Vigo County, Indiana. Journal of Herpetology **5**:127-150.

Wilbur H. M. 1987. Regulation of structure in complex-systems - experimental temporary pond communities. Ecology **68**:1437-1452.

Wilbur H. M. 1984. Complex life cycles and community organization in amphibians. Pages 195-224 *In* P. W. Price, C. N. Slobodchikoff, and W. S. Gaud, editors. A new ecology: novel approaches to interactive systems, Wiley, New York, New York.

Wilbur H. M. 1977. Density-dependent aspects of growth and metamorphosis in *Bufo americanus*. Ecology **58**:196-200.

Wilbur H. M., R. A. Alford. 1985. Priority effects in experimental pond communities - responses of Hyla to Bufo and Rana. Ecology **66**:1106-1114.

Wilbur H. M., J. P. Collins. 1973. Ecological aspects of amphibian metamorphosis. Science **182**:1305-1314.

Chapter 3: The effect of nutrients on tadpole performance

Introduction

Eutrophication, elevated levels of nutrients due to runoff from crops, livestock, and urban sources, is a growing threat to freshwater ecosystems (Carpenter et al. 1998). Heavy rains and snowmelt transport excess nutrients from terrestrial to aquatic habitats. Eutrophication may be of concern to amphibian populations because of these animals' permeable skin exposes them to the associated accumulation of nitrogenous compounds in wetlands. Aquatic amphibians can suffer lethal and non-lethal toxic effects from ammonia and other nitrogenous compounds released by fertilizers (Hecnar 1995, Rouse et al. 1999). Eutrophication has also been shown to promote pathogenic infections that cause severe limb deformities and mortality in anurans (Johnson and Chase 2004, Johnson et al. 2007). Wetlands near agricultural production are potentially further exposed to run-off carrying agrochemicals that reduced survival and performance of anurans (Relyea 2003, Hamer et al. 2004, Relyea et al. 2005). It is important to assess the effects of eutrophication on wetland-dependent species, especially amphibians whose complex life-histories include aquatic larval development.

North temperate pond-breeding amphibians, especially anurans, depend upon aquatic habitats for breeding, larval development, and may use wetlands to forage or maintain water balance. Northern amphibians, limited by short growing seasons and harsh winters, may have adapted their life history by having rapid development of larvae and utilize temporarily abundant resources. Fertilizer nutrients, sediments and other chemicals are transported by run-off from agricultural landscapes into

wetlands and are likely to affect the development and growth of resident amphibian larvae. Nitrogen and/or phosphorous can limit primary production and the addition of these nutrients can cause shifts in associated food webs (Bourassa and Cattaneo 2000, Chase 2003). Amphibian larvae, especially temperate anuran larvae, are dependent on aquatic production for growth and survival (Altig et al. 2007). Size at metamorphosis is positively influenced by larval growth, such that greater growth results in larger metamorphs. Size at metamorphosis is strongly related to metamorph survival and fitness of adult anurans (Smith 1987, Berven 1990). Anuran populations thus have the capacity to respond strongly to changes in larval environments due to changes to wetland condition.

Amphibians found in northern forests and grasslands may benefit from indirect changes to their habitat as a result of low-level eutrophication. Wetlands in Alberta are generally eutrophic (Allen et al. 2003) and phosphorous limited (N:P > 10:1, Eaves 2004; and upwards of 20:1 Prepas et al. 2001). Increases in phosphorous and nitrogen as a result of nutrient release have been shown to increase aquatic productivity several fold in streams (Spencer and Hauer 1991, Hauer and Spencer 1998) and lakes (Planas et al. 2000, Scrimgeour et al. 2001). Nutrient addition or fertilization has been used as a method to increase productivity of waters for fish production (Smith 1969) and has been suggested as a method to increase waterfowl production (Melanson and Payne 1988, Brylinsky 1993). Biomass of aquatic macro-invertebrates has been shown to increase as a result of nutrient enrichment (Scrimgeour et al. 2001). Herbivorous amphibian larvae are also likely to respond to increased productivity with better growth and survival.

Studies on the impacts of agriculture on amphibian populations have focused on post-metamorphic community structure and relative measures of abundance (Healey et al. 1997, Knutson et al. 2004, Burton et al. 2009). Few studies have examined the relationship of agricultural practices on aquatic environments and the success of larval amphibians (e.g. Loman and Lardner 2006, Jofre et al. 2007, Schmutzer et al. 2008). Understanding what conditions affect the recruitment of individuals from wetlands and persistence of amphibian populations in a landscape is important for their management.

My work focuses on amphibian populations in the Beaver Hills Region, located within the aspen parkland ecoregion of central Alberta, Canada. A large loss of wetlands within this ecoregion has occurred largely due to agriculture and urbanization with 21-48% of remaining wetlands disappearing between 1970 and 1990 (Alberta Environmental Protection 1996, but see Young et al. 2006). Sarah Eaves (2004) recorded reduced abundances of terrestrial amphibians in crop and pasture areas within the Beaver Hills Region as compared to natural areas. In addition to reduced abundances, she found that total nitrogen and total phosphorous were nearly five times higher in wetlands associated with agriculture than in protected park ponds. I have chosen to focus on the larval ecology of the boreal chorus frog, *Pseudacris maculata*, which is perhaps the most widespread and most abundant amphibian in Alberta (Russell and Bauer 2000), but we know little about the biology of this species' larvae. By monitoring the performance of larvae and production of metamorphs in mesocosms to which fertilizers have been added, I attempted to determine if the pattern observed by Eaves (2004) was a result of reduced tadpole performance (growth, survival and size at metamorphosis).

In addition to primary production, competition is believed to be important in structuring anuran communities (Alford 1999). Within the aspen parkland, wood frogs *Lithobates sylvaticus*, are common and co-occur with boreal chorus frogs at most ponds in the region, and may influence boreal chorus frog larval performance and success. Exploitative competition among anurans is often the mechanism proposed for reduced larval growth rates, reduced size of metamorphic frogs and reduced recruitment. There are a number of reports in the literature of anurans from the family Ranidae out-competing Hylidae larvae (Wilbur 1987, Morin and Johnson 1988, Alford 1989ab, Fauth and Resetarits 1991, Pehek 1995, Faragher and Jaeger 1998, but see Smith et al. 2004). The mechanism proposed for this dominance is that ranid tadpoles (such as wood frogs) consume a greater proportion of limiting resources than hylids (such as boreal chorus frogs) as a result of their typically larger size (Wilbur 1987, Morin and Johnson 1988). This is in contrast to work by Werner (1994) who showed that smaller size classes of tadpoles (both within and between two species: *Lithobates clamitans* and *L. catesbeianus*) have a greater effect, per unit biomass, on resources than larger size classes and tolerate competitive effects better (respond less to competitors). Further, small tadpoles are expected to be better competitors at low resource levels, as they have lower absolute metabolic demands than larger competitors. If competition occurs between wood and boreal chorus frog larvae for food resources, then addition of nutrients that foster increased primary production, and therefore increase tadpole foods, should increase tadpole growth, survival and size at metamorphosis.

I examined growth, survival and production of metamorphs in outdoor mesocosm with additions of nitrogen and phosphorous fertilizers, commonly found in run-off in agricultural landscapes. Along with the effects of nutrient additions, I

also examined the effects of the presence of wood frog tadpoles as potential competitors with chorus frog tadpoles for algal resources which are likely to increase as a result of fertilization. If fertilization increases primary production, such as periphyton, which boreal chorus frog larvae use, then larvae should respond with increased growth rates which if conditions are favourable will result in increased mass at metamorphosis. Survival will also increase if food limits larval survival. Resource competition with wood frog larvae, if it exists and wood frog are dominant, is likely to reduce chorus frog growth rates, and may limit chorus frog responses to increased nutrient levels. Through these comparisons I will investigate if increased nutrient inputs increase or reduce recruitment of anuran larvae from wetlands impacted by agricultural development in the aspen parkland of Alberta.

Methods

The experiment was conducted in mesocosms (cattle tanks) at University of Alberta's Meanook Biological Research Station near Athabasca, AB (MBRS; 54° 62' N, 113° 35' W) in 2007. I focused on growth, survival and size at metamorphosis of boreal chorus frog ("CF") tadpoles in a 2 × 2 factorial design manipulating presence of wood frog ("WF") tadpoles and fertilization with nitrogen and phosphorous. Cattle tanks were used to simplify communities and control environmental conditions and permit assessment of tadpole responses to increased nutrients while maintaining some realism by including the presence of phyto- and zooplankton. I used nitrogen (urea based) and phosphorous fertilizers, commonly applied to crops, to increase these nutrients and tadpole food resources in mesocosms.

Experimental animals and materials

Blue fibreglass cattle tanks (2.55 m in diameter and 75 cm tall) with sandy bottoms (5 cm deep) were filled to a depth of 27 cm with ~1300 L of water pumped from a nearby dugout on May 7 2007. Depth reflected typical average depths of ponds in Elk Island National Park (EINP) during the spring of 2005. The water pumped into the tanks contained algae and zooplankton, and macro-invertebrate predators (i.e. damselfly, dragonfly and dytiscid diving beetle larvae). Tanks lacked lids allowing colonization by invertebrates, which increased the naturalness of tanks, but I removed diving beetles and their larvae as predation in tanks could result in complete loss of tadpoles. Anisopteran nymphs found in tanks were small (<10 mm) and densities were similar across tanks and I assumed their effects were minimal. The rim of each tank had a narrow ledge and lip that prevented the escape of emerging chorus frogs. Cattle tanks were arranged in 4 rows of 4 tanks. Each treatment occurred in each row and treatments were randomly assigned among tanks within a row. Ten litres of dried (two days in the sun and microwaved for 10 minutes) detritus from the dugout were added to each tank. Tadpoles were added six days after filling to allow periphyton and other algae to establish.

I stocked tanks with WF and CF tadpoles and/or eggs collected on April 26 from ponds in EINP (53°35'34"N, 112°52'15"W). I placed 2000 CF eggs collected from one pond in an outdoor holding tank (89 cm in diameter filled with 25 cm of water) near the experimental tanks. Eggs hatched on May 6. On May 12, I randomly selected 99 CF hatchlings to add to each cattle tank. The tadpoles had a mean body length of 2.2 mm (range 1.6 – 2.9 mm from a sample of 40) and weighed on average 3.8 ± 0.6 mg (± SE from a sample of 24). The stocking density of CF tadpoles (0.077 tadpoles/L) was within the natural range of CF density from surveys in and around

EINP ($\bar{x}$ = 0.085, from 0.001 to 0.51 tadpoles/L, n = 40 pond-years over three years 2005-2007). WF eggs were collected from a pond in EINP 3.2 km away from the source of CF eggs. WF eggs hatched earlier than CF and tadpoles were placed in a rain barrel ($d \times h$ = 55 × 95 cm filled to about 80 cm depth) at the station until stocking. On May 12, I randomly selected eight groups of 50 WF tadpoles and added them to cattle tanks with the CF tadpoles. Mean body length of WF tadpoles was 3.7 mm (range 2.9 – 4.8 mm from a sample of 30) and mean body mass was 13.2 ± 1.1 mg (± SE). The density of WF tadpoles selected (0.038 tadpoles/L) was representative of the mean density of WF tadpoles from natural ponds in and around EINP ($\bar{x}$ = 0.059, from 0 to 0.31 tadpoles/L, n = 40 pond-years over three years 2005-2007). WF tadpoles from the holding tank were added to 3 replicates on June 6 (body length 8.93 ± 0.13 mm) to replace WF tadpole mortalities (n = 31, 19 and 7 tadpoles added).

Experimental Design

The 2 × 2 factorial design (Table 3-1) consisted of the following four treatments with four replicates per treatment: noFert CF = no fertilizer, CF tadpoles only; noFert + WF = no fertilizer, both WF and CF tadpoles; Fert CF = fertilizer, CF tadpoles only; and Fert + WF = fertilizer, both WF and CF tadpoles. There are various ways of designing competition experiments, for example, maintaining densities or biomass of the focal species, across treatments, reduces the confounding effect of increased densities in the interspecific treatments. However, collecting sufficient CF tadpoles and eggs for stocking proved to be difficult because eggs and hatchings are cryptic before tadpoles become free swimming. As a result, overall larval densities in the + WF treatments were greater than in those lacking

WF tadpoles, and any conclusions based on the effects of WF presence need to consider the effects of greater tadpole densities.

To determine if the fivefold increase in water concentrations of TN and TP found by Eaves (2004) affects the larval survival and performance I applied fertilizers to half of the experimental ponds. Initial concentrations prior to fertilization was 1024 µg/L TN and 79 µg/L TP (N:P = 13). Fertilizers were added as four equivalent pulses of approximately 2000 µg/L of N and 220 µg/L of P. A pilot study revealed that TN from a single pulse diminished by 569 µg/L after 18 days (31.6 µg/L/d). Fertilizers commonly applied to crops were purchased as pellets of either N (46% by weight) or P (52% by weight), and ground into a powder with mortar and pestle. The four pulses were on average 5.5 grams of N fertilizer and 0.55 g of P fertilizer and were added starting May 12 and subsequent pulses were added on May 28 and June 6 and 18. Powdered fertilizer was dissolved in 12 L of water from the appropriate tank and then poured into the tank. Addition of nutrients was based on individual tank measurements of TN and TP taken the previous week.

Responses measured

Water samples were collected every second week after setup (May 14, 29 and June 7) to monitor nutrient pulse decay and calculate mass of fertilizer to be added the following week. Water samples were analyzed for TN, TP, ammonium (NH_4^+) and Nitrogen ions ($NO_2^- + NO_3^-$) by the University of Alberta Biogeochemical Laboratory, Edmonton. Attached algae on the sides of cattle tanks were sampled using a home-made brush-syringe sampler (Loeb 1981). DO and pH were measured using a Fisher Accumet 119 digital handheld unit and an YSI 55 dissolved oxygen

meter on May 22 and 29 and again on June 5 and 18. Temperatures in all tanks were recorded every 15 minutes using temperature loggers (iButton DS1921G#F50, Maxim Integrated Products) starting shortly after tadpole additions.

Tadpoles were weighed every two weeks until they started to emerge (both forelimbs present, Gosner stage 42, Gosner 1960). Tank means were determined based on 10-15 tadpoles per tank from May 12 until June 18, whereas tank means on June 25 were calculated from all tadpoles remaining (range 11-80 tadpoles). Tadpole metamorphosis started on June 25 and those animals exiting tanks were excluded from all calculations of tadpole metrics. Tadpoles that emerged were measured and removed weekly. For fortnightly measurements, tadpoles (~15 CF and ~10 WF) were caught with aquarium dip nets and placed in a plastic tub. They were gently poured into an aquarium net, blotted dry with paper towel before being weighed as a group in a tared dish containing water on a Mettler PJ300 to the nearest mg (± 0.001 g).

To reduce accidental deaths, floating boards within tanks allowed emerging tadpoles to leave the water. I recorded date of emergence for each new froglet as the day of visit regardless of state of tail resorption; length and mass at emergence were recorded for all living froglets. I determined survival to metamorphosis as the number of emerging frogs removed (plus observed dead) divided by the number stocked minus tadpoles removed for isotopic analysis (see Chapter 2).

Data analysis

For water chemistry, individual repeated measures ANOVAs were performed on each of TN, TP, NH_4^+ and $NO_2^- + NO_3^-$ with fertilization and wood frog presence as main effects and sampling periods as the repeated trials. Only TN was

not normally distributed (natural log), but I report results on the raw data as it had no effect on the interpretation of main effects. No useful information was obtained from brushed algal samples, as material was lost during sampling. In future, strips of plastic or glass slides attached to the side of the tank would make sampling more effective.

As both tadpole species were stocked at comparable developmental stages and sizes, change in mass and length were used to assess growth. Average change in mass and length was calculated for each tank for analyses. For mass measures (change in mass, and mass at emergence), I used both averages and total mass produced in separate analyses to determine the influence individual and population biomass produced. A two-way ANOVA was used to examine the main effects and the interaction between WF presence and fertilization on CF responses (see Table 3-1). I tested the influence of surviving tadpole density and biomass (CF, WF and total tadpole) on CF responses using a two-way ANCOVA with either tadpole density or biomass as a covariate.

For WF, I tested only the influence of fertilization on the same set of responses as described for CF (Table 3-1). I used a one-way ANOVA for each response. I also tested for the influence of density on WF responses to fertilization using an ANCOVA with tadpole density or biomass as covariates (total and individual species).

I assessed the normality of responses based on Shapiro-Wilk normality tests and for homogeneity of variances with F tests prior to analysis of variances. Survival to metamorphosis was arcsine square root transformed to satisfy normality. All tests were performed using SYSTAT 12.02 (SYSTAT Software INC. 2007). As in

Chapter 2, I chose an alpha of 0.10 for detecting significant differences because competitive effects are expected to be weak and the identification of ecological mechanism was of interest.

Results

Environmental conditions: Water quality did not vary between treatments prior to the start of the experiment. The application of fertilizers resulted in a significant increase of TN and TP across treatments regardless of WF additions (Table 3-2, Figure 3-1). Dissolved oxygen also increased significantly with the addition of fertilizers ($F_{1,12}$=20.5, p=0.001), while indendently decreasing with the addition of wood frogs ($F_{1,12}$=3.44, p=0.09). Differences between treatments, though statistically significant, only represented a 1.2 mg/L difference in fertilizer treatments (10.8 vs 9.7mg/L) and 0.5 mg/L in WF treatments (10.5 vs. 10.0mg/L). Similarly, ammonium concentrations were higher in fertilized relative to unfertilized treatments (Table 3-2). However, N-ions (nitrate + nitrite) were significantly higher in WF treatments independent of fertilizer additions (Table 3-2). Concentrations of TN, TP and NH_4 decreased over time despite bi-weekly addition of nutrients, but differences in TN and TP among treatments were maintained throughout the experiment (Figure 3-1). Fertilization and WF presence had opposite effects on pH, with significantly higher pH in fertilized tanks (9.2 vs. 8.8, $F_{1,12}$ = 17.5, p = 0.001) and significantly lower pH in tanks containing WF (8.9 vs. 9.1, $F_{1,12}$ = 6.23, p = 0.028) independent of fertilization. A date × fertilization interaction was found as a result of an increase in pH in unfertilized tanks from 8.6 on May 28 to 9.2 on June 18 the last measurement, whereas fertilized tanks increased from 8.9 on May 28 to 9.3 on June 5 and then decreased slightly until June 18 (Table 3-2, Figure 3-2). The high pH in tanks may

reflect low buffering capacity of these systems due to low dissolved organic carbon and/or high rates of primary production which depletes carbon dioxide. Temperature initially decreased from 18.8° to 8.8°C in the first week and thereafter increased over the course of the experiment. The mean average temperature in tanks was 17.3° and the largest difference on a given day between tanks was 0.9°C, but differences were unrelated to treatment.

CF responses

Tadpole growth: CF tadpole growth rates (Figure 3-3) and total CF tadpole biomass within tanks were significantly higher in fertilized treatments just prior to metamorphosis on June 25 (Table 3-3) and was independent of WF presence. Use of covariates (# and total biomass of CF; WF; CF+WF) had no effect on the interpretation of change in CF mass, however, the effect of fertilization was stronger when the number of CF and CF+WF were used as covariates (p = 0.011 and p = 0.014 respectively compared to p = 0.030, although only the number of CF tadpoles was significant as a covariate).

Using the number of CF as a covariate with total CF tadpole biomass on June 25 slightly strengthened the effect of fertilization. After correcting total CF tadpole biomass for the total number of all tadpoles surviving to June 25 in each tank (CF+WF), WF presence significantly reduced total CF tadpole biomass (Table 3-3, Figure 3-4), and the positive effect of fertilization, though weaker, was still significant (p = 0.065). CF tadpole biomass was significantly lower in the presence of WF, when total tadpole biomass (CF+WF) was included as a covariate, and fertilization no longer affected CF biomass (Table 3-3). In contrast, including the biomass of WF tadpoles as a covariate weakened, but did not negate, the effect of

fertilization ($F_{1,11}$ = 3.17, p = 0.10 versus p = 0.04 without) with no effect of WF presence ($F_{1,11}$ = 1.01, p = 0.34).

Individual CF tadpoles in Fert CF were 28.7% heavier than those in Fert + WF. CF tadpole response to fertilization was reduced in the presence of WF tadpoles, as they only gained 0.089 g relative to noFert + WF treatments based on change in individual tadpole mass from the beginning of the experiment to June 25. In contrast, when alone, CF tadpoles in fertilized tanks gained 0.253 g compared to unfertilized tanks, representing 2.8 × greater increase in individual tadpole biomass (Table 3-3).

Tadpole survival: The survival rate of the total population of 1592 CF tadpoles added to tanks was 37% with 565 metamorphs emerging by the end of the study; 67 tadpoles were removed for stable isotope analysis (Chapter 2) and were excluded from the calculation of survival. Only one CF tadpole remained at the end of the experiment on July 17 (in a noFert + WF tank). Survival of CF tadpoles to emergence was significantly higher in fertilized treatments (46% vs 28%, $F_{1,12}$ = 3.644, p = 0.080) and independent of WF presence (Table 3-3).

Number and Mass at Metamorphosis: More CF tadpoles emerged and at greater mass from fertilized treatments. As I did not track emergence daily, I used the number of metamorphs that emerged by a given date to compare treatment effects. The number of CF metamorphs that emerged by July 9, the peak of emergence, was significantly greater in fertilized treatments ($F_{1,12}$ = 4.90, p = 0.047) independent of WF presence (p = 0.58). Individual CF emerged heavier in fertilized treatments than in unfertilized treatments (Table 3-3), but the difference was not statistically significant. A significant interaction between fertilization and WF presence was

found for mean individual mass at emergence for metamorphs emerging by July 3 (although it was statistically non-significant on July 9) (Table 3-3). By July 3, CF in Fert CF treatments were 37-149% heavier (and 45-59% by July 9) than in other treatments (Table 3-3). Mean individual mass at emergence was similar in WF treatments regardless of fertilization, whereas CF raised in the absence of WF showed a 149% and 59% increase in mass by July 3 and 9 respectively (Figure 3-5), suggesting that WF presence prevented CF from responding to fertilization. The relatively greater survival in fertilized treatments combined with greater mean individual mass at emergence resulted in significantly greater (1.8 × unfertilized) total biomass of CF emerging from fertilized treatments by experiment end, July 17 (Table 3-3). The greatest total biomass of CF metamorphs emerged from the Fert CF treatment (Table 3-3) despite producing on average 13 fewer metamorphs per replicate tank than the Fert + WF treatments (39.3 and 52.2 tadpoles surviving on average to metamorphosis, respectively).

WF responses

Tadpole growth: Fertilization resulted in greater average mass gains and survival of WF tadpoles. Total WF tadpole mass produced in fertilizer treatments was significantly greater than in unfertilized treatments (on June 25 treatment means ± SE = 34.97 ± 1.68 g versus 22.42 ± 4.90 g; $F_{1,6} = 4.401$, $p = 0.081$). However unlike total WF biomass, change in individual WF tadpole mass was never significantly higher in fertilized treatments ($F_{1,6} = 2.119$, $p = 0.196$ on June 25) and any difference decreased as metamorphosis neared ($F_{1,6} = 0.846$, $p = 0.393$ on July 9, the first WF metamorphs seen July 3). Mean mass gain in WF tadpoles was negatively affected by the number of CF tadpoles present in those treatment tanks (Figure 3-6); whereas the converse relationship, i.e., CF mass gain with number of WF tadpoles as

a covariate, did not show a comparable pattern. When treatment means were adjusted for the number of CF tadpoles, the effect of fertilizer became significant on July 9 and nearly so on June 25 (on July 9, adjusted treatment means were 1.104 g in fertilized versus 0.715 g in unfertilized tanks, Fertilizer: $F_{1,5} = 7.170$, $p = 0.044$ and number of CF covariate: $F_{1,5} = 8.609$, $p = 0.032$; on June 25 Fertilizer: $F_{1,5} = 3.879$, $p = 0.106$ and number CF tadpoles as covariate: $F_{1,5} = 1.644$, p = 0.256). Other covariates (number and total biomass of WF, CF+WF) were not related to WF growth.

Tadpole survival: The first WF metamorph emerged on July 3 and WF continued to emerge until the experiment was terminated. One tank in the unfertilized treatment only produced two metamorphs but as WF tadpoles remained at experiment end, this tank was not excluded from survival analyses. Survival estimates were based on total number of tadpoles added, and thus included those replacement tadpoles added to maintain WF densities. Fertilizer improved WF survival to emergence (30.9% vs 19.4%) and survival till the end of the experiment (33.5 % vs 24.5%) but differences were not statistically significant (% emergence $p = 0.272$; % survival $p = 0.331$).

Number and Mass at Metamorphosis: Individual mass at emergence and total WF tadpole mass were greater in fertilized tanks and the differences were significant when the number of CF tadpoles in tanks was considered as a covariate (Figure 3-7). The total mass of metamorphs produced in fertilizer treatments was significantly greater than in unfertilized treatment tanks (4.64 ± 0.56 g versus 2.06 ± 0.79 g, $F_{1,6} = 5.31$, $p = 0.061$) and was not affected by the number of CF tadpoles present.

Tadpole responses

In summary CF tadpole performance was enhanced by fertilization resulting in greater average mass gains in tadpoles, survival to metamorphosis and mass at metamorphosis. The presence of WF tadpoles did not affect CF survival, but appeared to prevent CF tadpoles in fertilizer treatments from experiencing increased growth linked to higher nutrient availability. WF performance was similarly enhanced by fertilization (greater growth, mass at metamorphosis and survival, even after accounting for replacement of WF tadpoles).

Discussion

I used fertilizer additions in cattle tanks to assess whether increased nutrient inputs to wetlands affect tadpole performance and survival and could thus influence recruitment into terrestrial populations. If fertilization decreased tadpole performance it might explain the lower abundances of terrestrial anurans at agricultural wetlands in the aspen parkland reported by Eaves (2004). However, as hypothesized, nutrient augmentation increased tadpole foods and improved tadpole performance. The inputs of nitrogen and phosphorous are unlikely the cause of reduced amphibian abundances observed by Eaves (2004). As expected, addition of nitrogen and phosphorous fertilizers to cattle tanks increased TN and TP, and these elevated nutrient concentrations resulted in increased CF tadpole growth, survival and mass at metamorphosis. Both mass gained and mass at metamorphosis increased with fertilization when WF tadpoles were absent. Fitness of terrestrial frogs is positively correlated with size at metamorphosis such that survival of metamorphs, and the life-time reproductive success of an individual, increases with size at metamorphosis (Smith 1987, Berven 1990). At higher nutrient levels, WF

prevented CF from realizing the potential benefits of greater food resources. This resulted in reduced total CF tadpole biomass (Figure 3-4) and reduced mass at emergence (Figure 3-5) compared to fertilized tanks lacking WF. In fact, total CF tadpole biomass was significantly lower in the presence of wood frogs independent of fertilization (Figure 3-4). I propose that exploitative or interference competition reduces the performance of CF tadpoles in the presence of WF. Thus, it does not appear that increased nutrient inputs in mesocosms negatively affected tadpole performance, but had the potential to improve tadpole performance, survival and ultimately lead to larger metamorphs with associated greater fitness.

CF response to fertilizer

Greater survival, larval growth and size at metamorphosis in fertilized treatments are assumed to be due to greater food availability and/or quality as a result of greater nutrient availability for food organisms. Periphyton is believed to be an important food resource for growing tadpoles (Dickman 1968, Kupferberg et al. 1994, Schiesari et al. 2009) and other aquatic herbivores. A number of studies have shown that periphyton biomass increases with the addition of nitrogen and phosphorous (e.g. Smith and Lee 2006, and others reviewed by Hillebrand 2002). For example, the addition of inorganic fertilizers increased periphyton biomass in a Vancouver Island stream in British Columbia by 2 to 10 fold (Perrin et al. 1987) which resulted in an 82% gain in mean weight of juvenile coho salmon (*Oncorhynchus kisutch*). Kiffney and Richardson (2001), similarly show that addition of fertilizers (NO_3-N and PO_4-P) to constructed channels resulted in a fourfold increase in tadpole (*Ascaphus truei*) growth rates. Improved tadpole growth, combined with greater survival in fertilized treatments, further demonstrated that food resources increased either in abundance or in quality resulting in better

growth for a greater number of tadpoles. Several studies have shown that food limitations are common in mesocosms and in nature and influence growth, survival and metamorph size (e.g. Leibold and Wilbur 1992). CF tadpoles therefore responded as expected to fertilization with greater growth, survival and greater size at metamorphosis.

CF response to WF

Competition between CF and WF tadpoles is suspected to be the cause of reducing CF performance in mesocosms. I will explore three lines of evidence that suggests that competition occurs between these two species. I will first examine the evidence for diet overlap, then the effect of density on CF responses, and lastly discuss interference mechanisms.

Firstly, competition may occur as tadpoles share food resources. I found that WF and CF tadpole isotopic carbon signatures were similar in mesocosms irrespective of treatment (Chapter 2). Others have used isotopic signatures to infer resource competition between species (e.g. Welch and Parsons 1993, Levesque et al. 2003, Syvaranta and Jones 2008). Increases in growth, survival and mass at emergence with fertilization suggest that resources were limited for both species. Competition for limited food resources with WF tadpoles in this experiment may be responsible for the reduced growth of tadpoles and smaller CF metamorphs. Oddly, the overlap in isotopic resources, or apparently limited shared food, did not affect CF growth in the unfertilized treatment despite the addition of WF tadpoles (1.68 times more tadpoles surviving to June 25, or 2.63 times more biomass in fertilized tanks). Rather WF tadpoles appear to enhance CF growth in unfertilized treatments (mean CF tadpole mass was greater from June 4 – June 18, Figure 3-3). Crowded

tadpoles can increase nutrient cycling rates and increase the availability of suspended particles (fecal and algal) and thereby improve growth of tadpoles (Gromko et al. 1973, Steinwascher 1978) or of aquatic invertebrates (e.g. Iwai and Kagaya 2007). Fish (Brown 1946, Jorgensen et al. 1993, Papoutsoglou et al. 1998, Celada et al. 2007) and anuran tadpoles (Gromko et al. 1973, Wilbur 1977, Smith-Gill and Gill 1978, Murray 1990) in moderately crowded conditions can have greater growth rates. Similarity in diet, based on isotopic similarity, and possible food limitation when the two species were together were insufficient to reduce the growth and survival of CF tadpoles. However, when crowding is too great, competition and decreased water quality has been shown to reduce growth and survival in tadpoles (Schmutzer et al. 2008, competition - Morin and Johnson 1988, water quality - Wood and Richardson 2009). The total density of tadpoles in Fert + WF was 2.58 times greater than in Fert CF treatments and combined with diet overlap may be responsible for the lack of response in CF performance associated with fertilization.

The greater stocking (1.5 times more tadpoles in WF tanks) and greater survival of tadpoles in fertilized WF treatments, combined with the overlap in shared food resources (based on isotopic signatures, Chapter 2), may have resulted in more intense food competition (Rosenzweig 1971). Though confounded by resulting greater overall densities of tadpoles, the addition of WF tadpoles in the presence of fertilization resulted in reduced CF tadpole growth rates and individual mass at metamorphosis which is consistent with interspecific competition. My design does not allow me to examine explicitly the effect of intraspecific competition. However, although the final total biomass of CF tadpoles was similar across fertilized tanks regardless of WF presence (Table 3-3), that biomass was

divided amongst the 20 additional CF tadpoles that survived in the Fert + WF treatments, whereas in Fert CF treatments the fewer remaining tadpoles were on average heavier. Fertilization may have increased foods allowing greater survival for both CF and WF tadpoles, but interspecific competition may have in turn reduced individual CF tadpole mass and ultimately metamorph mass at emergence. There was on average eight more WF tadpoles in the fertilized treatments (due to differential mortality), and each WF tadpole was on average 0.17 g heavier on June 25 in the fertilized treatments. The increased density and biomass of WF (see Table 3-3), combined with a shared diet, may be sufficient to have altered the abundance of food resources such that CF tadpoles in the Fert + WF treatment though having greater survival did not benefit from the addition of fertilizer in terms of individual growth and size at metamorphosis.

Asymmetries in competitive interactions are not uncommon, but the inherent greater size of WF tadpoles relative to CF may allow WF tadpoles to use interference mechanisms in competitive interactions. Interference in tadpole systems may operate either through resource diversion, aggressive behaviour, or by production of growth inhibitors. The combined grazing pressure of both tadpoles on periphyton might have caused nutrient inputs to be diverted to phytoplankton (Leibold and Wilbur 1992, but see Harris 1995). I did not observe any changes in water colour and did not measure water column Chl *a* and so cannot confirm that resources were diverted from periphyton to phytoplankton in WF or fertilizer treatments. Interference through aggression can decrease larval growth rates by inhibiting feeding rate or causing physical harm (Faragher and Jaeger 1998). I did not observe any aggressive behaviour in WF or CF tadpoles and generally tadpoles swam in groups (single and mixed species) or were clustered along the walls

grazing on attached periphyton. Chemical interference is the third possible mechanism that tadpoles might use to inhibit the growth of non-sibs and heterospecifics (cellular agents as in Richards 1958, or chemical products as in Akin 1966). My design did not test nor limit tadpole exposure to chemicals and this mechanism cannot be ruled out. In chapter 5, I describe laboratory experiments that demonstrated that WF tadpoles release some product into the water and in their feces that inhibits CF larval growth and mass at emergence. This chemical interference may be responsible for the competitive patterns observed in the cattle tanks. Interference might have been strongest in fertilized tanks as the gains in access to food by WF would have outweighed the energetic costs of interference, whereas costs might have outweighed gains in unfertilized tanks and prevented or reduced the production of inhibitors. The actual mechanism of chemical inhibition remains elusive and I can only infer that if it occurs in laboratory-raised tadpoles, it might also occur in larger artificial environments such as mesocosms.

WF responses to fertilizer and CF

Both WF and CF tadpoles responded positively to fertilizer addition in mesocosms. Significant increases in growth and metamorph mass support my view that fertilizers improved food resources availability and/or quality. Further, on average greater survival was also found in fertilized treatments suggesting that tadpole foods were limiting given that predators, a major source of mortality, were removed from tanks (or at least reduced). Differences in growth were not apparent early in the experiment, but significant increases in mass in fertilized treatments developed by June 25 and carried into metamorphosis. High densities of tadpoles present early in the experiment might have limited the response of WF to fertilizers,

but mortality of both species and the onset of metamorphosis in CF might have allowed compensatory growth by WF tadpoles in the final few weeks.

Interspecific competition is also indicated in WF responses as WF growth and size at metamorphosis were negatively affected by CF number. Of the covariates considered when assessing WF growth and size at metamorphosis only analyses including the number of CF in tanks produced a significant effect of fertilizer as WF mass was negatively related to CF numbers.

If growth inhibitors do operate, as discussed above, inhibitors may not be species specific (Licht 1967) or are by-products of normal metabolism (Runkova et al. 1974, Stepanova 1974, Glennemeier and Denver 2002), and the higher densities of tadpoles, and therefore inhibitors, in fertilized treatments would reduce growth for tadpoles of both species, explaining why WF did not respond early in fertilized treatments. Regardless of the effect of competition with CF or interference chemicals, fertilizers increased food resources and WF tadpoles displayed increased growth, survival, and metamorph size as a result.

Conclusions

The addition of nitrogen and phosphorous fertilizers increased tadpole growth rates, survival, and size at metamorphosis for both boreal chorus and wood frogs. These benefits lead to increased fitness of metamorphs and therefore it is unlikely that the reduced abundance of terrestrial amphibians in the aspen parkland observed by Eaves (2004) is a result of increased nutrient concentrations. If performance of aquatic larvae is affected in this region then the application of herbiceds may be responsible for the decrease (Harris et al. 1998, Relyea 2003, Griffis-Kyle 2007, and reviewed by Mann et al. 2009). However, decreases in larval

performance can result from other changes to the wetland such as reduction of riparian buffers or increased pond access to cattle which increases larval exposure to UV (Smith et al. 2000, Pahkala et al. 2003, Bancroft et al. 2008ab, Croteau et al. 2008), turbidity (Knutson et al. 2004, Wood and Richardson 2009), as well as the application of herbicides (Harris et al. 1998, Relyea 2003, Griffis-Kyle 2007, and reviewed by Mann et al. 2009). In addition to the above impacts, changes associated with cattle and the reduction of riparian buffers may decrease pond hydroperiods (Trauth et al. 2006) or pond quality (Schmutzer et al. 2008) which also can decrease larval performance. However, it is more likely that the fragmentation of populations (Kolozsvary and Swihart 1999, Semlitsch 2000, Marsh and Trenham 2001) and survival of terrestrial individuals may be reduced in these agricultural landscapes as a result of the intensity of upland use (Porej et al. 2004) or in crop lands the application agricultural chemicals (Oldham et al. 1997).

CF tadpoles in fertilized treatments grew slower and emerged lighter when WF tadpoles were present consistent with interspecific. When together, WF gained more mass with fertilization than did CF, though CF had greater survival. Further evidence of effects of interspecific competition are revealed by the negative relation between WF tadpole mass and CF density, suggesting that CF were able to slightly depress WF growth (Figure 3-6) and mass at emergence (Figure 3-7). The mechanism appears to be related to food exploitation as isotopic signatures overlap greatly in mesocosms. However, interference competition by the larger WF cannot be ruled out as CF growth in natural ponds was reduced in the absence of resource overlap based on stable isotopic signatures (Chapter 2).

Table 3-1: Mesocosm experimental design. Abbreviations for tadpole stocking are CF=boreal chorus frog and WF=wood frog, treatment names are as described in Methods.

	Fertilized		Unfertilized	
Treatment name	Fert CF	Fert + WF	noFert CF	noFert + WF
Tadpole stocking	99 CF	99 CF 50 WF	99 CF	99 CF 50 WF
Replication	4	4	4	4
Date (duration)	May 12–July 24 (74 days)			
Response variables	Survival to emergence Larval growth (mass & length) Mass & length at emergence			

Table 3-2: Repeated measures analysis of water chemistry variables from mesocosms with chorus frogs where wood frog presence (+ WF; CF) was crossed with fertilization (Fert; noFert). Variables tested were total nitrogen (TN), total phosphorous (TP), ammonium (NH_4), nitrogen ions of nitrate and nitrite (NO_2 + NO_3), pH and tank daily average over the course of the experiment. Values reported for each measure are the means (± 1 SE) over the course of the experiment (13 May – 30 June).

Treatment	**Response variables**					
	TN	**TP**	**NH_4^+**	**$NO_2^- + NO_3^-$**	**pH**	**Temperature**
	µg/L	**µg/L**	**µg/L**	**µg/L**		**°C**
noFert CF	630 ± 7	41 ± 2	10 ± 2	3 ± 0.1	8.85 ± 0.07	17.3 ± 0.2
noFert + WF	605 ± 27	34 ± 3	8 ± 3	4 ± 0.7	8.79 ± 0.09	17.4 ± 0.1
Fert CF	2169 ± 102	56 ± 2	48 ± 5	3 ± 0.1	9.33 ± 0.05	17.1 ± 0.2
Fert + WF	2230 ± 125	56 ± 3	42 ± 10	5 ± 0.7	8.98 ± 0.11	17.4 ± 0.1
Between subjects effects						
Fertilization	$F_{1,12} = 379.8^{***}$	$F_{1,12} = 59.1^{***}$	$F_{1,12} = 37.2^{***}$	$F_{1,12} = 2.01$	$F_{1,12} = 17.5^{***}$	$F_{1,12} = 1.89$
WF presence	$F_{1,12} = 0.06$	$F_{1,12} = 1.75$	$F_{1,12} = 0.55$	$F_{1,12} = 8.77^{**}$	$F_{1,12} = 6.23^{*}$	$F_{1,12} = 1.75$
Interaction	$F_{1,12} = 0.32$	$F_{1,12} = 2.18$	$F_{1,12} = 0.10$	$F_{1,12} = 1.34$	$F_{1,12} = 2.99$	$F_{1,12} = 0.05$
Within subjects effects						
Date	$F_{2,24} = 134.4^{***}$	$F_{1,12} = 580.6^{***}$	$F_{1,12} = 12.0^{**}$	$F_{1,12} = 76.0^{***}$	$F_{3,36} = 30.9^{***}$	$F_{58,638} = 2711^{***}$
Date × Fert	$F_{2,24} = 107.1^{***}$	$F_{1,12} = 43.8^{***}$	$F_{1,12} = 4.19^{\dagger}$	$F_{1,12} = 0.04$	$F_{3,36} = 7.22^{***}$	$F_{58,638} = 3.71^{***}$
Date × WF	$F_{2,24} = 0.23$	$F_{1,12} = 1.68$	$F_{1,12} = 0.17$	$F_{1,12} = 6.95^{*}$	$F_{3,36} = 0.63$	$F_{58,638} = 3.77^{***}$
Date × Fert × WF	$F_{2,24} = 0.20$	$F_{1,12} = 0.20$	$F_{1,12} = 0.001$	$F_{1,12} = 0.37$	$F_{3,36} = 0.38$	$F_{58,638} = 1.24$

$^{***}p \le 0.001$, $^{**}p \le 0.01$, $^{*}p \le 0.05$, $^{\dagger}p \le 0.10$

Table 3-3: Analysis of tadpole response variables for boreal chorus frogs in treatments with and without fertilization and wood frogs (CF only and +WF). Shown are treatment means ± 1 SE. Means are for individuals unless noted as total values for tadpole populations.

Response		Fertilized		Unfertilized		Effect [d]		
		CF only	+ WF	CF only	+ WF	Fertilization	WF presence	Interaction
Change in tadpole mass								
June 18		0.503 ± 0.038	0.376 ± 0.058	0.323 ± 0.066	0.415 ± 0.060	F=1.17, p=0.30	F=0.07, p=0.79	F=2.78, p=0.12
June 25		0.628 ± 0.059	0.488 ± 0.066	0.375 ± 0.059	0.399 ± 0.056	F=6.08, p=0.03	F=0.70, p=0.42	F=1.42, p=0.26
Total tadpole biomass								
June 25		22.53 ± 4.71	24.90 ± 3.91	14.00 ± 3.11	14.43 ± 1.91	F=5.32, p=0.04	F=0.11, p=0.74	F=0.06, p=0.82
covariates								
Number of tadpoles[a,b]		30.10 ± 3.11	13.44 ± 3.68	20.43 ± 2.98	11.88 ± 2.66	F=4.21, p=0.06	F=9.41, p=0.01	F=2.04, p=0.18
CF+WF tadpole biomass[a,c]		29.32 ± 2.61	8.17 ± 3.88	26.17 ± 3.22	12.20 ± 2.31	F=0.02, p=0.88	F=17.3, p < 0.01	F=2.19, p=0.17
Mass at emergence								
by July 3		0.265 ± 0.024	0.193 ± 0.036	0.106 ± 0.054	0.188 ± 0.031	F=3.07, p=0.11	F=0.01, p=0.92	F=3.55, p=0.08
by July 9		0.265 ± 0.024	0.180 ± 0.032	0.166 ± 0.025	0.183 ± 0.025	F=2.44, p=0.14	F=1.25, p=0.29	F=2.67, p=0.13
Total mass at emergence								
by July 3		6.52 ± 1.78	3.78 ± 0.70	1.69 ± 1.42	3.18 ± 1.43	F=1.91, p=0.19	F=0.49, p=0.50	F=1.01, p=0.34
by July 9		9.42 ± 1.74	7.66 ± 0.45	3.96 ± 1.97	4.66 ± 1.28	F=6.13, p=0.03	F=0.10, p=0.76	F=0.52, p=0.49
by July 17		9.47 ± 1.78	7.76 ± 0.45	4.78 ± 1.19	4.82 ± 1.19	F=5.65, p=0.03	F=0.27, p=0.61	F=0.29, p=0.60
% survival to metamorphosis		39.3 ± 8.2	52.2 ± 9.5	29.9 ± 7.2	27.1 ± 5.9	F=3.64, p=0.08	F=0.19, p=0.67	F=0.66, p=0.43
on June 25	# of CF tadpoles	36.8 ± 8.0	57.8 ± 12.9	40.3 ± 7.7	38.5 ± 3.6			
	# of WF tadpoles	0	37.3 ± 3.3	0	29.3 ± 5.2			
	biomass of WF (g)	0	35.0 ± 1.9	0	22.4 ± 5.4	t_6=2.10, p=0.081		

[a] means are adjusted for the effect of covariate

[b] covariate for number of tadpoles (CF+WF) $F_{1,11}$=19.29, p=0.001; whereas main effects were similar to basic model for WF covariate: $F_{1,11}$=1.36, p=0.27 and CF covariate: $F_{1,11}$=20.47, p=0.001

[c] covariate for the total tadpole biomass (CF+WF) $F_{1,11}$=28.41, p=0.001; whereas main effects were similar to basic model for WF covariate: $F_{1,11}$=1.36, p=0.27

[d] degrees of freedom for significance tests are 1,12 unless noted otherwise

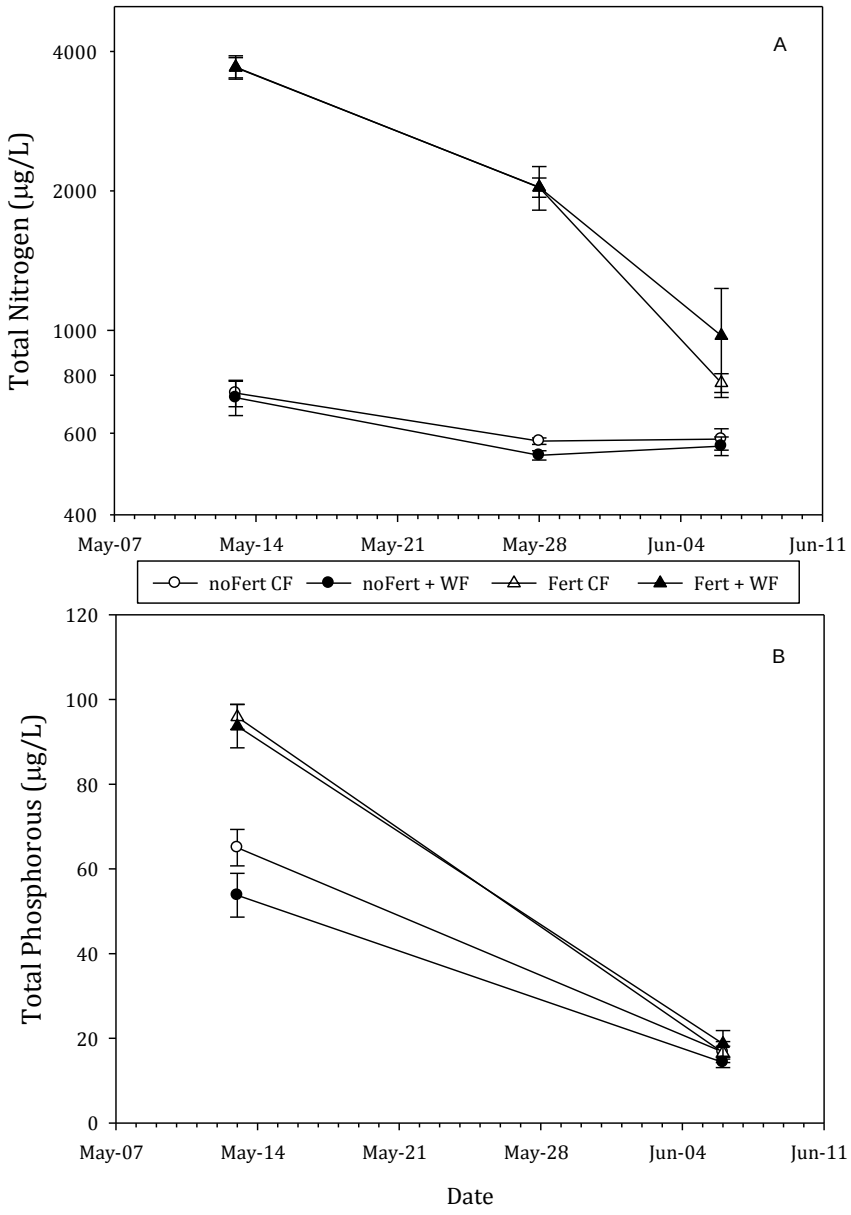


Figure 3-1: Changes in mean treatment total nitrogen A) and total phosphorous B) concentrations through time ± SE for four replicate tanks. Nitrogen and phosphorous fertilizers were added on May 12, 28 and June 6, 18. Water samples on May 28 were taken several hours after fertilizer addition, whereas others were taken before fertilizer addition. Initial concentrations prior to experiment start and fertilizer addition was 1024 µg/L TN and 79 µg/L TP based on two water samples from the reservoir used to fill tanks.

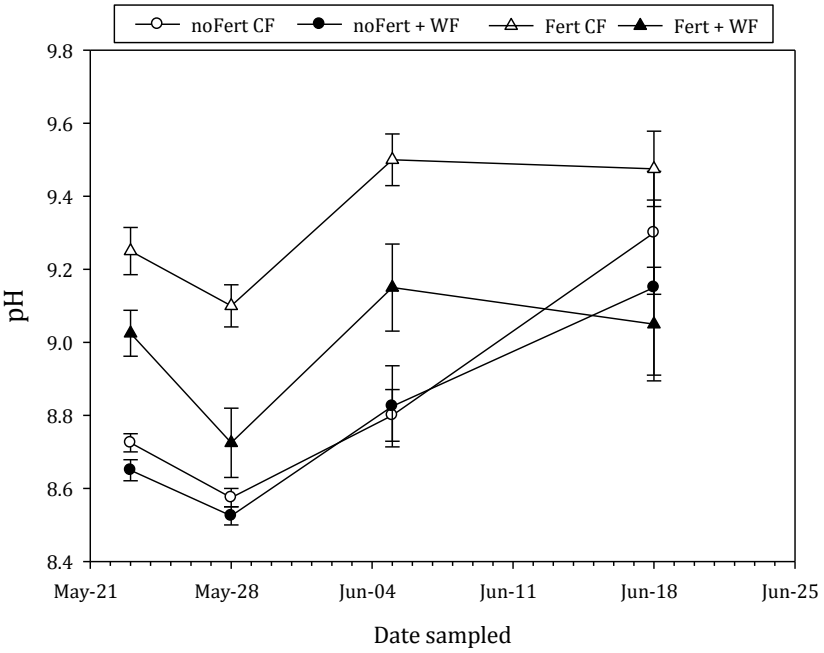


Figure 3-2: Relationship between pH and sample date across fertilizer and wood frog treatments. Shown are the mean treatment values ± SE. Water used to fill tanks had a pH of 7.2 on May 7 when added to mesocosms.

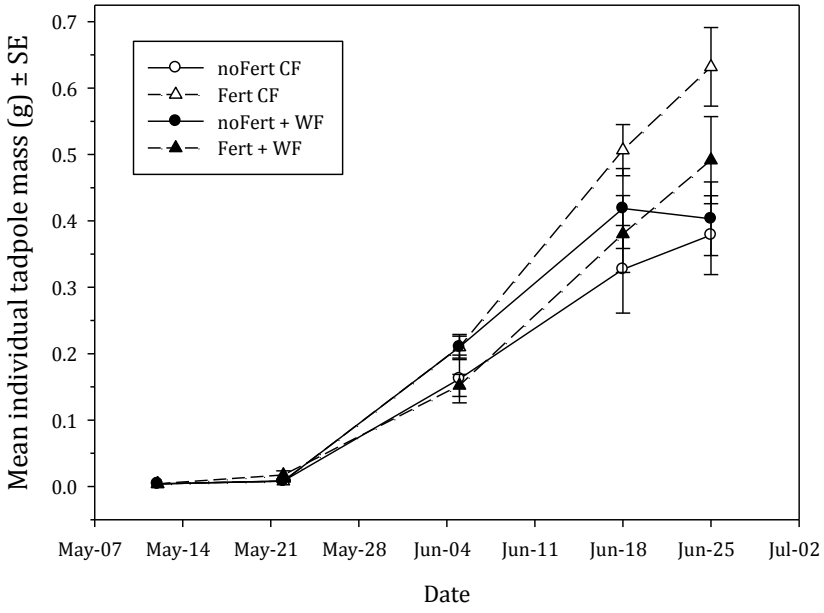


Figure 3-3: Mean individual boreal chorus frog tadpole mass (± 1 SE) from mesocosms until metamorphosis, June 25. Tank means (n = 4) until June 18 are based on 10-15 tadpoles per tank, whereas on June 25 tank means were calculated from all remaining tadpoles (range 11-80 tadpoles). Treatment names are as in text.

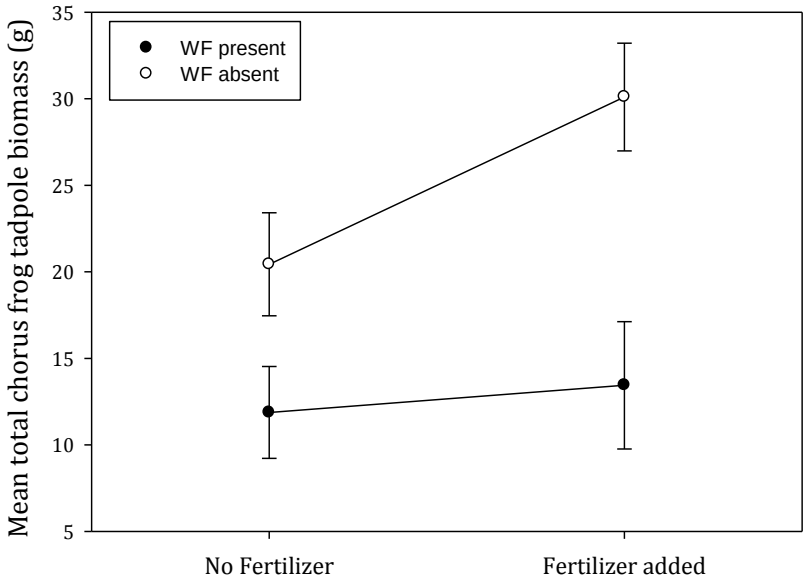


Figure 3-4: Mean boreal chorus frog tadpole biomass (± 1 SE) on June 25 across mesocosms that varied with fertilization crossed with presence of wood frog tadpoles (WF). Treatment means are based on four replicates per treatment.

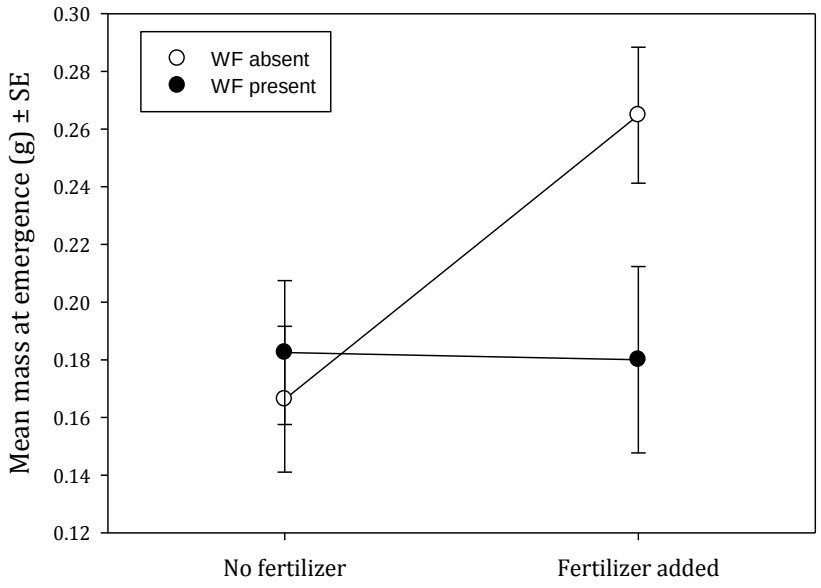


Figure 3-5: Mean boreal chorus frog mass at emergence (± 1 SE) for individual that emerged from mesocosms by July 9 from mesocosms that varied fertilization and presence of wood frog tadpoles (WF). Treatment means are based on four replicates per treatment.

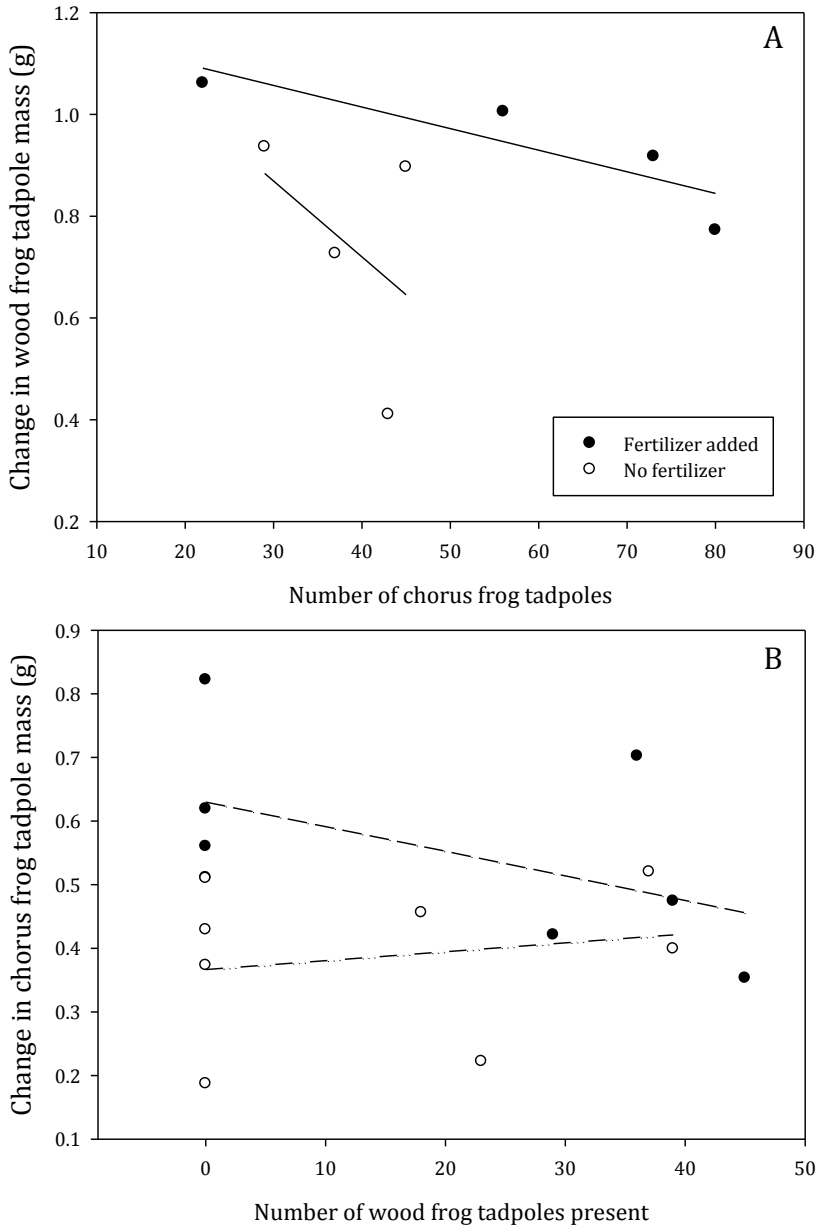


Figure 3-6: Relationship between mean change in tadpole mass, from May 13 to June 25, and interspecific tadpole abundance of surviving tadpoles on June 25 for A) wood frog tadpoles plotted against the number of chorus frog tadpoles, and B) chorus frog tadpoles plotted against the number of wood frog tadpoles. Points are replicate tanks from the different treatments, symbols in B) are the same as for A); slopes of lines shown in A) were homogeneous, and trend lines are shown by dotted lines in B).

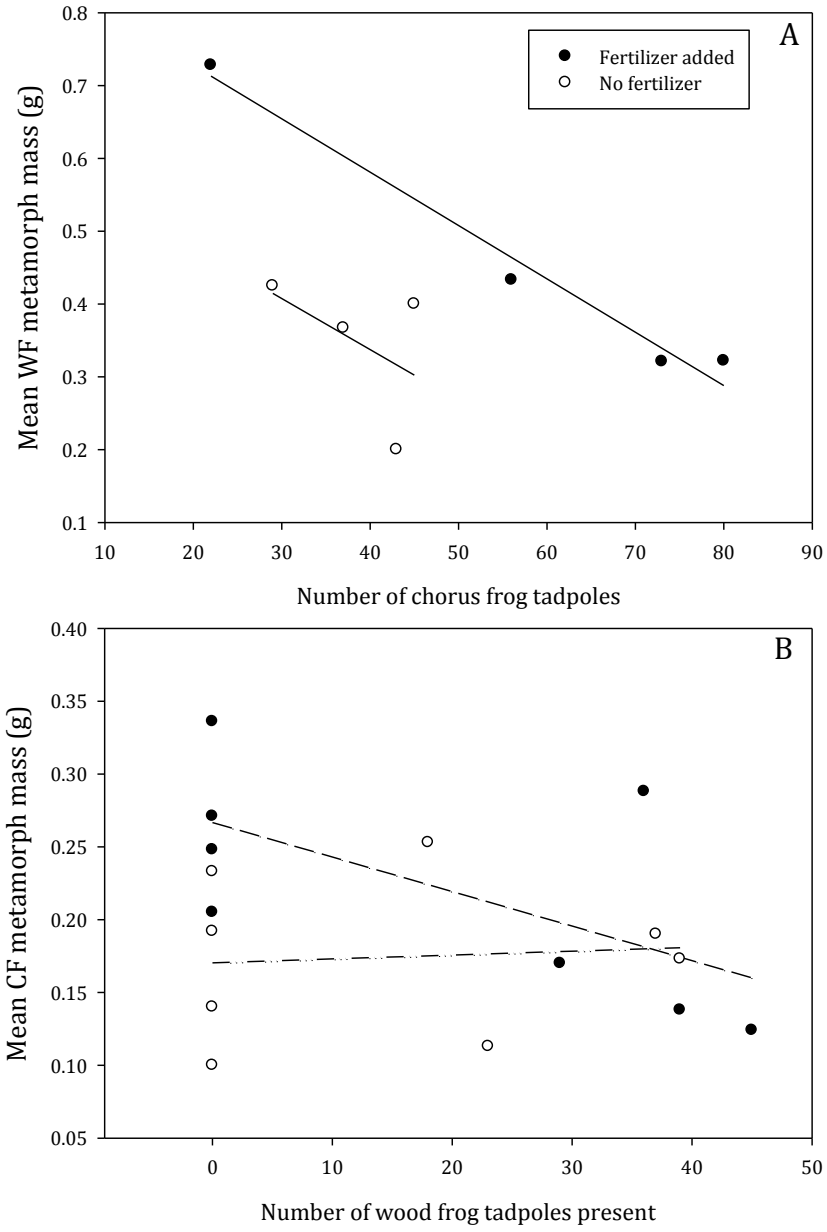


Figure 3-7: Relationship between mean mass at metamorphosis for individuals emerging by 9 July and interspecific tadpole abundance on June 25 for A) wood frogs as a function of the number of chorus frog tadpoles present, and B) chorus frogs as a function of the number of wood frog tadpoles present. Points are replicate tanks from the different treatments. Symbols in B) are as in A). Slopes in A) were homogeneous, and trend lines are shown by dotted lines in B).

Literature Cited

Akin G. W. 1966. Self-inhibition of growth in *Rana pipiens* tadpoles. Physiological Zoology **39**:341-356.

Alberta Environmental Protection. 1996 Alberta state of the environment report: aquatic ecosystems. Edmonton, Alberta: Alberta Environmental Protection; 1996.

Alford R. A. 1999. Ecology: resource use, competition, and predation. Pages 240-278 *In* R. W. McDiarmid, editor. Tadpoles: biology of anuran larvae, University of Chicago Press, Chicago.

Alford R. A. 1989a. Effects of parentage and competitor phenology on the growth of larval *Hyla chrysoscelis* . Oikos **54**:325-330.

Alford R. A. 1989b. Competition between larval *Rana palustris* and *Bufo americanus* is not affected by variation in reproductive phenology. Copeia **1989**:993-1000.

Allen E. W., E. E. Prepas, S. Gabos, W. Strachan, and W. Chen. 2003. Surface water chemistry of burned and undisturbed watersheds on the Boreal Plain: an ecoregion approach. Journal of Environmental Engineering and Science **2**:S73-S86.

Altig R., M. R. Whiles, and C. L. Taylor. 2007. What do tadpoles really eat? assessing the trophic status of an understudied and imperiled group of consumers in freshwater habitats. Freshwater Biology **52**:386-395.

Bancroft B. A., N. J. Baker, and A. R. Blaustein. 2008a. A meta-analysis of the effects of ultraviolet B radiation and its synergistic interactions with pH, contaminants, and disease on amphibian survival. Conservation Biology **22**:987-996.

Bancroft B. A., N. J. Baker, C. L. Searle, T. S. Garcia, and A. R. Blaustein. 2008b. Larval amphibians seek warm temperatures and do not avoid harmful UVB radiation. Behavioral Ecology **19**:879-886.

Berven K. A. 1990. Factors affecting population fluctuations in larval and adult stages of the wood frog (*Rana sylvatica*). Ecology **71**:1599-1608.

Bourassa N., A. Cattaneo. 2000. Responses of a lake outlet community to light and nutrient manipulation: effects on periphyton and invertebrate biomass and composition. Freshwater Biology **44**:629-639.

Brown M. E. 1946. The growth of brown trout (*Salmo trutta* Linn.) II. The growth of two-year-old trout at a constant temperature of 11.5°C. Journal of Experimental Biology **22**:130-144.

Brylinsky M. 1993. Evaluation of controlled fertilization of acidified wetlands for enhancement of waterfowl production. :.

Burton E. C., M. J. Gray, A. C. Schmutzer, and D. L. Miller. 2009. Differential responses of postmetamorphic amphibians to cattle grazing in wetlands. Journal of Wildlife Management **73**:269-277.

Carpenter S. R., N. F. Caraco, D. L. Correll, R. W. Howarth, A. N. Sharpley, and V. H. Smith. 1998. Nonpoint pollution of surface waters with phosphorus and nitrogen. Ecological Applications **8**:559-568.

Celada J. D., A. Aguilera, J. M. Carral, M. Saez-Royuela, P. M. Melendre, and J. R. Perez. 2007. Effects of stocking density on survival and growth of juvenile tench (Tinca tinca L.). Aquaculture International **15**:461-465.

Chase J. M. 2003. Strong and weak trophic cascades along a productivity gradient. Oikos **101**:187-195.

Croteau M. C., M. A. Davidson, D. R. S. Lean, and V. L. Trudeau. 2008. Global Increases in Ultraviolet B Radiation: Potential Impacts on Amphibian Development and Metamorphosis. Physiological and Biochemical Zoology **81**:743-761.

Dickman M. 1968. Effect of Grazing by Tadpoles on Structure of a Periphyton Community. Ecology **49**:1188-&.

Eaves SE. The distribution and abundance of amphibians across land-use types in Alberta's Aspen Parkland. University of Alberta; 2004. 134 p.

Faragher S. G., R. G. Jaeger. 1998. Tadpole bullies: examining mechanisms of competition in a community of larval anurans. Canadian Journal of Zoology **76**:144-153.

Fauth J. E., W. J. Resetarits. 1991. Interactions between the salamander *Siren intermedia* and the keystone predator *Notophthalmus viridescens* . Ecology **72**:827-838.

Glennemeier K. A., R. J. Denver. 2002. Small changes in whole-body corticosterone content affect larval Rana pipiens fitness components General and comparative endocrinology **127**:16-25.

Gosner K. L. 1960. A simplified table for staging anuran embryos and larvae with notes on identification. Herpetologea **16**:183-190.

Griffis-Kyle K. L. 2007. Sublethal effects of nitrite on eastern tiger salamander (Ambystoma tigrinum tigrinum) and wood frog (Rana sylvatica) embryos and larvae: implications for field populations. Aquatic Ecology **41**:119-127.

Gromko M. H., F. S. Mason, and S. J. Smith-Gill. 1973. Analysis of crowding effect in *Rana pipiens* tadpoles. Journal of Experimental Zoology **186**:63-71.

Hamer A. J., J. A. Makings, S. J. Lane, and M. J. Mahony. 2004. Amphibian decline and fertilizers used on agricultural land in south-eastern Australia. Agriculture Ecosystems & Environment **102**:299-305.

Harris M. L., C. A. Bishop, J. Struger, M. R. van den Heuvel, G. J. Van der Kraak, D. G. Dixon, B. Ripley, and J. P. Bogart. 1998. The functional integrity of northern leopard frog (Rana pipiens) and green frog (Rana clamitans) populations in orchard wetlands. I. Genetics, physiology, and biochemistry of breeding adults and young-of-the-year. Environmental Toxicology and Chemistry **17**:1338-1350.

Harris P. M. 1995. Are autoecologically similar species also functionally similar? A test in pond communities. Ecology **76**:544-552.

Hauer F. R., C. N. Spencer. 1998. Phosphorus and nitrogen dynamics in streams associated with wildfire: A study of immediate and longterm effects. International Journal of Wildland Fire **8**:183-198.

Healey M., D. Thompson, and A. Robertson. 1997. Amphibian communities associated with billabong habitats on the Murrumbidgee floodplain, Australia. Australian Journal of Ecology **22**:270-278.

Hecnar S. J. 1995. Acute and chronic toxicity of ammonium-nitrate fertilizer to amphibians from southern Ontario. Environmental Toxicology and Chemistry **14**:2131-2137.

Hillebrand H. 2002. Top-down versus bottom-up control of autotrophic biomass - a meta-analysis on experiments with periphyton. Journal of the North American Benthological Society **21**:349-369.

Iwai N., T. Kagaya. 2007. Positive indirect effect of tadpoles on a detritivore through nutrient regeneration. Oecologia **152**:685-694.

Jofre G., C. Reading, and I. di Tada. 2007. Habitat selection in the Pampa de Achala toad, Bufo achalensis. Amphibia-Reptilia **28**:129-138.

Johnson P. T. J., J. M. Chase. 2004. Parasites in the food web: linking amphibian malformations and aquatic eutrophication. Ecology Letters **7**:521-526.

Johnson P. T. J., J. M. Chase, K. L. Dosch, R. B. Hartson, J. A. Gross, D. J. Larson, D. R. Sutherland, and S. R. Carpenter. 2007. Aquatic eutrophication promotes pathogenic infection in amphibians. Proceedings of the National Academy of Sciences of the United States of America **104**:15781-15786.

Jorgensen E. H., J. S. Christiansen, and M. Jobling. 1993. Effects of Stocking Density on Food-Intake, Growth-Performance and Oxygen-Consumption in Arctic Charr (Salvelinus-Alpinus). Aquaculture **110**:191-204.

Kiffney P. M., J. S. Richardson. 2001. Interactions among nutrients, periphyton, and invertebrate and vertebrate (Ascaphus truei) grazers in experimental channels. Copeia :422-429.

Knutson M. G., W. B. Richardson, D. M. Reineke, B. R. Gray, J. R. Parmelee, and S. E. Weick. 2004. Agricultural ponds support amphibian populations. Ecological Applications **14**:669-684.

Kolozsvary M. B., R. K. Swihart. 1999. Habitat fragmentation and the distribution of amphibians: patch and landscape correlates in farmland. Canadian Journal of Zoology **77**:1288-1299.

Kupferberg S. J., J. C. Marks, and M. E. Power. 1994. Effects of Variation in Natural Algal and Detrital Diets on Larval Anuran (Hyla-Regilla) Life-History Traits. Copeia :446-457.

Leibold M. A., H. M. Wilbur. 1992. Interactions between food-web structure and nutrients on pond organisms. Nature **360**:341-343.

Levesque C., S. K. Juniper, and J. Marcus. 2003. Food resource partitioning and competition among alvinellid polychaetes of Juan de Fuca Ridge hydrothermal vents. Marine Ecology-Progress Series **246**:173-182.

Licht L. E. 1967. Growth inhibition in crowded tadpoles - intraspecific and interspecific effects. Ecology **48**:736-750.

Loeb S. L. 1981. An 'in situ' method for measuring the primary productivity and standing crop of the epilithic periphyton community in lentic systems. Limnology and Oceanography **26**:394-399.

Loman J., B. Lardner. 2006. Does pond quality limit frogs *Rana arvalis* and *Rana temporaria* in agricultural landscapes? A field experiment. Journal of Applied Ecology **43**:690-700.

Mann R. M., R. V. Hyne, C. B. Choung, and S. P. Wilson. 2009. Amphibians and agricultural chemicals: review of the risks in a complex environment. Environmental Pollution **157**:2903-2927.

Marsh D. M., P. C. Trenham. 2001. Metapopulation dynamics and amphibian conservation. Conservation Biology **15**:40-49.

Melanson R., F. Payne. 1988. Wetlands habitat management through nutrient enrichment. :.

Morin P. J., E. A. Johnson. 1988. Experimental studies of asymmetric competition among anurans. Oikos **53**:398-407.

Murray D. L. 1990. The Effects of Food and Density on Growth and Metamorphosis in Larval Wood Frogs (Rana-Sylvatica) from Central Labrador. Canadian Journal of Zoology-Revue Canadienne De Zoologie **68**:1221-1226.

Oldham R. S., D. M. Latham, D. HiltonBrown, M. Towns, A. S. Cooke, and A. Burn. 1997. The effect of ammonium nitrate fertilizer on frog (*Rana temporaria*) survival. Agriculture Ecosystems & Environment **61**:69-74.

Pahkala M., A. Laurila, and J. Merila. 2003. Effects of ultraviolet-B radiation on behaviour and growth of three species of amphibian larvae. Chemosphere **51**:197-204.

Papoutsoglou S. E., G. Tziha, X. Vrettos, and A. Athanasiou. 1998. Effects of stocking density on behavior and growth rate of European sea bass (Dicentrarchus labrax) juveniles reared in a closed circulated system. Aquacultural Engineering **18**:135-144.

Pehek E. L. 1995. Competition, pH, and the ecology of larval *Hyla andersonii*. Ecology **76**:1786-1793.

Perrin C. J., M. L. Bothwell, and P. A. Slaney. 1987. Experimental Enrichment of a Coastal Stream in British-Columbia - Effects of Organic and Inorganic Additions on Autotrophic Periphyton Production. Canadian Journal of Fisheries and Aquatic Sciences **44**:1247-1256.

Planas D., M. Desrosiers, S. R. Groulx, S. Paquet, and R. Carignan. 2000. Pelagic and benthic algal responses in eastern Canadian Boreal Shield lakes following harvesting and wildfires. Canadian Journal of Fisheries and Aquatic Sciences **57**:136-145.

Porej D., M. Micacchion, and T. E. Hetherington. 2004. Core terrestrial habitat for conservation of local populations of salamanders and wood frogs in agricultural landscapes. Biological Conservation **120**:399-409.

Prepas E. E., D. Planas, J. J. Gibson, D. H. Vitt, T. D. Prowse, W. P. Dinsmore, L. A. Halsey, P. M. McEachern, S. Paquet, G. J. Scrimgeour, W. M. Tonn, C. A. Paszkowski, and K. Wolfstein. 2001. Landscape variables influencing nutrients and phytoplankton communities in Boreal Plain lakes of northern Alberta: a comparison of wetland- and upland-dominated catchments. Canadian Journal of Fisheries and Aquatic Sciences **58**:1286-1299.

Relyea R. A. 2003. Predator cues and pesticides: A double dose of danger for amphibians. Ecological Applications **13**:1515-1521.

Relyea R. A., N. M. Schoeppner, and J. T. Hoverman. 2005. Pesticides and amphibians: The importance of community context. Ecological Applications **15**:1125-1134.

Richards C. M. 1958. The inhibition of growth in crowded *Rana pipiens* tadpoles. Physiological Zoology **31**:138-151.

Rouse J. D., C. A. Bishop, and J. Struger. 1999. Nitrogen pollution: An assessment of its threat to amphibian survival. Environmental Health Perspectives **107**:799-803.

Runkova G. G., Z. L. Stepanova, and L. A. Koval'chuk. 1974. Organ specificity in the action of metabolites of amphibian larvae on their endogenous metabolism under conditions of increased population density. Doklady Akademii Nauk SSSR **217**:328-329.

Russell A. P., A. M. Bauer. 2000. The amphibians and reptiles of Alberta: a field guide and primer of boreal herpetology. University of Calgary Press, Calgary.

Schiesari L., E. E. Werner, and G. W. Kling. 2009. Carnivory and resource-based niche differentiation in anuran larvae: implications for food web and experimental ecology. Freshwater Biology **54**:572-586.

Schmutzer A. C., M. J. Gray, E. C. Burton, and D. L. Miller. 2008. Impacts of cattle on amphibian larvae and the aquatic environment. Freshwater Biology **53**:2613-2625.

Scrimgeour G. J., W. M. Tonn, C. A. Paszkowski, and C. Goater. 2001. Benthic macroinvertebrate biomass and wildfires: evidence for enrichment of boreal subarctic lakes. Freshwater Biology **46**:367-378.

Semlitsch R. D. 2000. Principles for management of aquatic-breeding amphibians. Journal of Wildlife Management **64**:615-631.

Smith D. C. 1987. Adult recruitment in chorus frogs: effects of size and date at metamorphosis. Ecology **68**:344-350.

Smith G. R., H. A. Dingfelder, and D. A. Vaala. 2004. Asymmetric competition between *Rana clamitans* and *Hyla versicolor* tadpoles. Oikos **105**:626-632.

Smith G. R., M. A. Waters, and J. E. Rettig. 2000. Consequences of embryonic UV-B exposure for embryos and tadpoles of the plains leopard frog. Conservation Biology **14**:1903-1907.

Smith M. W. 1969. Changes in environment and biota of a natural lake after fertilization. Journal of the Fisheries Research Board of Canada **26**:3101-3132.

Smith S. M., K. D. Lee. 2006. Responses of periphyton to artificial nutrient enrichment in freshwater kettle ponds of Cape Cod National Seashore. Hydrobiologia **571**:201-211.

Smith-Gill S. J., D. E. Gill. 1978. Curvilinearities in the competition equations: an experiment with ranid tadpoles. American Naturalist **112**:557-570.

Spencer C. N., F. R. Hauer. 1991. Phosphorus and Nitrogen Dynamics in Streams during a Wildfire. Journal of the North American Benthological Society **10**:24-30.

Steinwascher K. 1978. The effect of coprophagy on the growth of *Rana catesbeiana* tadpoles. Copeia **1978**:130-134.

Stepanova Z. L. 1974. The chemical nature of the products of metabolism of amphibian larvae, excreted into the water. Soviet Journal of Ecology (Ekologiya) **5**:148-149.

SYSTAT Software INC. 2007. Systat. 12.02. San Jose, California.

Syvaranta J., R. I. Jones. 2008. Changes in feeding niche widths of perch and roach following biomanipulation, revealed by stable isotope analysis. Freshwater Biology **53**:425-434.

Trauth J. B., S. E. Trauth, and R. L. Johnson. 2006. Best management practices and drought combine to silence the Illinois chorus frog in Arkansas. Wildlife Society Bulletin **34**:514-518.

Welch D. W., T. R. Parsons. 1993. ^{13}C - ^{15}N values as indicators of trophic position and competitive overlap for Pacific salmon (*Oncorhynchus* spp.). Fisheries Oceanography **2**:11-23.

Werner E. E. 1994. Ontogenetic scaling of competitive relations: size-dependent effects and responses in two anuran larvae. Ecology **75**:197-213.

Wilbur H. M. 1987. Regulation of structure in complex-systems - experimental temporary pond communities. Ecology **68**:1437-1452.

Wilbur H. M. 1977. Density-dependent aspects of growth and metamorphosis in *Bufo americanus*. Ecology **58**:196-200.

Wood S. L. R., J. S. Richardson. 2009. Impact of sediment and nutrient inputs on growth and survival of tadpoles of the Western Toad. Freshwater Biology **54**:1120-1134.

Young J. E., G. A. Sanchez-Azofeifa, S. J. Hannon, and R. Chapman. 2006. Trends in land cover change and isolation of protected areas at the interface of the southern boreal mixedwood and aspen parkland in Alberta, Canada. Forest Ecology and Management **230**:151-161.

Chapter 4: The effect of agriculture on anuran larvae

Introduction

Canadian farmers managed 67.6 million hectares of land in 2006; this agricultural land comprises 7.3% of the Canadian landscape (Statistics Canada 2006). Within Alberta, agricultural land comprises 33% of the landscape (21.1 million ha); of which 9.7 million ha is cropland (46% of farmland), 6.5 million ha native pasture (31%), 2.5 million ha tame or seeded pasture (12%) and 1.5 million ha experiences other uses such as woodlots and wetlands (Statistics Canada 2006). These agro-landscapes support many species of Canada's native fauna (Javorek et al. 2007) but Vitousek et al. (1997) identified land transformation as the primary force driving global loss of biological diversity.

Among those species being lost, amphibians are declining faster than any other vertebrate group (Stuart et al. 2004). Collins and Storfer (2003) identified agriculture as one of the leading causes for the loss of amphibian species. Wetlands within agricultural lands have the capacity to support amphibian populations (Knutson et al. 2004), but agricultural practices have the capacity to reduce and impair critical amphibian habitats. Amphibians using wetlands on or near agricultural lands are exposed to a number of threats. Those threats include 1) changes to upland, terrestrial habitat, including loss and fragmentation; and 2) changes to wetlands themselves including loss, reduced water quality due to chemical and sediment run-off from the modified landscape or altered hydrology, and changes to aquatic and riparian vegetation.

Of these threats, changes to habitats surrounding wetlands directly affect terrestrial life-stages of amphibians. For northern temperate amphibians with biphasic life-cycles, the dependence on wetlands may require them to cross agricultural lands in order to reach breeding ponds. Draining or filling of wetlands in crop lands increases the distance between breeding ponds and suitable hibernation sites for terrestrial hibernators, and restricts population size and limits the number of populations. Alterations to vegetation due to intensive grazing and seeding of grasses and other grains may alter the value and permeability of grazed areas to amphibian movement (Turner 1989, Wiens 1997, Gibbs 1998, deMaynadier and Hunter 1999, Rothermel and Semlitsch 2002, Vos et al. 2007, Janin et al. 2009). Farmland intersecting dispersal routes for amphibians can fragment individual populations, and limit genetic exchange and the potential for recovery of isolated populations (Kolozsvary and Swihart 1999, Semlitsch 2000, Marsh and Trenham 2001). Terrestrial survival may also be reduced in farmlands as a result of reduced cover leading to greater predation risk, but also due to increased exposure to desiccation (Rothermel and Semlitsch 2002, Gray and Smith 2005, Mazerolle and Desrochers 2005), and increased exposure to harmful chemicals (Oldham et al. 1997) or machinery (Classen et al. 1996, Oppermann et al. 2000, Oppermann 2007, as cited in Humbert et al. 2009). In sum, amphibian breeding populations in agricultural landscapes may be reduced as a result of loss of required habitats or reduced connectivity with habitats or populations.

The effect of agriculture on water quality is of growing concern to aquatic life (Carpenter et al. 1998) and may impact amphibians using these aquatic habitats. Changes in water quality are likely to influence anuran larval performance (i.e. growth, survival and size at metamorphs) and for those that have terrestrial life

stages, indirectly the fitness of individuals that are recruited into terrestrial populations. Agriculture can affect water quality either through increased inputs of nutrients, sediments and agricultural chemicals, but can also change the hydrology and vegetation in the surrounding wetlands. Organic sediments provide a valuable source of nutrients for aquatic species, but high inputs of inorganic sediments increase turbidity and reduce tadpole food resources through reduced primary production (Wood and Richardson 2009). Agrochemicals such as pesticides (Relyea 2003), herbicides (Relyea et al. 2005) and fertilizers (Hamer et al. 2004) reduce survival of amphibians, but can also impair those that survive to metamorphosis, via feminization (Hayes et al. 2002), and limb deformities (Ouellet et al. 1997). In addition, combined stressors can further reduce survival of larvae; as vigilance in the presence of predators is reduced when exposed to pesticides (Berrill et al. 1993, Relyea 2003). Nitrogen and phosphorous inputs from fertilizers and cattle feces can lead to eutrophication of aquatic systems. Eutrophication promotes pathogenic infections, such as parasites, causing limb deformities and mortality in amphibians (Johnson et al. 2007). Growth and survival of anuran larvae are affected by resource abundance and quality but also through competition for resources (as reviewed by Alford 1999). If inputs from agriculture affect the growth and survival of larvae, it may also affect the fitness of metamorphs that are recruited into terrestrial populations, and ultimately reduce breeding populations and thereby subsequent larval densities.

Changes to hydrology of wetlands can reduce the growth and survival of anuran larvae. A reduction of pond hydroperiods can increase larval survival as the diversity and abundance of predators tends to increase with hydroperiod (Woodward 1983, Pearman 1995, Skelly 1995). However, these benefits may be lost

as a result of increased competition for resources (Relyea 2002, Resetarits et al. 2004), lower developmental rates, or declining water quality (as discussed above). Agricultural practices that promote drying and equal distribution of water across crop fields are eliminating ephemeral and temporary ponds from forming in the spring (Trauth et al. 2006). Similarly, these reductions to hydroperiods in other wetlands may create habitat sinks such that breeding efforts may result in failed recruitment as individuals are incapable of completing metamorphosis before pond drying. Cattle grazing, unlike crop production, may prolong hydroperiods due to livestock removal of wetland and upland plants effectively reducing evapotranspiration and increasing run-off (Bremer et al. 2001, Frank 2003). A simulation of the effects of land-use on water levels in the northern prairie pothole region (Voldseth et al. 2007) found that grazed sites had higher wetland water levels compared to ungrazed grasslands, while cultivated crops were intermediate. Other changes associated with agriculture include changes to wetland vegetation through effects of nutrients and hydrology (such as cattails - Newman et al. 1998, Vaccaro 2005). Reduction of emergent vegetation by mowing or grazing and trampling by cattle alters detritus that acts as feeding sites for larval amphibians, but also reduces attachment substrates for eggs and protected calling sites for breeding anurans (Healey et al. 1997). The loss of emergent vegetation can increase UV irradiation which may affect larval survival (Blaustein et al. 1998, Bancroft et al. 2008) and performance (Bruggeman et al. 1998, Smith et al. 2000, Pahkala et al. 2001, Belden and Blaustein 2002, Pahkala et al. 2003). Whether changes to the hydrology and vegetation affect anuran success depends upon the interplay of resource availability and the effects of water quality on larval development and survival.

My work focuses on amphibian populations in the Beaver Hills Region, located within the aspen parkland ecoregion of central Alberta, Canada. The soils, which are chernozems and luvisols, have a high organic content, and are thus excellent for agricultural production. The main land-uses in the Beaver Hills include livestock grazing (cattle and bison), cultivation of crops and rural residences. A large reduction in wetlands within this ecoregion has occurred owing to agriculture and urbanization with 21-48% of remaining wetlands disappearing between 1970 and 1990 (Alberta Environmental Protection 1996, but see Young et al. 2006). Eaves (2004) found that two anurans, *Pseudacris maculata* and *Lithobates sylvaticus*, in the Beaver Hills had reduced abundances of terrestrial stages at wetlands within crop and pasture lands compared to natural ponds surrounded by forested uplands. She concluded that landscape-level features influenced amphibian abundances but did not evaluate the performance of larvae at each location. Differences in wetland quality associated with land-use practices could have affected the survival of larvae or reduced the fitness of those that emerged which combined with landscape-level impacts could reduce the abundance and dispersal of terrestrial frogs.

The objectives of this study were to examine larval performances in relation to land-use and assess whether the reduced abundances of terrestrial amphibians observed by Eaves (2004) in the Beaver Hills region occurs as a result of reduced larval performance associated with land-use. Eaves (2004) found that both boreal chorus frog and wood frog abundance based on breeding calls and pitfall traps were lowest at crop ponds, then pasture sites, and highest at natural ponds surrounded by wetland vegetation. Studies on the impacts of agriculture on amphibian populations have generally focused on post-metamorphic community structure and measures of relative abundance (Healey et al. 1997, Knutson et al. 2004, Gagne and

Fahrig 2007, Burton et al. 2009). Few studies have examined the linkages between agricultural practices, aquatic environments, and the performance of larval amphibians (Loman and Lardner 2006, Schmutzer et al. 2008) and how they might affect terrestrial populations. To address this lack of knowledge, I measured larval densities and larval performance at ponds surrounded by cropland and pastures, and compared those to ponds located in Elk Island National Park to determine if agriculture affects anuran populations in the aspen parkland using temporary wetlands. I have chosen to focus on the larval ecology of the boreal chorus frog, *Pseudacris maculata*. Despite this species being the most widespread, and likely most abundant, amphibian in Alberta (Russell and Bauer 2000), we know little about its larvae. As wood frogs, *Lithobates sylvaticus*, are common and co-occur at most agricultural ponds in this region. I compared patterns observed for chorus frogs with those of wood frogs to determine the generality of effects of land-use on anurans. Together, larval density and performance surveys were aimed at determining whether wetlands in the agricultural area of the aspen parkland of Alberta serve as sites for successful amphibian reproduction and whether larval performance is affected by wetland quality.

If agricultural practices fragment amphibian populations (Kolozsvary and Swihart 1999) and limit adult survival, then breeding populations should be lower and should correspond to lower larval densities at agricultural sites compared to those in the national park. I used larval densities at each wetland as they reflect successful breeding attempts. Though egg number or masses may be a better indicator of adult abundance, CF masses were difficult to locate prior to hatching. Larval densities should correlate to adult abundances given the number of eggs deposited depends on the number of females at a location, at least for wood frogs

(Crouch and Paton 2000, Stevens and Paszkowski 2004). This relationship appears to hold true for chorus frogs, based on calling code and number of egg masses found at six sites in 2007 (R^2 = 0.71, p = 0.036). As cattle grazing and crop cultivation are associated with changes in vegetation, and in the case of crops reduced abundance or proportion of area as wetlands, I expected agricultural landscapes to have reduced abundance of wetlands, or the amount of water on the landscape, along with reduced unmanaged vegetation, such as forested patches. Wetlands and forested patches are known to be important to both boreal chorus frogs and to wood frogs for breeding, foraging and overwintering (Heatwole 1961, Whitaker 1971, Smith 1983, deMaynadier and Hunter 1998, Knutson et al. 1999, Constible et al. 2001, Regosin et al. 2003, Porej et al. 2004, Gibbs et al. 2005, Baldwin et al. 2006). The area covered by these two habitats within 1 km of breeding ponds are expected to be lowest in croplands, followed by pastures, and highest in natural protected areas, whereas habitats such as crop and pastures surrounding wetlands would be higher at agricultural ponds. In addition to wetlands and forested patches being reduced around survey ponds, I predicted that the distance (absolute or effective) from the breeding pond to the nearest neighbouring wetland and to the nearest forested patch would be higher in agricultural lands as a result of wetland loss. Again I predicted that these distances would be greater in crop lands and lower in the park, as found by Eaves (2004) in the same area. Together, through a reduction of suitable habitat, reduced connectivity, and greater intensity of upland disturbance surrounding wetlands (such as mowing as reviewed by Humbert et al. 2009), I expect terrestrial survival to be lower in agricultural lands and therefore fewer animals use them as breeding sites which will result in lower larval densities at these agricultural ponds.

Performance of larvae within agricultural ponds may contribute to reduced survival and fitness of individuals emerging from these sites, and therefore result in further reduction in density. I used the performance of anuran larvae, as measured by growth, survival and size at metamorphosis, to determine whether agriculture affects anuran density as these metrics are related to the number and fitness of metamorphs entering terrestrial populations (Smith 1987, Berven 1990, Altwegg and Reyer 2003). Anuran larval growth rates are determined by the allocation of resources to growth, but are also dependent upon the aquatic environments in which tadpoles are developing (Arendt 1997). Survival of anuran larvae is difficult to measure without the use of enclosures or accurate counts of deposited eggs. To address survival, I used relative survival of larvae to metamorphosis using catch per unit effort of metamorphs divided by larval densities to compare effects of pond environment as influenced by agriculture and tadpole densities. I also used metamorph size to assess the impacts of agriculture and tadpole densities, because it is a good predictor of survival of metamorphs (Smith 1987, Altwegg and Reyer 2003, Chelgren et al. 2006) and fitness (Smith 1987, Berven 1990, Altwegg and Reyer 2003). As both grazing and crop cultivation increase sediment and fertilizer inputs into associated wetlands, I expect to find that nutrient concentrations are higher in agricultural ponds. Increased sedimentation and runoff may result in potentially toxic nitrogen loads that decrease tadpole growth, survival and result in smaller metamorphs (Gillespie 2002, Schmutzer et al. 2008, Wood and Richardson 2009). Therefore I expected that larval performance and metamorph production to be reduced at agricultural ponds relative to park ponds. Together these would produce smaller populations which may persist, but are at greater risk of extirpation (Gilpin and Soule 1986).

Agriculture may negatively impact larval performance through wetland quality, but predicted lower larval densities can also alleviate the negative effects of density within larval assemblages (Berven 1990, Wilbur 1997, Altwegg and Reyer 2003, Loman 2004) and, therefore, larval performance may not be reduced as predicted above. Smith (1987, 1983, 1990) showed that greater intraspecific densities of *P. maculata*, formerly *P. triseriata* (Moriarty and Cannatella 2004, Lemmon et al. 2007), reduced growth, survival, and size at metamorphosis. Interspecific competition, which is suspected to occur with sympatric wood frog tadpoles (see Chapters 2 and 3), is also expected to reduce boreal chorus frog larval performance. Therefore, to assess the effects of density on performance, I also compared the performance of chorus frogs against intraspecific densities and against wood frog densities. I predict higher larval growth, survival and size at metamorphosis at lower larval densities. If interspecific competition is weaker than intraspecific competition as expected, then I also predict that intraspecific densities will have a greater effect on performance of chorus frog larvae.

This study addresses whether larval anuran performance is impacted by agricultural practices and provides insight into the carry-over effects of larval-rearing environments on the abundance of terrestrial anurans and ultimately larval densities at wetlands within the Beaver Hills region of Alberta.

Methods

Study sites

I conducted this study over two years (2006-2007) within the aspen parkland ecoregion of central Alberta, Canada. I selected six temporary ponds (Class II in Stewart and Kantrud 1971) within Elk Island National Park (EINP 53°35’34”N,

112°52’15”W), hereafter referred to as park ponds (Figure 4-1). Ponds were chosen (Appendix Table 1-1) based on four criteria: ease of access, generally next to a paved or gravel road; size (< 0.1 ha); presence of calling boreal chorus frogs (CF) and wood frogs (WF) with evidence of eggs or larvae; and prior sampling in 2005 (Chapter 2). Calling frogs were first heard on April 13 and 24 in 2006 and 2007, respectively. Wood frog eggs were more obvious than chorus frog eggs and were found on April 15 2006 and April 24 2007. Chorus frog eggs were more difficult to locate but were found on April 28 2006 near hatching and on April 26 2007 which had recently been deposited within some of the ponds. All ponds had emergent sedge vegetation (Cyperaceae – *Carex* spp.) and were often bordered by tall grasses (Poaceae – *Poa* spp. and *Glyceria* spp.), and only one pond had cattails (*Typha latifolia*). Upland vegetation consisted mainly of trembling aspen (*Populus tremuloides*) and balsam poplar (*Populus balsamifera*) with some willows (*Salix* spp.) within the pond or along the shoreline.

I also surveyed a total of nine (≤ eight in a given year, Appendix Table 1-1, Figure 4-1) temporary to semi-permanent ponds (Class II to IV in Stewart and Kantrud 1971) in or near agricultural land (collectively referred to as agriculture ponds): four pasture ponds (three with cattle access, one with sheep access) and five crop ponds. Two of the crop ponds occurred within lands used for haying, and the other three bordered row crops (canola, barley, and corn). I selected ponds based on the same criteria as for park ponds, however, these ponds also needed to be either within or within 20 m of either crop field or a pasture. Difficulties in finding ponds with frogs or larvae, and that fit the other criteria, resulted in three ponds with larger areas, 0.16 to 0.25 ha. Agriculture ponds differed in extent of riparian

vegetation, but all contained emergent shoreline vegetation composed of sedges, tall grasses or cattails (Appendix Table 2-2).

Larval amphibians

Each year amphibian larval density was measured once at every pond: 21-28 June 2006 and 13-14 June 2007. Eight to 15 random locations were selected in each pond based on a 1 m^2 grid; the location had to contain water and be > 5 m from another sample location. I used a circular sweep net, 37 cm wide, and made three 1 m long sweeps passing over the same area. The contents of sweeps were placed in buckets and contents were identified after the last sample. The volume sampled was determined from depth markings on the sweep net using the equation from Chapter 2. When water was higher than the net, a volume of 107.5 L was assigned and the sweep was along the pond bottom, as tadpoles usually flee into the sediment (pers. observation). Pond tadpole density (CF and WF) for each date was calculated as the average density across sample locations.

Larval performance metrics measured were growth rates, survival, and size at metamorphosis. To estimate growth, I conducted additional haphazard larval sampling every other week. I measured tadpole body length (tip of snout to where limbs emerge at base of tail) using callipers or from digital photographs for the first 10 larvae per species captured with dip nets. Measurements from photographs were done relative to a 1 mm grid laid below tadpoles using Gimp 4.2.3 photo editor. Growth rate was determined from linear regressions of body length over date; generally up to the two leg stage (Gosner stages 39-40) as growth ceased to be linear after this point. To measure relative survivorship of tadpoles to metamorphosis and size at metamorphosis at each location, I conducted surveys for

metamorphs starting the last week of June through the end of July in each year. For size at metamorphosis, I included only those metamorphs that still showed signs of tail resorbtion as those without tail buds were likely to have grown since metamorphosis. If the pond held water long enough to produce metamorphs, surveyors counted the number of metamorphs by walking the pond perimeter (entire perimeter for small ponds or up to 50% of the largest ponds). However, at dry ponds surveyors walked a transect (< 50 m) through the middle of the pond. Size (snout-to-urostyle length – SUL) and developmental stage were recorded for each metamorph captured during these surveys. Relative production of metamorphs across ponds was estimated by catch per unit effort (CPUE = # metamorphs divided by minutes searched). To evaluate proportional survival at a location I adjusted CPUE by tadpole density (adj. CPUE = CPUE/tadpole density), so that I could compare production based on tadpole density.

Landscape variables

Upland habitat composition and the connectivity of wetlands to upland habitat were assessed using ArcMAP 9.3 to classify land cover types building on work by Eaves (2004). I digitized land cover from digital orthophotos taken in the spring of 2007 of Strathcona and Lamont counties and Elk Island National Park (1:20,000). For the 15 ponds (four pasture, five crop, and six park sites), I created a 1 km circular buffer from the center of the wetland and classified land within these buffers based on seven cover classes: forest (tree stands of greater than 0.01 ha in size, including fence rows), crop, pasture (native or tame pasture), road (paved and gravel treated equally), water (lakes, ponds, dugouts and streams), non-forested vegetation (shrubs and tall grasses which might have included wet meadows) and human constructions (houses, barns, and lane ways). I chose a 1 km buffer as it

matched that used by Eaves (2004), and CF abundance is also best predicted at this scale (Browne et al. 2009). The 1 km buffer also encompasses the terrestrial migration ranges of the two anuran species of interest (Spencer 1964, Berven and Grudzien 1990, Regosin et al. 2005, Baldwin et al. 2006). Wetland connectivity and connectivity to natural habitat features was assessed by nearest neighbour distance between the edge of a survey pond to the edge of the nearest water body and the nearest forested patch using ArcMap 9.3. I did not assess the distance to roads as I had used them in selecting wetlands.

Local pond variables

Abiotic environment

Variables considered part of the abiotic environment included those associated with water quality and those associated with pond morphometry and hydroperiod. I measured water quality variables twice in 2006 (May 30-June 7 and June 21-27) and once in 2007 (June 11-13). Sampling occurred where there was open water, generally the middle of the pond, though in the large agriculture ponds I sampled where tadpoles were most commonly seen. I measured the following water quality variables: total nitrogen (TN), total phosphorous (TP), ammonium (NH_4), nitrate plus nitrite (NO_2 + NO_3), pH, temperature (°C), and dissolved oxygen (DO). I used a Fisher Accumet 119 portable digital pH meter and an YSI 55 DO meter for all field measurements of pH and DO. Other water chemistry analysis was done at the University of Alberta Biogeochemical Laboratory (Edmonton, Alberta) using standard procedures. For analyses, limnological variables in 2006 were averaged over the two sampling periods as all variables except TN had means that did not differ between sampling periods (paired *t*-test $p > 0.2$, whereas TN was significantly higher in the June 21-27 samples). Temperature loggers (Thermochron® iButton

DS-1921G – Maxim Integrated Products), placed in plastic bags with rocks recorded water temperatures every 30 minutes from the pond bottom from June 6 until pond drying or the last week of July in both years. For analyses, I calculated daily average temperatures from June 6 to 22; the period when loggers were present and ponds held water. Loggers were attached to a meter stick from which I monitored pond depth at each visit. Pond area was previously determined during the classification of landscape variables using ArcMAP 9.3. Lastly, hydroperiod was measured from the beginning of frog calling in each year (April 15 2006 and April 20 2007) until the pond dried.

Biotic environment

Biotic variables measured included pond vegetation, chlorophyll-a (Chl- *a*), and aquatic invertebrate density (Appendix Table 2-1). I conducted vegetation surveys at each pond between July 12 and 20, 2007 to determine the extent and identity of emergent vegetation (list of species see Appendix Table 2-2). Hecnar and M'Closkey (1998) found that emergent vegetation was positively associated with amphibian species richness in Ontario, and thus may also affect the abundance of species in Alberta. The proportion of pond area covered by emergent vegetation, based on 1 m^2 grids, was estimated from pond maps drawn on graph paper. I also determined the proportion of pond area that was cattails which was also included in the measure of emergent vegetation. Water column Chl- *a*, which is a measure of phytoplankton, a possible food source for tadpoles (Wassersug 1972, Seale 1980), was assessed in 2006 from samples collected for water quality and values obtained were averaged over two measurement periods. During tadpole density surveys, I also estimated predatory invertebrate abundance including larval and adult Coleoptera from the family Dytiscidae, and larval Odonata, classified as either

Zygoptera or Anisoptera (Appendix 2). Although Notonectidae can attack and kill tadpole larvae (Van Buskirk 2001 and personal observation), they were rare within samples and excluded from predator density estimates. In 2006 only, I also determined the density of snails, as they may compete for attached periphyton with tadpoles (Bronmark et al. 1991, Holomuzki and Hemphill 1996). A complete list of invertebrates identified from samples is included in Appendix Table 2-1. I attempted to estimate periphyton biomass in 2007, as another food source for tadpoles, but failed.

Data analysis

As in previous chapters I considered an alpha of 0.10 for detecting significant differences. Given the small number of ponds surveyed, the probability of finding an effect of agricultural practices may be weak and concluding that agriculture has no effect is of interest, and thus I chose a less conservative alpha for detecting differences.

Landscape and local habitat variables

To assess if the assigned land-use categories (park, pasture and crop) were distinct based on upland habitat or pond characteristics, I used Principal Components Analysis (PCA), using correlation matrices in PC-ORD 5.0 (MjM Software Design 2006). Eaves (2004) had conducted a PCA similar analysis and I wanted to assess whether a focus on small wetlands (0.1 ha vs. 1.0 ha used by Eaves) would also distinguish land-use types. I conducted one PCA on landscape variables from the 15 survey ponds and then separate PCAs on pond water quality and pond biotic variables for each year, resulting in 5 separate PCAs. As abiotic and biotic conditions varied between years, separate PCAs were generated as larval

performance likely reflects conditions animasl are exposed to during a given growing season. For the ordinations of pond biotic variables, I chose to include individual measures of tadpole-predator densities, as opposed to a single predator measure, to account for differences that might be related to individual pond environments. Pond and landscape variables were assessed for normality and equality of variances across land-use categories. When necessary, data transformations were done to satisfy normality (Table 4-1) and missing values were replaced with that category's mean. As variables were measured in different units, and to create equality in variances across variables, I standardized each variable before ordinations were conducted. As the ratio of observations (n) to variables measured (K) exceeded the ratio of 3:1 (suggested to assure reasonable stability and reliability;(Gibson et al. 1984, Grossman et al. 1991, Williams and Titus 1988), I used method B4 described by Joliffe (1972) to reduce the number of variables used in the final ordinations. After an initial ordination is conducted with all variables, variables are reduced by assigning variables to each of the Principal Components based on their coefficients and then selecting those components (and associated variables) whose eigenvalues are greater than $\lambda_0 \geq 0.70$ or up to $p = n/3$ (where n is the number of sites). Only those variables on the first p components are then used in the final ordination; in my case up to four variables were included in the final ordinations. To evaluate if the three land-use categories differed based on the environmental variables retained in each ordination (landscape, abiotic, biotic) I used the Multi-Response Permutation Procedure (MRPP) in PC-ORD to test for pair-wise differences among categories based on the original data matrix. I also tested if park ponds differed from agricultural ponds (crop + pasture). In addition to ordinations, I determined whether landscape, abiotic and biotic measures differed

across land-use categories within each year using MANOVA followed by univariate ANOVAs or Kruskal-Wallis one-way analysis on ranks if variances were not equal among land-use types. A Bonferroni correction was not used as I did not want to make inferences about the lack of effects of agriculture which may exist but would not be apparent given my low sample size.

Tadpole-habitat relationships

For density, growth and metamorph data I analyzed years separately, in part because sampling occurred two weeks earlier in 2007 and tadpole densities decline through time owing to mortality and metamorphosis (see Appendix 5:). The other reason to treat years separately was that chorus frogs breeding effort varied among years and as mentioned above density affects performance. As tadpole densities were not normal distributed (based on the Shapiro-Wilk criteria), they were $x^{1/4}$ transformed, whereas growth, size at metamorphosis and CPUE and adjusted CPUE were normal. To test for differences among land-use categories, I used ANOVA, or a Kruskal-Wallis test if equality of variances was violated, and when differences were significant I used post hoc comparisons. To determine if relationships between tadpole density, growth, or metamorphs were related to landscape or local pond variables, I performed Pearson correlations between density or performance metrics and the PCA scores from the first two PC axes for each ordination. Further, I used Pearson correlations to evaluate the relationships between larval performance and density, and also between larval performance and individual landscape and local pond variables. All analyses were done using Systat 12.02 (SYSTAT Software INC. 2007) or SigmaPlot 11.0 (SYSTAT Software INC. 2008).

Results

Habitat characteristics

Landscape variables

Following the selection process, variables used in PCA were proportion of area in water (WATER), proportion of area in road (ROAD), distance from breeding pond to nearest wetland (DIST. WATER), and distance to nearest forested patch (DIST. FOREST). The ordination of landscape variables (Figure 4-2) showed good separation of the park ponds from the two agriculture pond types (pasture and crop) and those differences were significant (MRPP - $p \leq 0.005$ for both comparisons). Differences among land-uses with respect to landscape variables was also confirmed by MANOVA. The first PC axis (Land 1) explained 34% of the variation among ponds, with WATER (+) and DIST. FOREST (–) as the main loadings (Figure 4-2). The second axis (Land 2) had negative loadings of DIST. WATER and ROAD (Figure 4-2), and explained nearly 30% of the remaining variation. Landscape variables retained within the ordination were significantly correlated to others not in the final ordination (Appendix Table 4-2): Water correlated with FORESTED (+), NON-FORESTED (+), CROP (–), PASTURE (–) and CONSTRUCTED (–); DIST. FOREST correlated with CONSTRUCTED (+); and DIST. WATER correlated with NON-FORESTED (+). Crop and pasture ponds overlapped in the ordination and was confirmed that they did not differ based on the reduced variable set (MRPP, $p = 0.39$). The proportion of lands that were CROP, PASTURE or CONSTRUCTED was similar among crop and pasture ponds (based on separate *t-tests,* $p \geq 0.28$) and significantly higher than for park ponds (Table 4-3, Figure 4-1). Land 1 separated park ponds from agricultural ponds as they had significantly higher proportions of water within the 1km radius and tended

to be closer to forest compared to both crop and pasture ponds (Table 4-3, Figure 4-1).

Local pond variables

With respect to abiotic conditions, following the selection process, variables retained were total nitrogen (TN), combined concentration of nitrate and nitrite (NIONS), average daily temperature (TEMPERATURE) and pond hydroperiod (HYDROPERIOD). These variables were significantly correlated to those that were not retained in the final ordination (Appendix Table 4-3): TN correlated to TP (+), NH_4 (+) and DEPTH (–); NIONS correlated to pH (–) in 2007 where all ponds were sampled; and HYDROPERIOD correlated to pond DEPTH (–). Ordinations of abiotic variables (Figure 4-3) showed no separation of ponds amongst land-use categories in either year and was confirmed by pairwise comparisons (MRPP $p = 0.32$ in 2006 and $p = 0.16$ in 2007) and by MANOVA (Wilk's lambda $p \geq 0.15$ in either year). The first PC axis, Abiotic 1, in 2006 explained 43.6% of the variation among ponds with TN (+) and NIONS (+) and HYDROPERIOD (+) as the main loadings (Table 4-4). The second PC axis, Abiotic 2, in 2006 had positive loadings for TN and TEMPERATURE (Table 4-4) and explained about 35% of the remaining variation. In 2007, PC axis Abiotic 1 explained 45.3% of the variation among ponds with positive loadings of NIONS and HYDROPERIOD and a negative loading for TN (Table 4-4). PC axis Abiotic 2 explained a further 23% of the variation among ponds with TEMPERATURE (+) as the major loading (Table 4-4). Only pond HYDROPERIOD and DEPTH differed significantly among ponds with respect to land-use in 2006 (Table 4-3), as all park ponds dried significantly earlier compared to agricultural ponds, resulting in hydroperiods being significantly shorter than in crop ponds (Tukey $p = 0.004$), and shorter than pasture ponds, although not significantly (Tukey $p = 0.11$). Park ponds in 2006 were

significantly shallower than all agricultural ponds ($t_{12} = -2.76$, $p = 0.017$), by 18 cm on average, which likely contributed to the reduced hydroperiods. There was also a tendency for park ponds to be cooler than both crop and pasture ponds in 2006 (Table 4-3). Of the differences seen in 2006, only the trend for cooler park ponds was found in 2007 (comparing park to agricultural ponds grouped $t_{11} = -2.45$, $p = 0.03$) as both pond depth and hydroperiod were similar among land-uses (Table 4-3).

With respect to ordinations of biotic variables, following the selection process, variables used in the final PCA were anisopteran density (ANISOPTERA), dytiscid density (DYTISCIDAE), and the proportion of pond covered by cattails (CATTAILS). These variables were significantly correlated to variables not retained in the final ordination (Appendix Table 4-4): CATTAILS correlated with *PHYSA* sp. density in 2006 (–); DYTISCIDAE correlated with both EMERGENT vegetation (+) and ZYGOPTERA density (–) in 2007; and ANISOPTERA correlated with EMERGENT vegetation (+) but only in 2006. I found significant separation among ponds with respect to land-use based on the biotic variables retained in the ordination using MRPP ($p < 0.01$ each year), as crop ponds differed significantly from park ponds each year, and from pasture ponds in 2006, whereas park and pasture ponds were similar each year (Table 4-4, Figure 4-3). MANOVA supported the existence of biotic differences amongst land-use in 2006 (Wilk's lambda $p = 0.034$) whereas differences were not significant in 2007 (p=0.13). In 2006, the first PC axis, Biotic 1, explained 52% of the variation among ponds with CATTAILS (+), and ANISOPTERA (–) as the main loadings (Table 4-4), while the second PC axis, Biotic 2 explained a further 36.7% of the remaining variation with DYTISCIDAE (+) as the main loading (Table 4-4). In 2007, PC axis, Biotic 1 explained 48% of the variation among ponds with CATTAILS (+) and

DYTISCIDAE (–) as main loadings (Table 4-4), and ANISOPTERA was the main loading on PC axis, Biotic 2 (Table 4-4) which explained a further 34% of the remaining variation. Crop ponds, in 2006, had significantly lower densities of Anisopteran larvae and *Physa* sp. compared to park ponds and significantly greater areas of *Typha* stands compared to both park and pasture ponds (Table 4-3). The proportion of the ponds covered by emergent vegetation also varied significantly across land-use (Table 4-3). In relation to park ponds, pasture ponds had significantly lower proportions of emergent vegetation (Tukey $p = 0.07$), whereas crop ponds also had lower proportions ($p = 0.12$) but did not differ significantly from either park or pasture. Of note was the change in density of dragonfly larvae (Anisoptera) in 2007 which decreased significantly in park ponds from the previous year (*paired* $t_5 = 2.41$, $p = 0.03$), while there was a significant increase in damselfly larvae (Zygoptera) across all ponds (Wilcoxon signed rank test $Z = 2.67$, $p = 0.005$, $n = 12$). The changes in predator densities in 2007 increased the similarity of park and crop ponds, while decreasing the similarity of park and pasture ponds resulting in decreased overall differences among land-use types. It is unclear if these shifts in invertebrate communities were related to land-use or small differences in abioitic conditions.

Tadpole-habitat relationships: difference across land-use categories

Tadpole density

Observed differences found in the landscape ordination with respect to land-use did not translate into significant patterns for CF tadpole density, as density did not differ with land-use in either year (Table 4-5, Figure 4-4). CF tadpole densities tended to be higher in pasture ponds, but this pattern was driven by one pond which

had the highest density of any pond in both years. Removal of this pond had no effect on the among land use comparisons, but did result in CF densities tending to be highest in park ponds in 2007. Compared to only crop ponds, CF densities in park ponds tended to be higher (Figure 4-4, $t_5 = 2.26$, one tailed $p = 0.03$ in 2006 and $t_7 = 0.98$, $p = 0.17$ in 2007). With respect to landscape variables, CF density was weakly positively related to the Land 2 axis in 2007 (Table 4-6) and thus showed a negative relationship with road density. In fact, in both years, CF density was significantly negatively correlated to road density (Table 4-7).

WF density, as was true of CF density, did not differ among land-use categories (Table 4-5, Figure 4-4). WF density was positively correlated with CF density across locations and was also higher within pasture ponds, but only on account of the same pasture pond that increased mean CF density. Removal of this pond had no effect on the among land use comparison, but did result in WF densities tending to be higher in park ponds both years. WF density also tended to be higher in park ponds compared to crop ponds (Figure 4-4, $t_5 = 1.34$, one tailed $p = 0.12$ in 2006 and $t_7 = 2.20$, $p = 0.03$ in 2007). WF density tended to increase with the Land 1 axis in 2007 (Table 4-6) which characterizes ponds that are nearer to forested areas (Table 4-7), and was confirmed by a significant negative relationship between WF density and distance to forest in 2007 (Table 4-7); although still negative, this relationship was very weak in 2006 ($p = 0.70$). WF density, as did CF density, appeared to be affected by the pond biotic environment, but unlike CF, WF density increased with dytiscid density (Table 4-7) which corresponds to the negative relationship between WF density and the Biotic 2 axis in 2006 (Table 4-6).

Tadpole growth

CF growth rates did not differ among the land-uses (Table 4-5) as was confirmed by weak relationships to all landscape PC axes (Table 4-6). CF tadpole growth rates were consistently higher within crop ponds (Figure 4-4), but large variation in rates within both agriculture pond types negated any differences with land-use. CF growth rates tended to be higher in park ponds compared to pasture ponds only in 2006 (Figure 4-4, $t_8 = 2.21$, $p = 0.06$), coincident with reduced hydroperiods observed in park ponds relative to the other land uses. Though unrelated to local pond environmental variables, CF growth rates were negatively related to both intra-, interspecific and total tadpole density, and significantly so in 2007 (Table 4-7, Figure 4-5) when tadpole densities were higher (Table 4-5).

WF growth rates, unlike CF growth, differed among land-use, although only in 2006 (Table 4-5, Figure 4-4), with crop ponds having greater rates compared to park ponds (Tukey $p = 0.006$). WF growth rates, again unlike CF responses, were related to local pond variables (Table 4-6 and Table 4-7) and increased with pond temperatures and decreased with herbivorous snails in 2006 and the proportion of pond that supported emergent vegetation each year. Dytiscid larvae were observed predating both WF and CF tadpoles during stable isotope sampling (Chapter 2). Dytiscid larvae were common within stands of emergent vegetation and their density increased with the percent of pond that supported emergent vegetation, and significantly so in 2007($r_{10} = 0.55$, $p = 0.05$ in 2007). Growth rates of WF tadpoles were not significantly affected by intraspecific density ($p \geq 0.49$) or by total tadpole density ($p \geq 0.17$), but I did find WF growth decreased significantly with CF densities in 2007 (Table 4-7, Figure 4-5).

Recruitment of metamorphs and tadpole survivorship

Based on CPUE values adjusted for tadpole densities, survivorship to metamorphosis for CF was generally similar across land-use categories (Figure 4-4). However, CF survival like CF growth, tended to be greater in crop ponds in 2007, despite similarities in abiotic and biotic conditions among pond types. CF survival, much like CF growth, was negatively related to tadpole densities, but unlike growth, survival decreased strongly only with density of WF (Figure 4-5), and significantly so in 2007 (Table 4-7). CF survival also decreased significantly with total tadpole density but only in 2007, but the relationship was weaker than for just WF density (Table 4-7).

WF survival to metamorphosis was highly variable and did not differ significantly with respect to land-use (Table 4-5, Figure 4-4). WF survival showed stronger responses to both abiotic and biotic pond environments in 2007 (Table 4-6) than CF survival as a result of decreased WF survival with the proportion of pond that was covered by emergent vegetation (Table 4-7) which was positively related to dytiscid abundance (as noted above). WF survival also increased with pond temperatures (Table 4-7). WF survival was, however, more strongly related to tadpole densities than pond features and, like CF survival, decreased significantly with WF tadpole density (Table 4-7, Figure 4-5) and decreased weakly with total tadpole density in 2007. Relationships between WF survival and CF density varied in direction between years and were not significant (Table 4-7, Figure 4-5).

Metamorph size

Size at metamorphosis for CF was similar across land-use categories in both years (Figure 4-4). Unfortunately I was unable to measure any CF metamorphs from

crop ponds in 2006 owing to timing and thick riparian vegetation that impeded capture and detectability of metamorphs. CF size at metamorphosis, though significantly positively related to WF size at metamorphosis (Table 4-7), was weakly related to both landscape and local pond variables (Table 4-7). Relationships with tadpole density, though negative, were weak (Figure 4-5) or unreliable (as only four ponds were considered in the analysis in 2006, Table 4-7).

WF size at metamorphosis, though not significantly different across land-use categories, tended to be smaller at park ponds compared to agriculture ponds (Figure 3; $t_{10} = -1.60$, $p = 0.14$ in 2006, $t_{10} = -2.23$, $p = 0.05$ in 2007) and largest at crop ponds. WF size at metamorphosis was negatively correlated with the PC axis Biotic 1 in 2007, thus it decreased with the proportion of pond that was covered by emergent vegetation. WF size at metamorphosis was also negatively related to WF and total tadpole density, significantly in 2006, but not in 2007 (Table 4-7, Figure 4-5). The relationships of WF size with WF density and CF+WF density were almost identical.

Summary of responses

Ponds in the national park differed from agricultural ponds in upland vegetation and abundance of wetlands on the landscape. There were, however, no consistent differences in ponds' abiotic conditions, but biotic conditions in crop ponds differed relative to the park and pasture sites.

Tadpole densities though not significantly different across land-use types were higher in park ponds. Larval growth tended to be greatest in crop ponds where densities were lowest. Both CF and WF larval growth rates were reduced at higher interspecific densities, however, survival for both species was only negatively

related to WF density. CF metamorphs were of similar size across land-use types, but WF metamorphs were significantly larger from crop relative to park ponds.

Discussion

If agriculture is expected to negatively affect pond environments and reduce larval performance and/or anuran density at the scale of a pond, it was not observed in the temporary ponds I surveyed in the Beaver Hills of Alberta. Significantly reduced larval densities were not observed despite suitable upland habitats, such as forested areas, unmanaged non-forested vegetation and wetlands being significantly reduced in agricultural lands. However, the total density of anurans on the landscape surrounding ponds in agricultural areas were likely lower than in the park as a result of a reduced density of ponds and their lower suitability for breeding and development of larvae (Figure 4-1). The absence of a significant land-use effect on *Pseudacris* sp. density has also been reported in Texas (Gray et al. 2004), southern Minnesota (Knutson et al. 2004) and in Tennessee (Schmutzer et al. 2008). In my study there was, however, a tendency for larval densities of both species to be reduced at crop ponds, which may be related to reduced survival of individuals emerging from those ponds or survivorship of larvae and/or adults using those ponds. I found that the effects of agriculture on ponds failed to translate into significant negative impacts on the performance of either CF or WF larvae. This finding may relate to the observation that both CF and WF are commonly found in agricultural landscapes (Gibbs et al. 2005). Ultimatley the lack of impacts may be due to an absence of significantly different abiotic conditions which could reduce larval performance. Rather than observing significant effects of land-use, I found that larval performance was affected by density of larvae in ponds. Together, the

lack of significantly lower larval densities and changes in the performance of either anuran at surveyed ponds, might suggest that CF and WF using temporary ponds are capable of tolerating current disturbance levels in the agricultural lands within the Beaver Hills region of Alberta.

Effects of land-use on pond environments

The absence of significantly different abiotic pond conditions was somewhat unexpected given Eaves (2004) found significantly elevated TN and TP in agricultural ponds within the Beaver Hills combined with reduced abundances of breeding frogs. Contrary to Eaves (2004), I only found a trend for greater TN and TP concentrations at crop ponds compared to park ponds, whereas she found concentrations were five times higher in crop ponds (from (Eaves 2004), crop vs. park TN = 9805 ± 2889 vs. 2757 ± 410 µg/L; TP = 1030 ± 481 vs. 186 ± 58 µg/L, n = 6 in each category. In my surveys, TN = 4241 vs. 4332 µg/L and TP = 2782 vs. 1676 µg/L with eight crop and 13 park ponds with pond-years combined. Ponds and conditions differed between the studies as Eaves' surveys were conducted during a severe drought and encompassed larger ponds 1.0 ha versus 0.1 ha). Though Eaves (2004) did not measure ammonia, nitrate or nitrite, I found that these nitrogen compounds, though tending to be higher in crop and pasture ponds, were not significantly higher than in park ponds. Concentrations at crop ponds are an order of magnitude lower than lower than those that affect survival and larval performance (2.5 mg/L for nitrate, 0.44 mg/L for nitrite, 0.6 mg/L ammonia - Hecnar 1995, Rouse et al. 1999, Jofre and Karasov 1999, Marco et al. 1999 even after converting ammonium to ammonia Thurston et al. 1979). Dissolved oxygen levels recorded did not differ with respect to land-use, though not all ponds were sampled in a given year. Differences in sampling times amongst land-use may have confounded results,

as agriculture ponds were generally sampled in the afternoon, whereas park ponds were sampled in the morning. The effects of phosphorous remain largely untested, survival and growth of WF (Smith 2007) and other anurans (Hamer et al. 2004, Earl 2010) are not significantly affected by elevated phosphate concentrations. However, I found that WF growth rates and CF size at metamorphosis decreased significantly with phosphorous concentrations in 2007 and 2006, respectively (Table 4-6). Whether these relationships were due to phosphorous itself or due to factors associated with high phosphorous concentration are not clear. Prairie ponds in the aspen parkland of Alberta are all typically rich in phosphorous and classified as eutrophic to hypereutrophic (Casey et al. 1999, Anderson et al. 2002) and may be effective at removing nutrients from inflowing water by sedimentation, degassing and uptake by biota (White et al. 2000, White and Bayley 2001, Foote and Hornung 2005). These factors, combined with my choice of smaller ponds compared to Eaves (0.1 ha versus 1.0 ha) might have prevented the effects of landscape disturbances being reflected in abiotic measures.

Effects of land-use on larval performance

Most studies examining the effects of agricultural practices on amphibians have focused on post-metamorphic community structure, measures of relative abundance and controlled exposures to chemical contaminants. This study addresses a gap in our knowledge about the effects of agricultural practices on anuran larval performance in ponds. I have demonstrated that the larval performance of both CF and WF were not significantly reduced in agricultural ponds. My results on performance correspond to Loman and Lardner (2006), as they found that larval performance of two ranids was not reduced at farmland ponds compared to natural ponds in Sweden. With respect to metamorph size only, I

found a non-significant tendency for CF to be smaller from pasture ponds consistent with patterns seen by Burton et al. (2009) for *Pseudacris crucifer*. Burton et al. (2009) also found that ranid metamorphs were larger at pasture ponds in Tennessee, which corresponds to my result on WF (a ranid), however, this was likely due to lower larval densities in Tennessee pasture ponds. My results contrast with those of Gray and Smith (2005) who found that metamorph size for two spadefoot toads (Genus *Spea*), a true toad (*Anaxyrus*) and a mole salamander (*Ambystoma*) at crop ponds were significantly reduced compared to grassland ponds in Texas. However, the densities of amphibians (terrestrial stages) observed in Gray and Smith's crop ponds were almost twice as high compared to grassland ponds. Overall patterns of reduced larval performance in my study were consistent with effects of density dependence and therefore competition. Together, these studies with mine suggest that the negative effects of density and competition may obscure effects of agricultural practices.

To sustain populations, individuals must be recruited into the population through metamorphosis. Size at metamorphosis is correlated with metamorph survival (Altwegg and Reyer 2003, Chelgren et al. 2006) and lifetime fitness of individuals (Smith 1987, Berven 1990). My results suggest that CF metamorphs would have similar survival and fitness across pond types as metamorph size was similar (pasture ponds only reduced by 2.7-3.2% relative to park ponds). WF metamorphs in contrast were significantly larger at crop compared to park ponds (12.8 and 7.6% larger relative to those from park ponds, 2.0 and 1.5 mm in SUL, in 2006 and 2007, respectively). Metamorphs emerging from crop ponds should have a greater probability of survival (Altwegg and Reyer 2003, Chelgren et al. 2006) and greater relative fitness to those from park ponds (Smith 1987, Berven 1990). With

respect to WF, my hypothesis that larval performance would be reduced in agricultural ponds relative to natural park ponds is rejected, and I hypothesize that greater rates of growth for WF larvae in agricultural ponds, owing to lower larval densities and warmer temperatures, combined with generally longer hydroperiods, resulted in significantly larger metamorphs (as predicted by Wilbur and Collins 1973).

Overall, I found WF performance exhibited comparable to enhanced performance in agricultural ponds compared to park ponds and responses had stronger associations with abiotic and biotic conditions than did CF's. Size at metamorphosis in the agricultural ponds surveyed is greater than reported for CF throughout their range (Table 4-8) and was similarly greater for WF compared to studies in more forested landscapes (Table 4-8). These findings provide further evidence that the effect of agriculture on the performance of these frogs appears to be weak compared to the effects of density.

Patterns of larval performance in my study demonstrated that species specific requirements affect responses to pond conditions. CF performance was unrelated to pond conditions but decreased with intra- and interspecific larval densities. WF performance conversely increased with lower proportions of emergent vegetation, warmer temperatures, and like CF, with lower larval densities (conspecific and total). The relationship with emergent vegetation may stem from a relationship between emergent vegetation and densities of predatory dytiscids. WF larvae have been observed to reduce feeding and activity in the presence of predators in mesocosms (Schoeppner and Relyea 2009) and consequently it decreases WF growth and may correspond to my results. In contrast, CF growth in

my surveys did not respond to predator density, or emergent vegetation, as previously demonstrated for CF in natural ponds (Smith and Vanbuskirk 1995) and in mesocosms (Van Buskirk et al. 1997). Though both CF and WF larvae growth rates increased with temperature at surveyed ponds (in line with results for wood frogs Smith-Gill and Berven 1979, Skelly et al. 2002, and for *Pseudacris* Harkey and Semlitsch 1988), only the change in WF rates was significant. Increased temperature, combined with reduced emergent vegetation and lower tadpole densities, in agricultural ponds may account for both greater WF growth rates and size at metamorphosis in agriculture ponds. Both larvae and terrestrial phases of CF are smaller than WF, and therefore have lower absolute requirements for food and growth prior to metamorphosis. As such, CF may be released from strong effects of pond conditions due to lower resource requirements to reach metamorphosis; whereas WF larvae grow faster but develop slower and must attain greater size prior to metamorphosis which may result in greater dependence on pond abiotic and biotic environments. The difference in the strength of correlations between performance and pond environment suggest a possible mechanism to explain why WF abundance (for example, in northern Alberta - Browne et al. 2009, and in New Hampshire - Herrmann et al. 2005) has stronger associations with local pond features than landscape variables (but see Hecnar and MCloskey 1997, Porej et al. 2004, Rubbo and Kiesecker 2005). The abundance of CF, in contrast to WF, tend to be correlated with landscape variables (*P. maculata* - Browne et al. 2009, either *P. maculata* or *triseriata* - Gibbs et al. 2005, *P. triseriata* - Price et al. 2005) likely owing to their dependence on connectivity to other wetlands (*P. triseriata* - Werner et al. 2009).

In summary, density dependent interactions appeared to be the main mechanism reducing larval performance as abiotic and biotic conditions showed few strong, consistent relationships with performance. With respect to larval growth, significant negative relationships between CF growth with WF density (Figure 4-5) suggests that interspecific competition, common among larval anurans (Alford 1999) occurs in these prairie ponds, as also indicated by results presented in Chapters 2 and 3. However, intraspecific effects were also detected in 2007 when CF densities were highest. Smith (1987, 1983, 1990) similarly found that CF (*P. maculata* sensu Lemmon et al. 2007) growth, in addition to survival and metamorph size, decreased with intraspecific densities in rock pools on Isle Royale, Michigan. WF growth did not respond significantly to intraspecific density, in contrast to patterns reported in natural populations in Maryland (Berven 1990) and in mesocosms (Relyea 2002). WF growth decreased significantly with increased interspecific density in 2007 when both species' larval densities were about six times higher than 2006 levels (mean factor of difference between years at same ponds for CF = 6.0 ± 2.4 SE and for WF = 5.6 ± 2.2).

Survival of CF showed significant negative relationships with WF density, whereas WF survival was significantly related to intraspecific density. This pattern is consistent with asymmetric interspecific interactions and may be related to species' size differences. However, survival estimates reported here are not especially robust given the timing of metamorphosis and date of survey combined with detectability differed among ponds which would affect the number of metamorphs found at each location. Size at metamorphosis was the least responsive to tadpole density, at least for CF. CF size at metamorphosis decreased with temperature in 2007, which may reflect constricted larval periods in response to

pond drying, but showed weak relationships with tadpole densities. WF, in contrast, emerged smaller at higher intraspecific densities and responded weakly to interspecific densities.

To my knowledge only Relyea (2002) has identified *P. maculata* and *L. sylvaticus* as interspecific competitors based on a lab study, but there are examples of *Pseudacris* competing with ranids and specifically with the wood frog *L. sylvaticus* in mesocosms (Morin and Johnson 1988, Sours and Petranka 2007) and at both mesocosms and field venues in Connecticut (Skelly 2002). Each of these studies suggested that interspecific competition affected larval performance and tended to be asymmetric with WF being the dominant competitor. The conclusions of these studies are in accordance with the results from my surveys that indicate competition operates between these species. My findings agree with the literature on competitive interactions between *Hyla* and *Rana* as ranids are believed to dominate hylid larvae (Wilbur 1987, Alford 1989ab, Fauth and Resetarits 1991, Pehek 1995, Faragher and Jaeger 1998, but see Smith et al. 2004).

Factors affecting larval abundance in crop ponds

I expected larval densities to be lower at both crop and pasture ponds through reduced larval survival associated with pond conditions. Alternatively lower larval densities might reflect reduced breeding output owing to lower adult densities at agricultural ponds as observed by Eaves (2004). Unfortunately I can not rule out the possibility that the number of adults was comparable among pond types and that survivorship from egg to tadpole was lower at crop ponds. Larval performance at pasture ponds was not significantly different than at park ponds and corresponded with the similar densities observed. However the results for larval

performance do not correspond with observed lower larval densities found at crop compared to park ponds. This lack of agreement between performance and larval density suggests that survival of terrestrial stages, although expected to be greater initially due to greater metamorph size in crop lands, is lower owing to effects of land-use, habitat suitability and/or and landscape resistance, a measure of the landscape's permeability to movements. Although I did not monitor terrestrial survival, I present evidence below on the effects of land-use, habitat suitability and landscape resistance that support the hypothesis that terrestrial survival is reduced in crop lands.

As expected by definition, landscapes surrounding agricultural ponds had significantly lower proportions of water, tree cover and non-forested vegetation (such as meadows), and greater areas of disturbed lands such as tilled fields, pastures, and human constructions. Further, survival in these agricultural landscapes may be reduced if there are few suitable habitats for individuals to use, which may interact with density dependence in the terrestrial stage (Berven 2009). If suitable habitats are rarer in crop lands, or are more difficult to get to, metamorph density may result in decreased survival or condition of individuals (Harper and Semlitsch 2007). Contrary to expectations, crop ponds were not more isolated from other wetland populations or further from favourable terrestrial habitats (forested patch) as the distance to the forested patch and nearest wetland was similar and did not exceed 125 m, which is well within the limits of both CF and WF migration capabilities (Spencer 1964, Kramer 1973, Berven and Grudzien 1990, Regosin et al. 2005, Baldwin et al. 2006). However, many of the wetlands within 1 km of the agricultural wetlands that had calling anurans (mostly CF) did not hold water long enough to permit larval development (50-80 days for both species based on my

observations). In contrast there were many more wetlands within 1 km of park ponds that recruited individuals. Marsh and Trenham's (2001) review of anuran metapopulations suggests that inter-pond movements are significantly reduced in disturbed lands as evidenced by greater genetic divergence among inter-pond populations.

Cultivated crops and/or lack of cover, especially surrounding crop ponds, decrease the survival of terrestrial stages choosing to settle or cross these habitats and prevents or impedes movement into adjacent areas (deMaynadier and Hunter 1999, Rothermel and Semlitsch 2002, Joly et al. 2003, Mazerolle and Desrochers 2005, Vos et al. 2007, Janin et al. 2009). In the absence of cover, common among the ponds surveyed in crop lands, frogs lose more water (Mazerolle and Desrochers 2005), which leads to water stress and desiccation, reducing the condition and survival of individuals (Constible et al. 2001, Rothermel and Semlitsch 2002). It follows, that metamorphs in crop lands face greater risks of water loss and are less likely to survive to breeding.

Because I did not measure terrestrial survival or adult abundance at these locations, I can only hypothesize the reasons behind reduced abundance of tadpoles at crop ponds. Production of metamorphs was similar between crop and park ponds (see unadjusted CPUE Table 4-5) and CF metamorphs were of similar size across land-use types, or in the case of WF significantly larger at crop ponds. Thus assuming individuals surviving to breeding show similar site philopatry (Spencer 1964, Berven and Grudzien 1990), and that production of metamorphs is similar across land-use and year, densities ought to be similar unless survival of individuals is lower in crop lands or production varies annually. However, as WF metamorphs

do not return to breed for up to 2 years (Berven 1990), I cannot confirm this hypothesis. I have shown that production varies annually within these species (Figure 2-5) and does so for anurans in general (Green 2003, Berven 2009). Notwithstanding effects of annual variation, it seems probable that densities were lower at crop ponds due to lower survival in surrounding crop lands. I argue that given WF metamorphs were significantly larger at crop ponds and their probable dependence on forested patches (Baldwin et al. 2006), that the case for lower terrestrial survival of WF emerging from crop ponds is even stronger than for CF.

Conclusions

In summary, it appears that larval performance of both boreal CF and WF are significantly affected by density dependence, as is common among larval anurans. In addition to density dependence, species differences in requirements and life-history strategies lead to different responses to pond environments. If those environments are a product of human land-use, they may limit terrestrial populations and affect the persistence of anuran species in agricultural lands. Models relating to anuran persistence generally identify juvenile and adult survival as the most important in maintaining populations (Biek et al. 2002, Vonesh and De la Cruz 2000). However, a model I developed for *P. triseriata*, now *P. maculata* sensu Lemmon et al. (2007), suggested that CF population size and persistence showed greater sensitivity to recruitment from the aquatic habitat (Whiting 2004) and therefore on the performance of larvae. I do not know of comparable models related to WF persistence, however WF populations are believed to respond to changes in land use (Hecnar and MCloskey 1997, Porej et al. 2004, Rubbo and Kiesecker 2005), although adult abundance at sites appears to be related to local pond characteristics (Herrmann et al. 2005, Browne et al. 2009). Though population size and persistence

for different species may show greater sensitivity at different life stages, I think that carry-over effects of larval environments (Chelgren et al. 2006) are important to consider, especially when evaluating the effect of disturbances surrounding wetlands. Populations may be sandwiched between poor larval rearing environments and inhospitable or marginal terrestrial habitats.

I found little evidence to suggest that environmental conditions in agricultural ponds are significantly reducing the performance of larvae or density of frogs. My mesocosm experiment together with these surveys (Chapter 3) demonstrate that CF and WF larvae tolerate conditions representative of those found in agricultural ponds and that ponds in agricultural landscapes are used by amphibians and allow successful recruitment of individuals into terrestrial populations and are not directly limiting populations. Rather, it appears that the structure of these landscapes may be affecting terrestrial survival and connectivity to required habitats, ultimately reducing larval densities which in turn affect larval performance. Greater disturbance such as tilling, pond drainage, crop harvesting, and chemical application to fields are likely reasons for lower abundance of frogs occurring at crop ponds as observed by Eaves (2004). Future investigations should focus on terrestrial habitat use and survival of frogs using agricultural wetlands as breeding sites to determine if land disturbance is limiting population persistence in the aspen parkland and perhaps for amphibians globally.

Table 4-1: Names and descriptions of landscape habitat variables and local pond variables that were used in Principal Component Analyses.

Habitat Variable			
Abbrevation	Description	Units	Transf. type
Landscape Variables			
CROP	Proportion of area that is crop	%	$(\sin^{-1}\sqrt{x})$
FOREST	is forest	%	$(\sin^{-1}\sqrt{x})$
NON FOREST	is shrub and tall grasses	%	$(\sin^{-1}\sqrt{x})$
ROAD	is road	%	$(\sin^{-1}\sqrt{x})$
WATER	is water	%	$(\sin^{-1}\sqrt{x})$
PASTURE	is pasture	%	$(\sin^{-1}\sqrt{x})$
CONSTRUCTED	is homes, barns, and lawns	%	$(\sin^{-1}\sqrt{x})$
DIST. WATER	Distance to nearest water body	m	
DIST. FOREST	Distance to nearest forested area > 0.1 ha	m	
Local Pond Abiotic Variables			
AREA	Pond area	ha	$\ln x$
DEPTH	Average depth of pond	cm	
HYRDOPERIOD	Hydroperiod (days from start of peak breeding)	days	
TEMPERATURE	Daily average temperature (June 6-22)	°C	
PH	pH	-	
TN	Total nitrogen	µg/L	$\ln x$
TP	Total phosphorous	µg/L	$\ln x$
NH4	Ammonium	µg/L	$\ln x$
NIONS	Nitrate + nitrate	µg/L	$\ln x$
Local Pond Biotic Variables			
CHLA	Water column chlorophyll a	µg/L	$\ln x$
EMERGENT	Proportion of area in emergent vegetation	%	$(\sin^{-1}\sqrt{x})$
CATTAILS	Proportion of pond areas in cattails	%	$(\sin^{-1}\sqrt{x})$
PHYSA	Density of *Physa* sp. snails	#/L	$\sqrt{x}$
ZYGOPTERA	Density of zygopteran larvae	#/L	$\sqrt{x}$
ANISOPTERA	Density of anisopteran larvae	#/L	$\sqrt{x}$ in '06 x in '07
DYTISCIDAE	Density of dytiscid larvae and adults	#/L	$\sqrt{x}$ in '06 x in '07
PREDATOR	Density of Zygoptera+Anisoptera+dytiscid	#/L	$\sqrt{x}$ in '06 x in '07

Table 4-2: Results of Principal Component Analysis (Figure 4-2) and Multiple Response Permutation Procedures (MRPP) comparing landscape feature around 15 ponds within 3 different land-use types. Eigenvalues and the percent variance captured by the principal components, along with each principal component's loadings and the proportion of the variance (r^2) each variable shared with PC scores for sites on each axis.

	Land1		Land2	
Landscape metrics	Loading	r^2	Loading	r^2
WATER	1.45	0.54	−0.82	0.15
DIST. WATER	−0.12	0.00	−1.76	0.70
DIST. FOREST	−1.51	0.59	0.14	0.00
ROAD	−0.96	0.24	−1.24	0.34
Eigenvalue	1.37		1.19	
% of variance	34.2		29.9	
Cumulative %	34.2		64.1	

Land-use comparison using MRPP			***t***	***p*-value**
Effect of land-use			−3.03	0.01
Park	vs.	Crop	−4.00	0.0033
Park	vs.	Pasture	−3.30	0.0049
Pasture	vs.	Crop	0.08	0.39
Park	vs.	Agriculture	−4.57	0.002

Table 4-3: Summary of landscape and local habitat features collected from 15 ponds (six park, four pasture, five crop) in the Beaver Hills Region near Elk Island National Park. Variables are described in Table 4-1. For each variable, I tested the difference in means either using ANOVA on transformed values or using Kruskal-Wallis one-way analysis on ranks. Significant differences existed among land-use types, then difference between groups were compared using Tukey's Post Hoc Test and group membership is shown by superscript letters.

			Land-use category means and (ranges)						Test for difference across land-use
	units	year	Natural (n = 6)		Pasture (n = 4)		Crop (n = 5)		
Landscape variables									
FOREST	%	n/a	[a] 55.1	(35 - 71)	[ab] 35.4	(27 - 44)	[b] 25.7	(3 - 41)	$F_{2,12}$=5.22, p = 0.023
CROP	%	n/a	[a] 0	(0)	[b] 28.2	(7.6 – 43.9)	[b] 47.9	(25.6 – 86.7)	$F_{2,12}$=19.7, p < 0.001
PASTURE	%	n/a	[a] 2.7	(0 – 15.6)	[b] 24.0	(18.9 – 35.0)	[b] 16.4	(3.5 – 21.0)	$F_{2,12}$=13.1, p < 0.001
WATER	%	n/a	[a] 18.0	(9.4 – 27.4)	[b] 2.2	(1.5 – 4.3)	[b] 0.8	(0.1 – 1.3)	$F_{2,12}$=38.0, p < 0.001
NON FORESTED	%	n/a	[a] 23.0	(14.5 – 53.1)	[b] 5.7	(4.1 - 7.2)	[b] 5.8	(4.3 – 8.6)	$F_{2,12}$=9.71, p = 0.003
ROAD	%	n/a	1.1	(0.6 – 1.8)	0.8	(0 – 1.3)	1.5	(1.3 – 2.1)	$F_{2,12}$=2.55, p = 0.12
CONSTRUCTED	%	n/a	[a] 0.05	(0 – 0.3)	[b] 2.0	(0.4 – 2.1)	[b] 1.9	(0.6 – 4.5)	$F_{2,12}$=11.1, p = 0.002
DIST. WATER	m	n/a	81.4	(30.1 – 148.4)	57.0	(25.1 – 99.7)	70.8	(47.5 – 121.3)	$F_{2,12}$=0.42, p = 0.66
DIST. FOREST	m	n/a	0.7	(0 – 4.1)	3.1	(0 – 10.4)	10.4	(0 – 31.9)	H_2=3.08, p = 0.21
Local abiotic variables									
TN	µg/L	2006	4775	(2445 – 12650)	3538	(2555 – 4575)	4665	(1775 – 9715)	$F_{2,11}$=0.08, p = 0.93
		2007	3462	(1760 – 7250)	2703	(2130 – 3770)	4440	(2790 – 8390)	$F_{2,10}$=0.72, p = 0.51
TP	µg/L	2006	938	(127 – 1627)	1223	(279 - 3202)	2009	(143 – 4247)	$F_{2,11}$=0.03, p = 0.97
		2007	1176	(550 – 2890)	904	(307 – 2055)	3372	(268 – 8720)	$F_{2,10}$=1.03, p = 0.39
NH_4	µg/L	2006	49	(12 – 114)	74	(13 -187)	205	(22 – 473)	$F_{2,11}$=0.93, p = 0.42
		2007	83	(20 – 349)	61	(54 – 65)	111	(55 – 216)	$F_{2,10}$=1.16, p = 0.39
NO_2 + NO_3	µg/L	2006	2.1	(0 - 4)	4.8	(0 – 15)	3.1	(3 – 4)	$F_{2,11}$=0.67, p = 0.53
		2007	2.3	(0 – 4)	2.3	(55 - 216)	1.0	(0 – 4)	$F_{2,10}$=1.12, p = 0.36
pH		2006	6.10	(5.7 – 6.5)	7.38	(6.6 – 8.2)	7.25	(6.7 -7.8)	H_2=4.25, p = 0.12
		2007	6.88	(6.3 – 7.7)	6.67	(6.5 – 6.9)	7.16	(7.0 – 7.6)	$F_{2,10}$=1.34, p = 0.30
D.O.	mg/L	Mix	4.91	(1.2 – 11.2)[n=10]	8.60	(5.2 – 13.5)[n=4]	7.10	(1.2 – 13.0)[n=2]	$F_{2,13}$=1.20, p = 0.33
Temperature	°C	2006	15.3	(12.9 – 16.5)	17.1	(16.0 – 19.3)	16.1	(15.6 – 16.5)	$F_{2,11}$=2.64, p = 0.12
		2007	15.3	(13.4 – 17.3)	17.8	(16.5 – 19.8)	16.8	(16.4 – 17.6)	H_2 = 3.25, p= 0.21
Pond area	m^2		542	(454 – 682)	974	(251 – 1604)	887	(424 – 2479)	$F_{2,12}$=0.67, p = 0.53
Depth	cm	2006	[a] 22.3	(8.0 – 30.5)	[ab] 40.3	(23 – 60)	[b] 43.4	(26 – 63)	$F_{2,11}$=3.57, p = 0.06
		2007	33.5	(22 – 43)	34.3	(31 – 37)	29.7	(22 – 37)	$F_{2,10}$=0.63, p = 0.55
Hydroperiod	days	2006	[a] 81.7	(74 – 89)	[ab] 93.0	(83 - 107)	[b] 103.5	(93 – 107)	$F_{2,11}$=9.32, p = 0.004
		2007	91.5	(81 – 102)	93.0	(87 – 102)	94.8	(87 – 102)	$F_{2,10}$=0.17, p = 0.84
Local biotic variables									
Emergent	%	2007	[a] 79.4	(67 - 86)	[b] 51.5	(13 - 90)	[ab] 56.8	(43 - 70)	$F_{2,12}$=3.47, p = 0.065
Cattails	%	2007	[a] 0.5	(0 – 3)	[a] 1.2	(0 - 3)	[b] 16.1	(2 - 24)	$F_{2,12}$=9.21, p = 0.004
Chl-*a*	µg/L	2006	48	(9 -111)	26	(1 - 54)	78	(5 - 269)	$F_{2,11}$=0.69, p = 0.52
Physa	#/L	2006	[a] 0.57	(0.01 – 1.28)	[ab] 0.29	(0.04 – 0.69)	[b] 0.01	(0 – 0.03)	$F_{2,11}$=4.30, p = 0.042
Anisoptera	#/L	2006	[a] 0.23	(0.05 – 0.50)	[ab] 0.12	(0.01 – 0.20)	[b] 0.05	(0.02 – 0.08)	$F_{2,11}$=3.82, p = 0.055
		2007	0.04	(0.01 – 0.09)	0.10	(0.05 – 0.16)	0.05	(0.01 – 0.12)	$F_{2,10}$=2.45, p = 0.14
Zygoptera	#/L	2006	0.006	(0 – 0.011)	0.015	(0.001 – 0.027)	0.011	(0.001 – 0.024)	$F_{2,11}$=1.74, p = 0.22
		2007	[a] 0.01	(0 – 0.03)	[ab] 0.06	(0.03 – 0.10)	[b] 0.17	(0.02 – 0.39)	$F_{2,10}$=6.02, p = 0.019
Dytiscidae	#/L	2006	0.10	(0.05 – 0.14)	0.16	(0.05 – 0.035)	0.15	(0.10 – 0.18)	$F_{2,11}$=0.92, p = 0.43
		2007	0.04	(0.02 – 0.07)	0.04	(0.02 – 0.05)	0.02	(0.02 -0 .03)	$F_{2,10}$=1.55, p = 0.26
Predator	#/L	2006	0.33	(0.12 – 0.64)	0.30	(0.08 – 0.58)	0.21	(0.18 – 0.23)	$F_{2,11}$=0.57, p = 0.58
		2007	[a] 0.09	(0.02 – 0.11)	[ab] 0.20	(0.14 – 0.19)	[b] 0.25	(0.05 -0 .40)	$F_{2,10}$=4.34, p = 0.044

Table 4-4: Results of Principal Components Analysis (Figure 4-3) and Multiple Response Permutation Procedures (MRPP) on the local abiotic and biotic pond variables within three different land-use types in 2006 and 2007. Only those variables retained after variable selection are shown. Eigenvalues and the percent variance captured by the principal components, along with the loading of each variable on the first two principal components and the correlation between those variable and the PC scores. Only those axes with eigenvalues > 1.0 are shown.

2006	Abiotic1		Abiotic2		Biotic1		Biotic2	
Local variables	Loading	r^2	Loading	r^2	Loading	r^2	Loading	r^2
TN	−0.54	0.46	0.60	0.38				
NIONS	0.59	0.56	0.00	0				
Hydroperiod	0.46	0.34	0.03	0				
Temperature	0.39	0.24	0.80	0.66				
Anisoptera					−0.68	0.73	0.35	0.13
Dytiscidae					0.12	0.02	0.92	0.93
% Cattails					0.72	0.81	0.18	0.04
Eigenvalue	1.31		1.04		1.56		1.10	
% of variance	43.57		34.81		52.07		36.65	
Cumulative % of variance	43.57		78.38		52.07		88.72	

Land-use comparison using MRPP			***t***	***p*-value**	***t***	***p*-value**
Effect of land-use			−0.29	0.32	−3.26	0.008
Park	vs.	Crop	−1.45	0.09	−3.76	0.004
Park	vs.	Pasture	0.78	0.78	0.23	0.51
Pasture	vs.	Crop	−0.98	0.15	−1.74	0.05
Park	vs.	Agriculture	−0.77	0.37	−2.40	0.03

2007	Abiotic1		Abiotic2		Biotic1		Biotic2	
Local variables	Loading	r^2	Loading	r^2	Loading	r^2	Loading	r^2
TN	−1.47	0.59	−0.46	0.03				
NIONS	1.33	0.48	−0.41	0.02				
Hydroperiod	1.33	0.48	1.44	0.29				
Temperature	−1.00	0.27	2.06	0.60				
Anisoptera					0.01	0	0.98	0.98
Dytiscidae					−0.71	0.72	0.15	0.02
% Cattails					0.71	0.72	0.14	0.02
Eigenvalue	1.81		0.94		1.56		1.03	
% of variance	45.28		23.55		51.88		34.22	
Cumulative % of variance	45.28		68.83		51.88		86.10	

Land-use comparison using MRPP			***t***	***p*-value**	***t***	***p*-value**
Effect of land-use			−0.92	0.16	−2.93	0.01
Park	vs.	Crop	−0.73	0.18	−3.49	0.01
Park	vs.	Pasture	0.37	0.55	−1.22	0.12
Pasture	vs.	Crop	−1.22	0.11	−0.86	0.19
Park	vs.	Agriculture	0.19	0.44	−3.44	0.01

Table 4-5: Results of tadpole density and performance from surveys of 15 ponds surround by three land-uses. Values are reported as $\bar{x}$ ± SE (n ponds) ANOVA or Kruskal-Wallis's comparison of ranks was used to evaluate differences among land-use and significant differences between land-use is denoted by letters. "Meta. CPUE" = metamorph catch per unit effort. Survival = Meta. CPUE/density. Size @ Meta. is the snout-to-urostyle length of metamorphs.

Metric	units	year	Upland type			Test for difference among sites
			Park	Pasture	Crop	
Boreal Chorus Frog						
Density	#/L	2006	0.017 ± 0.005 (6)	0.078 ± 0.061 (4)	0.005 ± 0.001 (4)	H_2=2.46, p = 0.35
		2007	0.099 ± 0.044 (6)	0.217 ± 0.146 (3)	0.037 ± 0.012 (3)	$F_{2,9}$=1.40, p = 0.30
Growth rate	mm/d	2006	0.287 ± 0.019 (6)	0.233 ± 0.009 (4)	0.317 ± 0.051 (3)	$F_{2,10}$=2.45, p = 0.14
		2007	0.270 ± 0.027 (5)	0.254 ± 0.058 (3)	0.306 ± 0.030 (4)	$F_{2,9}$=0.53, p = 0.61
Larval period	days	2006	66.0 ± 3.0 (2)	73.0 ± 3.0 (3)	72.5 ± 3.5 (2)	$F_{2,4}$=1.38, p = 0.35
		2007	67.5 ± 3.5 (6)	55.7 ± 2.3 (3)	61.5 ± 3.5 (4)	$F_{2,10}$=2.67, p = 0.12
Meta. CPUE	#/min	2006	0.41 ± 0.32 (6)	0.94 ± 0.36 (4)	0.03 ± 0.03 (3)	H_2=2.61, p = 0.31
		2007	4.40 ± 2.11 (6)	3.33 ± 1.20 (3)	6.89 ± 4.26 (3)	$F_{2,9}$=0.37, p = 0.70
Survival		2006	15.27 ± 8.46 (6)	12.61 ± 8.52 (3)	0.00 ± 0 (2)	H_2=2.20, p = 0.43
		2007	48.50 ± 21.24 (6)	32.65 ± 22.18 (3)	157.44 ± 78.81 (3)	$F_{2,9}$=2.50, p = 0.14
Size @ Meta.	mm	2006	12.70 ± 0.30 (2)	12.36 ± 0.52 (3)	-	$F_{1,3}$=0.23, p = 0.66
		2007	13.18 ± 0.52 (5)	12.76 ± 0.31 (2)	13.26 ± 0.46 (3)	$F_{2,7}$=0.17, p = 0.85
Wood Frog						
Density	#/L	2006	0.058 ± 0.023 (5)	0.077 ± 0.055 (3)	0.017 ± 0.007 (3)	$F_{2,8}$=0.40, p = 0.69
		2007	0.133 ± 0.008 (6)	0.171 ± 0.083 (3)	0.075 ± 0.042 (3)	$F_{2,9}$=1.14, p = 0.36
Growth rate	mm/d	2006	[a]0.295 ± 0.021 (6)	[ab]0.360 ± 0.034 (4)	[b]0.451 ± 0.034 (4)	$F_{2,11}$=7.78, p = 0.01
		2007	0.426 ± 0.027 (6)	0.423 ± 0.119 (3)	0.378 ± 0.053 (4)	$F_{2,10}$=0.23, p = 0.80
Meta. CPUE	#/min	2006	0.53 ± 0.22 (6)	0.49 ± 0.04 (4)	0.74 ± 0.27 (4)	H_2=0.50, p = 0.80
		2007	4.06 ± 1.52 (6)	10.52 ± 3.68 (3)	4.78 ± 2.42 (3)	$F_{2,9}$=2.13, p = 0.18
Survival		2006	121.86 ± 115.65 (6)	18.99 ± 9.83 (3)	33.80 ± 4.92 (3)	H_2=2.91, p = 0.25
		2007	32.06 ± 11.95 (6)	187.81 ± 150.02 (3)	183.76 ± 132.02 (2)	$F_{2,9}$=1.31, p = 0.32
Size @ Meta.	mm	2006	17.02 ± 0.78 (4)	17.87 ± 0.93 (4)	19.20 ± 0.49 (4)	$F_{2,11}$=2.12, p = 0.18
		2007	19.48 ± 0.51 (6)	20.82 ± 1.17 (2)	20.97 ± 0.41 (4)	$F_{2,11}$=2.25, p = 0.16

Table 4-6: Results of correlations of tadpole density and performance metrics against Principle Component scores for the first two axes from the landscape (Figure 4-2), abiotic (Figure 4-3) and biotic (Figure 4-3) ordinations. The numbers of ponds in each comparison are noted. Abbreviations are as in Table 4-5.

			Landscape		Local pond			
	year	N	Land1	Land 2	Abiotic 1	Abiotic 2	Biotic 1	Biotic 2
Chorus frog								
Density	`06	11	0.40	0.36	−0.13	0.36	0.57*	−0.22
	`07	12	0.42	0.50†	−0.14	−0.29	0.42	−0.39
Growth rate	`06	13	−0.42	−0.04	−0.06	0.14	−0.13	−0.02
	`07	12	−0.39	−0.07	−0.01	−0.19	−0.09	0.07
Survival	`06	11	0.20	0.24	0.18	0.10	0.20	0.36
	`07	12	−0.62*	0.12	−0.44	−0.41	−0.17	0.11
Size @ Meta	`06	5	−0.83†	−0.55	0.25	−0.30	−0.27	0.78
	`07	10	0.15	0.10	−0.09	0.47	−0.003	0.03
Wood frog								
Density	`06	11	0.06	0.30	−0.12	0.21	−0.03	−0.62*
	`07	12	0.55†	−0.14	0.47	0.15	0.53†	−0.22
Growth rate	`06	14	−0.76**	0.27	0.44	0.56*	−0.61*	−0.02
	`07	13	0.001	−0.38	0.13	0.48†	−0.18	−0.007
Survival	`06	12	0.07	0.16	−0.002	−0.10	0.24	0.32
	`07	12	−0.37	0.35	−0.57†	−0.02	−0.53†	0.21
Size @ Meta	`06	12	−0.59*	0.21	0.06	0.08	−0.25	0.01
	`07	12	−0.42	0.34	−0.40	0.39	−0.55†	−0.18

** = p ≤ 0.01, * = p ≤ 0.05, and † = p ≤ 0.10.

Table 4-7: Correlations between tadpole density, growth rate, survival and size at metamorphosis (size at meta.) with each other, and with landscape and local pond variables across ponds; (the number of ponds considered in each comparison is given in Table 4-6. Variables correlated with tadpole performance metrics with $p \leq 0.10$ are listed to the right of the table in order of decreasing significance.

			Tadpole density			Tadpole growth rate		Size at metamorphosis		Other Correlations of note	
	Metric	Year	CF	WF	CF+WF	CF	WF	CF	WF	Variable	*r*
Chorus Frog	Density	2006	-	0.70*		-	-	-	-	% Road	−0.76**
										% Cattails	−0.63*
										Hydroperiod	−0.63*
										Physa sp.	0.51†
		2007	-	0.39		-	-	-	-	% Road	−0.76**
										Hydroperiod	−0.53†
	Growth rate	2006	−0.19	−0.33	−0.30	-	0.42	-	-	Dist. Forest	0.69**
		2007	−0.69*	−0.62*	−0.75**	-	0.53†	-	-	% Road	0.57*
	Survival	2006	0.40	-0.45	0.13	0.08	0.10	0.80[a]	-0.04	% Forest	0.60†
		2007	-0.26	−0.72**	−0.61*	0.40	−0.34	−0.27	−0.05	TP	0.78**
										Dist. Forest	0.72**
										TN	0.62*
										Zygopteran	0.61*
	Size at meta.	2006	−0.78[a]	−0.78[a]	−0.99**[a]	0.39[b]	0.62[b]	-	0.59[a]	% Road	0.99**[a]
										TP	−0.85†[a]
										Dytiscidae	−0.85†[a]
		2007	−0.25	−0.09	−0.23	0.36	0.53	-	0.81**	% Forest	0.63*
										Temperature	−0.55†
Wood Frog	Density	2006	0.70*	-		-	-	-	-	Dytiscidae	0.63*
		2007	0.39	-		-	-	-	-	Zygopteran	−0.64*
										Dist. Forest	−0.59*
										% Emergent	0.57*
										Predators	−0.55†
	Growth rate	2006	−0.09	−0.16	−0.16	0.42	-	-	-	Urban	0.79**
										NH_4	0.68**
										Snail	−0.63*
										Dist. Forest	0.62*
										% Water	−0.60*
										% Emergent	−0.58*
										Temperature	0.57*
		2007	−0.54†	−0.12	−0.42	0.53†	-	-	-	TP	−0.70**
										Depth	0.62*
										% Road	0.51†
										% Emergent	−0.49†
	Survival	2006	0.19	−0.75**	−0.13	0.22	0.06	0.58[a]	0.36	None	
		2007	−0.12	−0.85**	−0.57†	0.39	<0.01	−0.04	0.42	Zygopteran	0.77**
										Predators	0.67*
										% Emergent	−0.61*
										Area	0.56*
										% Pasture	0.55†
	Size at meta.	2006	−0.46	−0.69*	−0.68*	0.32	0.57†	0.59[a]	-	% Water	−0.61*
										Urban	0.58*
		2007	−0.37	−0.40	−0.47	0.38	0.43	0.81**	-	% Emergent	−0.75**
										% Non Forest	−0.67*
										Area	0.55†
										Hydroperiod	0.54†

** $p \leq 0.01$, * $p \leq 0.05$, † $p \leq 0.1$;

[a] =only four ponds in comparisons

[b] = only five ponds in comparisons

Table 4-8: Comparison of size at metamorphosis from chorus and wood frog individuals from agricultural ponds in the Beaver Hills region of Alberta to literature values.

size at metamorphosis (mm)	Range	N	Study	Location	Landscape
Chorus frog					
12.91	10.4-16.0	150	Current	Alberta	Agriculture
10.37	9.4-12.7	77	Whitaker 1971[c]	Indiana	Forested, agriculture
11.54	9-15	771	Smith 1987[c]	Michigan	Forested
10.4-14.5 [a]			Smith 1983[c]	Michigan	Forested
Wood frog					
21.0	18.0-24.7	33	Current	Alberta	Agriculture
16.92	14.6-19.0	97	DeBenedictis 1974	Michigan	Forested
16 [b]	14.2-18.1	-	Berven 1990	Michigan	Forested

[a] means from pens 16 with different densities

[b] lengths are snout to ischium which would be slightly shorter than snout to urostyle.

[c] previously classified as *Pseudacris triseriata,* now considered *P. maculata* sensu Lemmon et al. 2007.

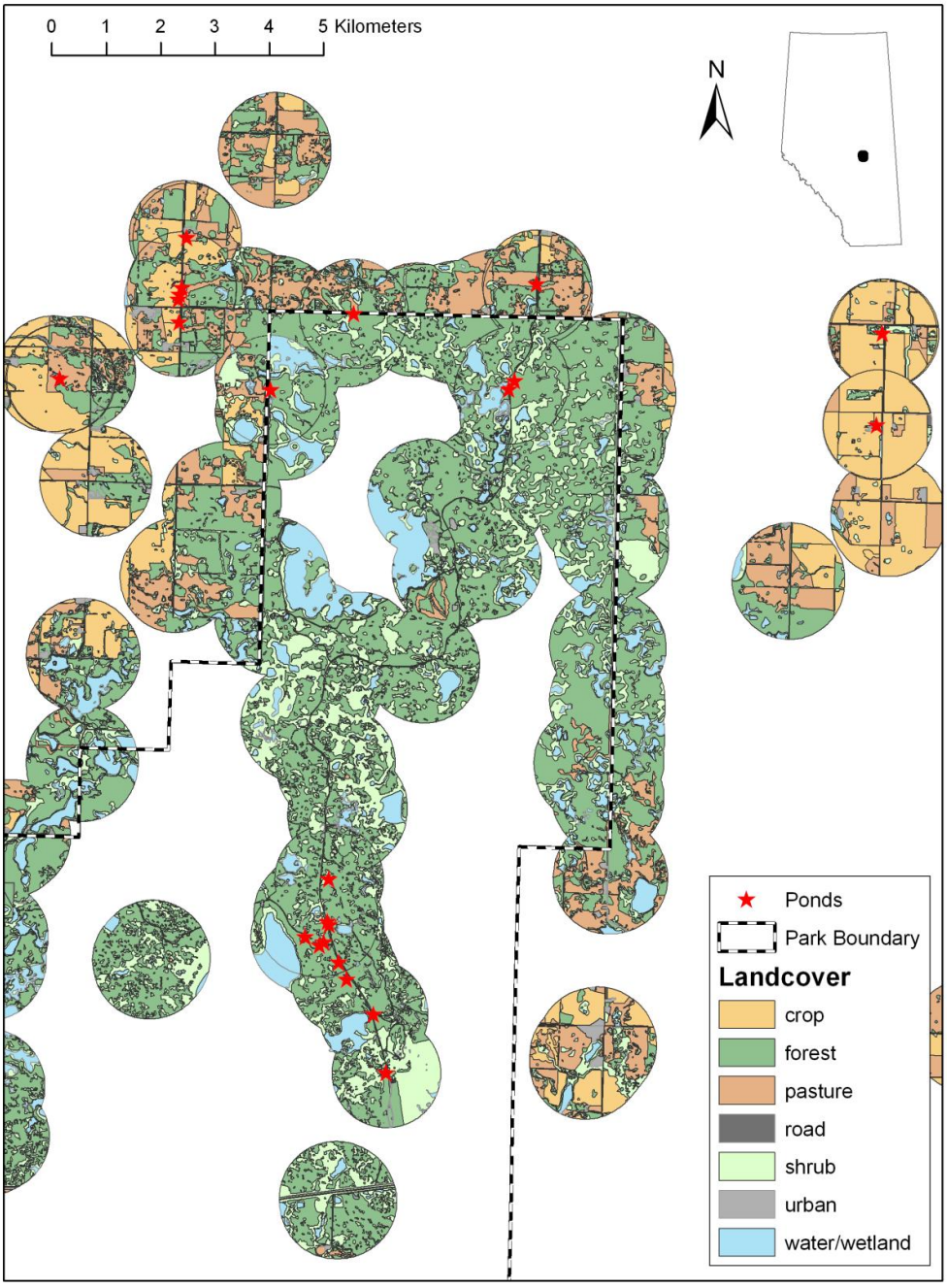


Figure 4-1: Land-cover map created in ArcGIS for ponds in and around Elk Island National Park, Alberta, showing the distribution of study ponds and dominant land-cover classes surrounding them.

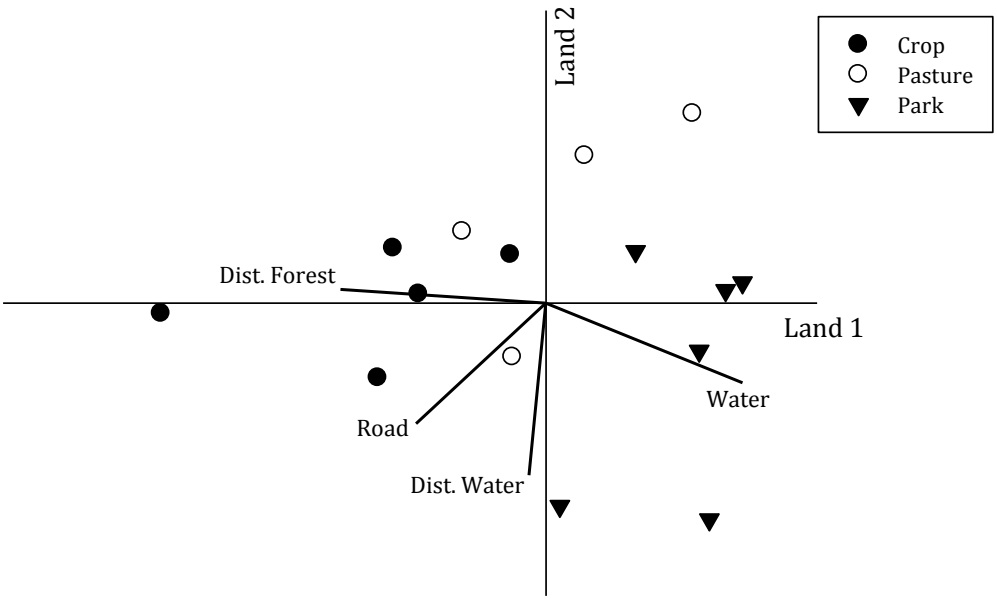


Figure 4-2: Biplot of the first two ordination axes from a Principal Component Analysis of landscape variables (Water, Road, Dist. Forest, Dist. Water) measured at 15 ponds located in different land-use types (five crop, 4 pasture, 6 park) in the Beaver Hills region of Alberta. Refer to Table 4-1 for definition of habitat variables; variables appearing were those selected using method B4 described by Joliffe (1972). Each symbol represents a pond, and each vector represents the correlation between a habitat variable and the two ordination axes.

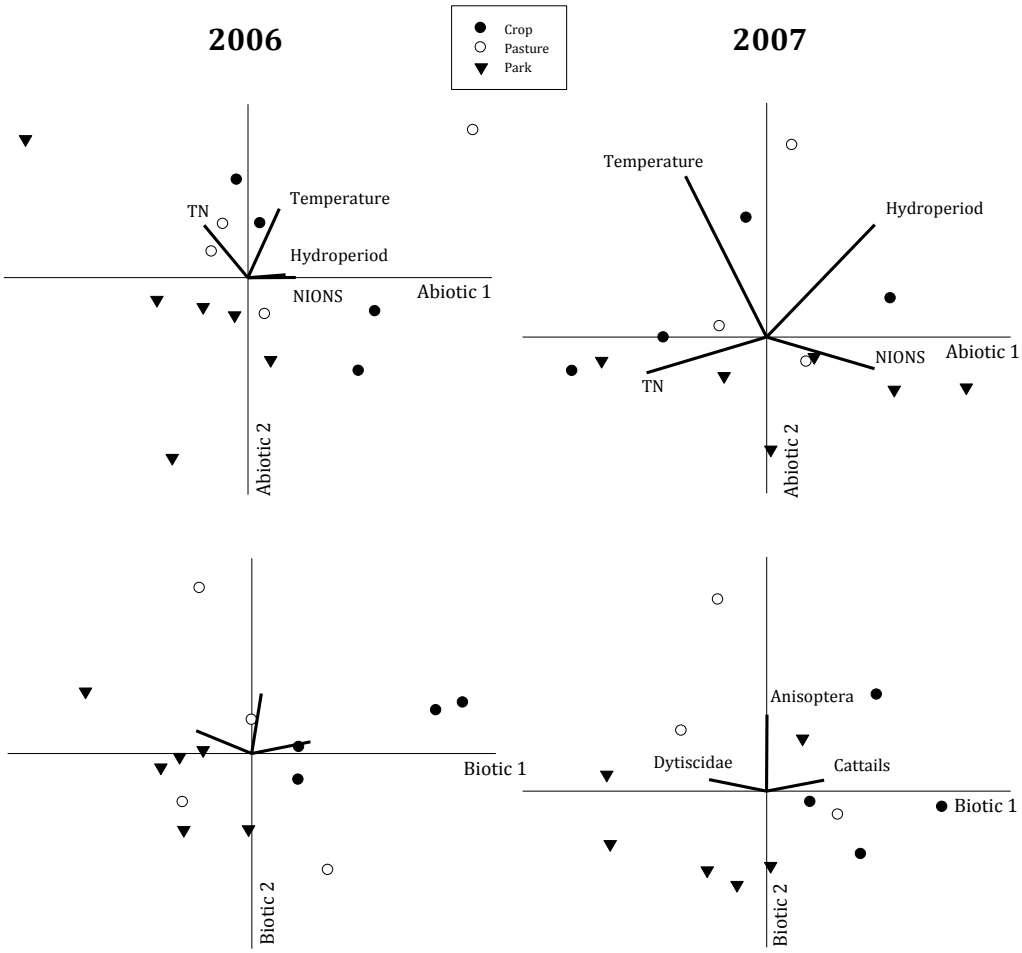


Figure 4-3: Biplots of the first two ordination axes from a Principal Component Analysis of either abiotic (upper) or biotic (lower) variables measured at 14 ponds in 2006 (left) and at 13 ponds in 2007 (right) in the Beaver Hills region of Alberta. Displayed are the vectors of variables that were retained after selection. Refer to Table 4-1 for definition of abiotic and biotic variables. Each symbol represents a pond, and each vector represents the correlation between that variable and the two ordination axes.

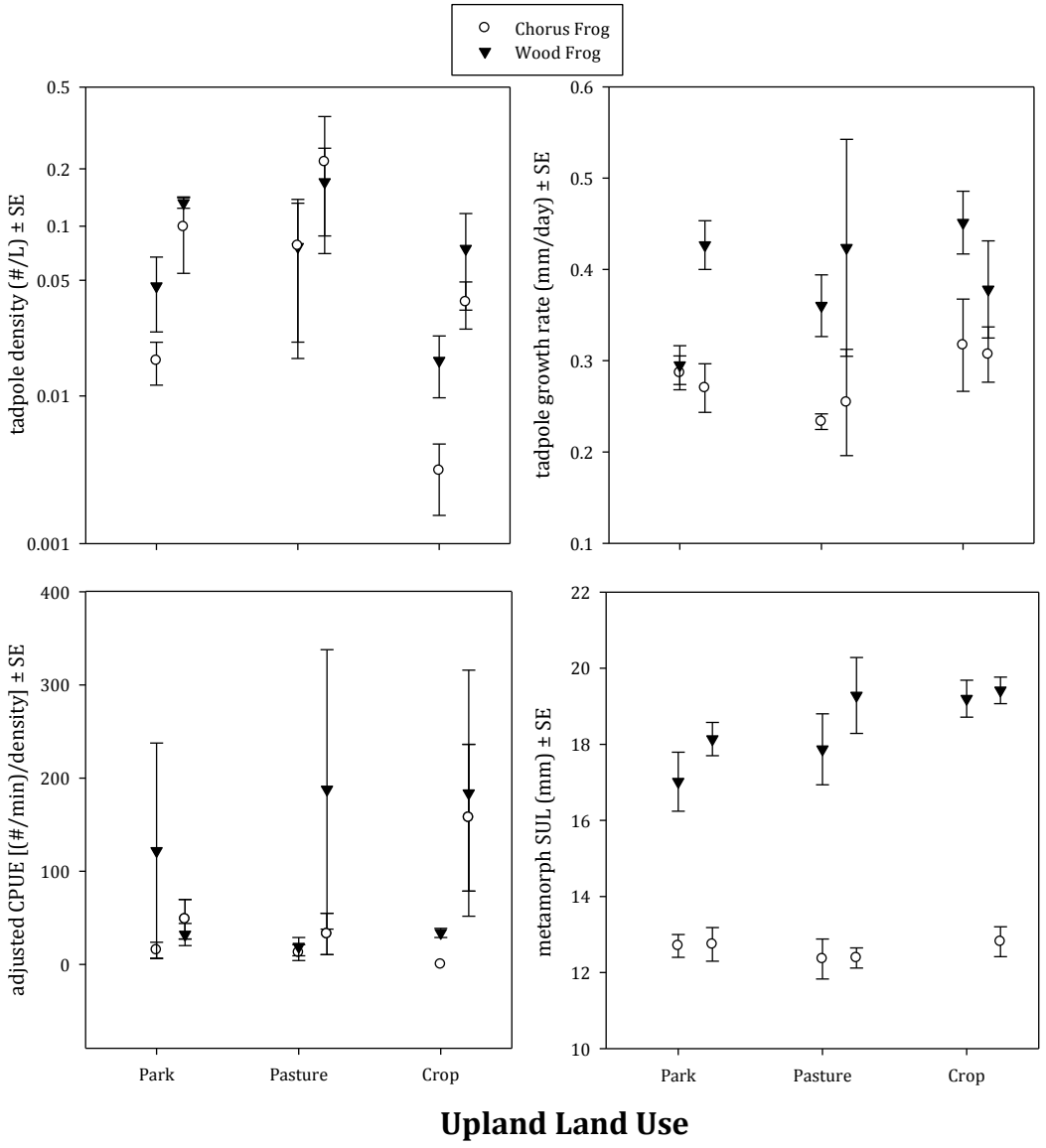


Figure 4-4: Comparison of anuran larval density and performance metrics across 14 ponds surrounded by three land-uses (six park, four[a] pasture, four crop) in the Beaver Hills of Alberta. Mean ± 1 SE for both chorus frog and wood frog for abundance, growth rate, survival and metamorph size. Results for 2007 are offset from those in 2006. Results of statistical comparisons are reported in Table 4-5.

[a] only three pasture ponds were used for all measures in 2007, and for larval density in 2006

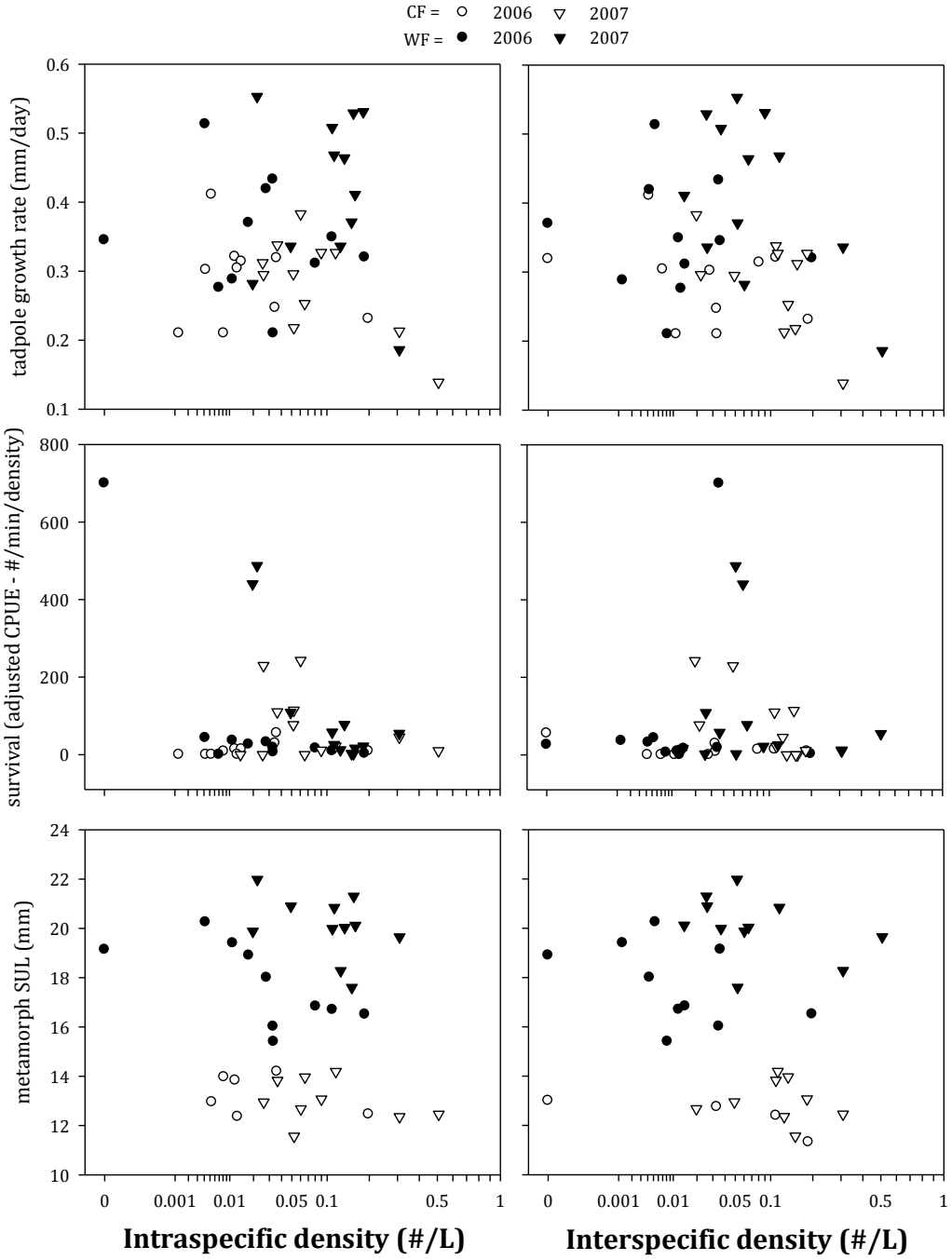


Figure 4-5: Comparison of tadpole performance metrics (growth, survival and size at metamorphosis) against intraspecific (left) and interspecific (right) tadpole densities at 11 ponds in 2006 and 12 ponds in 2007 in the Beaver Hills region of Alberta. Species are distinguished by shading, and year by shape.

Literature Cited

Alberta Environmental Protection. 1996 Alberta state of the environment report: aquatic ecosystems. Edmonton, Alberta: Alberta Environmental Protection; 1996.

Alford R. A. 1999. Ecology: resource use, competition, and predation. Pages 240-278 *In* R. W. McDiarmid, editor. Tadpoles: biology of anuran larvae, University of Chicago Press, Chicago.

Alford R. A. 1989a. Effects of parentage and competitor phenology on the growth of larval *Hyla chrysoscelis* . Oikos **54**:325-330.

Alford R. A. 1989b. Competition between larval *Rana palustris* and *Bufo americanus* is not affected by variation in reproductive phenology. Copeia **1989**:993-1000.

Altwegg R., H. U. Reyer. 2003. Patterns of natural selection on size at metamorphosis in water frogs. Evolution **57**:872-882.

Anderson A, Byrtus G, Thompson J, Humphries D, Hill B, Bilyk M. Baseline pesticide data for semi-permanent wetlands in the aspen parkland of Alberta. Edmonton: Alberta Environment; 2002.

Arendt J. D. 1997. Adaptive intrinsic growth rates: An integration across taxa. Quarterly Review of Biology **72**:149-177.

Baldwin R. F., A. J. K. Calhoun, and P. G. deMaynadier. 2006. Conservation planning for amphibian species with complex habitat requirements: A case study using movements and habitat selection of the Wood Frog *Rana sylvatica*. Journal of Herpetology **40**:442-453.

Bancroft B. A., N. J. Baker, and A. R. Blaustein. 2008. A meta-analysis of the effects of ultraviolet B radiation and its synergistic interactions with pH, contaminants, and disease on amphibian survival. Conservation Biology **22**:987-996.

Belden L. K., A. R. Blaustein. 2002. Exposure of red-legged frog embryos to ambient UV-B radiation in the field negatively affects larval growth and development. Oecologia **130**:551-554.

Berrill M., S. Bertram, A. Wilson, S. Louis, D. Brigham, and C. Stromberg. 1993. Lethal and sublethal impacts of pyrethroid insecticides on amphibian embryos and tadpoles. Environmental Toxicology and Chemistry **12**:525-539.

Berven K. A. 2009. Density dependence in the terrestrial stage of wood frogs: evidence from a 21-year population study. Copeia **2009**:328-338.

Berven K. A. 1990. Factors affecting population fluctuations in larval and adult stages of the wood frog (*Rana sylvatica*). Ecology **71**:1599-1608.

Berven K. A., T. A. Grudzien. 1990. Dispersal in the wood frog (*Rana sylvatica*): implications for genetic population structure. Evolution **44**:2047-2056.

Biek R., W. C. Funk, B. A. Maxell, and L. S. Mills. 2002. What is missing in amphibian decline research: insights from ecological sensitivity analysis. Conservation Biology **16**:728-734.

Blaustein A. R., J. M. Kiesecker, D. P. Chivers, D. G. Hokit, A. Marco, L. K. Belden, and A. Hatch. 1998. Effects of ultraviolet radiation on amphibians: field experiments. American Zoologist **38**:799-812.

Bremer D. J., L. M. Auen, J. M. Ham, and C. E. Owensby. 2001. Evapotranspiration in a prairie ecosystem: effects of grazing by cattle. Agronomy Journal **93**:338-348.

Bronmark C., S. D. Rundle, and A. Erlandsson. 1991. Interactions between fresh-water snails and tadpoles - competition and facilitation. Oecologia **87**:8-18.

Browne C. L., C. A. Paszkowski, A. L. Foote, A. Moenting, and S. M. Boss. 2009. The relationship of amphibian abundance to habitat features across spatial scales in the Boreal Plains. Ecoscience **16**:209-223.

Bruggeman D. J., J. A. Bantle, and C. Goad. 1998. Linking teratogenesis, growth, and DNA photodamage to artificial ultraviolet B radiation in *Xenopus laevis* larvae. Environmental Toxicology and Chemistry **17**:2114-2121.

Burton E. C., M. J. Gray, A. C. Schmutzer, and D. L. Miller. 2009. Differential responses of postmetamorphic amphibians to cattle grazing in wetlands. Journal of Wildlife Management **73**:269-277.

Carpenter S. R., N. F. Caraco, D. L. Correll, R. W. Howarth, A. N. Sharpley, and V. H. Smith. 1998. Nonpoint pollution of surface waters with phosphorus and nitrogen. Ecological Applications **8**:559-568.

Casey RJ, Paszkowski CA, Kendall SA, Ambrose N, Gingras B. Effects of cattle grazing intensity on water chemistry, aquatic invertebrates, waterbirds, songbirds and amphibians of pothole ponds of the Aspen Parkland, Central Alberta. Institute for Wetland and Waterfowl Research; 1999.

Chelgren N. D., D. K. Rosenberg, S. S. Heppell, and A. I. Gitelman. 2006. Carryover aquatic effects on survival of metamorphic frogs during pond emigration. Ecological Applications **16**:250-261.

Classen A., A. Hirler, and R. Oppermann. 1996. Auskirkungen unterschiedlicher Maehgeraete auf die wiesenfauna in Nordost-Polen. Naturschutz und Landschaftspanung **28**:139-144.

Collins J. P., A. Storfer. 2003. Global amphibian declines: sorting the hypotheses. Diversity and Distributions **9**:89-98.

Constible J. M., P. T. Gregory, and B. R. Anholt. 2001. Patterns of distribution, relative abundance, and microhabitat use of anurans in a boreal landscape influenced by fire and timber harvest. Ecoscience **8**:462-470.

Crouch W. B., P. W. C. Paton. 2000. Using egg-mass counts to monitor wood frog populations. Wildlife Society Bulletin **28**:895-901.

DeBenedictis P. A. 1974. Interspecific competition between tadpoles of *Rana pipiens* and *Rana sylvatica*: an experimental field study. Ecological Monographs **44**:129-151.

deMaynadier P. G., M. L. Hunter. 1999. Forest canopy closure and juvenile emigration by pool-breeding amphibians in Maine. Journal of Wildlife Management **63**:441-450.

Earl J. E., H. H. Whiteman. 2010. Evaluation of phosphate toxicity in Cope's gray treefrog (*Hyla chrysoscelis*) tadpoles. Journal of Herpetology **44**:201-208.

Eaves S. E. The distribution and abundance of amphibians across land-use types in Alberta's Aspen Parkland. University of Alberta; 2004. 134 p.

Faragher S. G., R. G. Jaeger. 1998. Tadpole bullies: examining mechanisms of competition in a community of larval anurans. Canadian Journal of Zoology **76**:144-153.

Fauth J. E., W. J. Resetarits. 1991. Interactions between the salamander *Siren intermedia* and the keystone predator *Notophthalmus viridescens* . Ecology **72**:827-838.

Foote A. L., C. L. R. Hornung. 2005. Odonates as biological indicators of grazing effects on Canadian prairie wetlands. Ecological Entomology **30**:273-283.

Frank A. B. 2003. Evapotranspiration from northern semiarid grasslands. Agronomy Journal **95**:1504-1509.

Gagne S. A., L. Fahrig. 2007. Effect of landscape context on anuran communities in breeding ponds in the National Capital Region, Canada. Landscape Ecology **22**:205-215.

Gibbs J. P. 1998. Amphibian movements in response to forest edges, roads, and streambeds in southern New England. Journal of Wildlife Management **62**:584-589.

Gibbs J. P., K. K. Whiteleather, and F. W. Schueler. 2005. Changes in frog and, toad populations over 30 years in New York State. Ecological Applications **15**:1148-1157.

Gibson A. R., A. J. Baker, and A. Moeed. 1984. Morphometric variation in introduced populations of the common Myna (*Acridotheres tristis*) - an application of the jackknife to principal component analysis. Systematic Zoology **33**:408-421.

Gillespie G. R. 2002. Impacts of sediment loads, tadpole density, and food type on the growth and development of tadpoles of the spotted tree frog *Litoria spenceri*: an in-stream experiment. Biological Conservation **106**:141-150.

Gilpin M. E., M. E. Soule. 1986. Minimum viable populations: processes of species extinction. Pages 19-34 *In* M. E. Soule, editor. Conservation Biology: The Science of Scarcity and Diversity, Sinauer Associates, Sunderland, Mass.

Gray M. J., L. M. Smith. 2005. Influence of land use on postmetamorphic body size of playa lake amphibians. Journal of Wildlife Management **69**:515-524.

Gray M. J., L. M. Smith, and R. I. Leyva. 2004. Influence of agricultural landscape structure on a Southern High Plains, USA, amphibian assemblage. Landscape Ecology **19**:719-729.

Green D. M. 2003. The ecology of extinction: population fluctuation and decline in amphibians. Biological Conservation **111**:331-343.

Grossman G. D., D. M. Nickerson, and M. C. Freeman. 1991. Principal component analyses of assemblage structure data - utility of tests based on eigenvalues. Ecology **72**:341-347.

Hamer A. J., J. A. Makings, S. J. Lane, and M. J. Mahony. 2004. Amphibian decline and fertilizers used on agricultural land in south-eastern Australia. Agriculture Ecosystems & Environment **102**:299-305.

Harkey G. A., R. D. Semlitsch. 1988. Effects of temperature on growth, development, and color polymorphism in the ornate chorus frog *Pseudacris ornata*. Copeia **1988**:1001-1007.

Harper E. B., R. D. Semlitsch. 2007. Density dependence in the terrestrial life history stage of two anurans. Oecologia **153**:879-889.

Hayes T., K. Haston, M. Tsui, A. Hoang, C. Haeffele, and A. Vonk. 2002. Herbicides: feminization of male frogs in the wild. Nature **419**:895-896.

Healey M., D. Thompson, and A. Robertson. 1997. Amphibian communities associated with billabong habitats on the Murrumbidgee floodplain, Australia. Australian Journal of Ecology **22**:270-278.

Heatwole, H. 1961. Habitat selection and activity of the wood frog, *Rana sylvatica* Le Conte. American Midland Naturalist **66**:301-313.

Hecnar S. J. 1995. Acute and chronic toxicity of ammonium-nitrate fertilizer to amphibians from southern Ontario. Environmental Toxicology and Chemistry **14**:2131-2137.

Hecnar S. J., R. T. M'Closkey. 1998. Species richness patterns of amphibians in southwestern Ontario ponds. Journal of Biogeography **25**:763-772.

Hecnar S. J., R. T. MCloskey. 1997. Patterns of nestedness and species association in a pond-dwelling amphibian fauna. Oikos **80**:371-381.

Herrmann H. L., K. J. Babbitt, M. J. Baber, and R. G. Congalton. 2005. Effects of landscape characteristics on amphibian distribution in a forest-dominated landscape. Biological Conservation **123**:139-149.

Holomuzki J. R., N. Hemphill. 1996. Snail-tadpole interactions in streamside pools. American Midland Naturalist **136**:315-327.

Humbert J., J. Ghazoul, and T. Walter. 2009. Meadow harvesting techniques and their impacts on field fauna. Agriculture Ecosystems & Environment **130**:1-8.

Janin A., J. Lena, N. Ray, C. Delacourt, P. Allemand, and P. Joly. 2009. Assessing landscape connectivity with calibrated cost-distance modelling: predicting common toad distribution in a context of spreading agriculture. Journal of Applied Ecology **46**:833-841.

Javorek S. K., R. Antonowitsch, C. Callaghan, M. Grant, and T. Weins. 2007. Changes to wildlife habitat on agricultural land in Canada, 1981-2001. Canadian Journal of Soil Science **87**:225-233.

Jofre M. B., W. H. Karasov. 1999. Direct effect of ammonia on three species of North American anuran amphibians. Environmental Toxicology and Chemistry **18**:1806-1812.

Johnson P. T. J., J. M. Chase, K. L. Dosch, R. B. Hartson, J. A. Gross, D. J. Larson, D. R. Sutherland, and S. R. Carpenter. 2007. Aquatic eutrophication promotes pathogenic infection in amphibians. Proceedings of the National Academy of Sciences of the United States of America **104**:15781-15786.

Jolliffe I. T. 1972. Discarding variables in a principal component analysis I: artificial data. Journal of the Royal Statisical Society. Series C (applied statistics) **21**:160-173.

Joly P., C. Morand, and A. Cohas. 2003. Habitat fragmentation and amphibian conservation: building a tool for assessing landscape matrix connectivity. Comptes Rendus Biologies **326**:S132-S139.

Knutson M. G., W. B. Richardson, D. M. Reineke, B. R. Gray, J. R. Parmelee, and S. E. Weick. 2004. Agricultural ponds support amphibian populations. Ecological Applications **14**:669-684.

Knutson M. G., J. R. Sauer, D. A. Olsen, M. J. Mossman, L. M. Hemesath, and M. J. Lannoo. 1999. Effects of landscape composition and wetland fragmentation on frog and toad abundance and species richness in Iowa and Wisconsin, USA. Conservation Biology **13**:1437-1446.

Kolozsvary M. B., R. K. Swihart. 1999. Habitat fragmentation and the distribution of amphibians: patch and landscape correlates in farmland. Canadian Journal of Zoology **77**:1288-1299.

Kramer D. C. 1973. Movements of western chorus frogs *Pseudacris triseriata triseriata* tagged with Co^{60}. Journal of Herpetology **7**:231-235.

Lemmon E. M., A. R. Lemmon, J. T. Collins, J. A. Lee-Yaw, and D. C. Cannatella. 2007. Phylogeny-based delimitation of species boundaries and contact zones in the trilling chorus frogs (*Pseudacris*). Molecular Phylogenetics and Evolution **44**:1068-1082.

Loman J. 2004. Density regulation in tadpoles of *Rana temporaria*: A full pond field experiment. Ecology **85**:1611-1618.

Loman J., B. Lardner. 2006. Does pond quality limit frogs *Rana arvalis* and *Rana temporaria* in agricultural landscapes? A field experiment. Journal of Applied Ecology **43**:690-700.

Marco A., C. Quilchano, and A. R. Blaustein. 1999. Sensitivity to nitrate and nitrite in pond-breeding amphibians from the Pacific northwest, USA. Environmental Toxicology and Chemistry **18**:2836-2839.

Marsh D. M., P. C. Trenham. 2001. Metapopulation dynamics and amphibian conservation. Conservation Biology **15**:40-49.

Mazerolle M. J., A. Desrochers. 2005. Landscape resistance to frog movements. Canadian Journal of Zoology **83**:455-464.

MjM Software Design. 2006. PC-ORD. Multivariated analysis of ecological data. **5.30**:.

Moriarty E. C., D. C. Cannatella. 2004. Phylogenetic relationships of the North American chorus frogs (Pseudacris : Hylidae). Molecular Phylogenetics and Evolution **30**:409-420.

Morin P. J., E. A. Johnson. 1988. Experimental studies of asymmetric competition among anurans. Oikos **53**:398-407.

Newman S., J. Schuette, J. B. Grace, K. Rutchey, T. Fontaine, K. R. Reddy, and M. Pietrucha. 1998. Factors influencing cattail abundance in the northern Everglades. Aquatic Botany **60**:265-280.

Oldham R. S., D. M. Latham, D. HiltonBrown, M. Towns, A. S. Cooke, and A. Burn. 1997. The effect of ammonium nitrate fertilizer on frog (*Rana temporaria*) survival. Agriculture Ecosystems & Environment **61**:69-74.

Oppermann R. 2007. Auswirkungen landwirtschaftlicher mähgeräte auf amphibien. Pages 102-108 *In* H. Laufer, K. Fritz, and P. Sowig, editors. Die amphibien und reptilien Baden-Württemberg, Ulmer, Stuttgart.

Oppermann R., J. Handwerk, M. Hosten, and A. Krismann. 2000. Naturverträgliche Mähtechnik für das Feuchtgrünland. *In* Anonymous Voruntersuchung für das E & E vorhaben, ILN Singen, Bonn, Germany.

Ouellet M., J. Bonin, J. Rodrigue, J. L. DesGranges, and S. Lair. 1997. Hindlimb deformities (ectromelia, ectrodactyly) in free-living anurans from agricultural habitats. Journal of Wildlife Diseases **33**:95-104.

Pahkala M., A. Laurila, and J. Merila. 2003. Effects of ultraviolet-B radiation on behaviour and growth of three species of amphibian larvae. Chemosphere **51**:197-204.

Pahkala M., A. Laurila, L. O. Bjorn, and J. Merila. 2001. Effects of ultraviolet-B radiation and pH on early development of the moor frog *Rana arvalis* . Journal of Applied Ecology **38**:628-636.

Pearman P. B. 1995. Effects of pond size and consequent predator density on 2 species of tadpoles. Oecologia **102**:1-8.

Pehek E. L. 1995. Competition, pH, and the ecology of larval *Hyla andersonii*. Ecology **76**:1786-1793.

Porej D., M. Micacchion, and T. E. Hetherington. 2004. Core terrestrial habitat for conservation of local populations of salamanders and wood frogs in agricultural landscapes. Biological Conservation **120**:399-409.

Price S. J., D. R. Marks, R. W. Howe, J. M. Hanowski, and G. J. Niemi. 2005. The importance of spatial scale for conservation and assessment of anuran populations in coastal wetlands of the western Great Lakes, USA. Landscape Ecology **20**:441-454.

Regosin J. V., B. S. Windmiller, R. N. Homan, and J. M. Reed. 2005. Variation in terrestrial habitat use by four poolbreeding amphibian species. Journal of Wildlife Management **69**:1481-1493.

Regosin J. V., B. S. Windmiller, and J. M. Reed. 2003. Terrestrial habitat use and winter densities of the wood frog (*Rana sylvatica*). Journal of Herpetology **37**:390-394.

Relyea R. A. 2003. Predator cues and pesticides: A double dose of danger for amphibians. Ecological Applications **13**:1515-1521.

Relyea R. A. 2002. Competitor-induced plasticity in tadpoles: consequences, cues, and connections to predator-induced plasticity. Ecological Monographs **72**:523-540.

Relyea R. A., N. M. Schoeppner, and J. T. Hoverman. 2005. Pesticides and amphibians: The importance of community context. Ecological Applications **15**:1125-1134.

Resetarits W. J., J. F. Rieger, and C. A. Binckley. 2004. Threat of predation negates density effects in larval gray treefrogs. Oecologia **138**:532-538.

Rothermel B. B., R. D. Semlitsch. 2002. An experimental investigation of landscape resistance of forest versus old-field habitats to emigrating juvenile amphibians. Conservation Biology **16**:1324-1332.

Rouse J. D., C. A. Bishop, and J. Struger. 1999. Nitrogen pollution: An assessment of its threat to amphibian survival. Environmental Health Perspectives **107**:799-803.

Rubbo M. J., J. M. Kiesecker. 2005. Amphibian breeding distribution in an urbanized landscape. Conservation Biology **19**:504-511.

Russell A. P., A. M. Bauer. 2000. The amphibians and reptiles of Alberta: a field guide and primer of boreal herpetology. University of Calgary Press, Calgary.

Schmutzer A. C., M. J. Gray, E. C. Burton, and D. L. Miller. 2008. Impacts of cattle on amphibian larvae and the aquatic environment. Freshwater Biology **53**:2613-2625.

Schoeppner N. M., R. A. Relyea. 2009. Phenotypic plasticity in response to fine-grained environmental variation in predation. Functional Ecology **23**:587-594.

Seale D. B. 1980. Influence of amphibian larvae on primary production, nutrient flux, and competition in a pond ecosystem. Ecology **61**:1531-1550.

Semlitsch R. D. 2000. Principles for management of aquatic-breeding amphibians. Journal of Wildlife Management **64**:615-631.

Skelly D. K. 2002. Experimental venue and estimation of interaction strength. Ecology **83**:2097-2101.

Skelly D. K. 1995. A behavioral trade-off and its consequences for the distribution of Pseudacris treefrog larvae. Ecology **76**:150-164.

Skelly D. K., L. K. Freidenburg, and J. M. Kiesecker. 2002. Forest canopy and the performance of larval amphibians. Ecology **83**:983-992.

Smith D. C. 1990. Population-structure and competition among kin in the chorus frog (*Pseudacris triseriata*). Evolution **44**:1529-1541.

Smith D. C. 1987. Adult recruitment in chorus frogs: effects of size and date at metamorphosis. Ecology **68**:344-350.

Smith D. C. 1983. Factors controlling tadpole populations of the chorus frog (*Pseudacris triseriata*) on Isle Royale, Michigan. Ecology **64**:501-510.

Smith D. C., J. Vanbuskirk. 1995. Phenotypic design, plasticity, and ecological performance in two tadpole species. American Naturalist **145**:211-233.

Smith G. R. 2007. Lack of effect of nitrate, nitrite, and phosphate on wood frog (*Rana sylvatica*) tadpoles. Applied Herpetology **4**:287-291.

Smith G. R., H. A. Dingfelder, and D. A. Vaala. 2004. Asymmetric competition between *Rana clamitans* and *Hyla versicolor* tadpoles. Oikos **105**:626-632.

Smith G. R., M. A. Waters, and J. E. Rettig. 2000. Consequences of embryonic UV-B exposure for embryos and tadpoles of the plains leopard frog. Conservation Biology **14**:1903-1907.

Smith-Gill S. J., K. A. Berven. 1979. Predicting amphibian metamorphosis. American Naturalist **113**:563-585.

Sours G. N., J. W. Petranka. 2007. Intraguild predation and competition mediate stage-structured interactions between wood frog (*Rana sylvatica*) and upland chorus frog (*Pseudacris feriarum*) larvae. Copeia **2007**:131-139.

Spencer AW. The relationship of dispersal and migration to gene flow in the Boreal Chorus Frog. Fort Collins, Colorado: Colorado State University; 1964.

Statistics Canada. Census of agriculture 2006. Ottawa, ON: Statistics Canada; 2006.

Stevens C. E., C. A. Paszkowski. 2004. Using chorus-size ranks from call surveys to estimate reproductive activity of the wood frog (*Rana sylvatica*). Journal of Herpetology **38**:404-410.

Stewart R. E., H. A. Kantrud. 1971. Classification of natural ponds and lakes in the glaciated prairie region. U.S. Fisheries and Wildlife Service Resource Publication **92**:57.

Stuart S., J. S. Chanson, N. A. Cox, B. E. Young, A. S. L. Rodrigues, D. L. Fishman, and R. W. Waller. 2004. Status and trends of amphibian declines and extinctions worldwide. Science **306**:1783-1786.

SYSTAT Software INC. 2008. SigmaPlot. 11.0. Richmond, California.

SYSTAT Software INC. 2007. Systat. 12.02. San Jose, California.

Thurston RV, Russo RC, Emerson K. Aqueous ammonia equilibrium. Tabulation of percent un-ionized ammonia. Duluth, MN: U.S. Environmental Protection Agency; 1979. Report nr EPA-600/3-79091.

Trauth J. B., S. E. Trauth, and R. L. Johnson. 2006. Best management practices and drought combine to silence the Illinois chorus frog in Arkansas. Wildlife Society Bulletin **34**:514-518.

Turner M. G. 1989. Landscape ecology - the effect of pattern on process. Annual Review of Ecology and Systematics **20**:171-197.

Vaccaro LE. Patterns, mechanisms, and ecological implications of cattail (*Typha* spp.) dominance in great lakes wetlands. Cornell University; 2005.

Van Buskirk J. 2001. Specific induced responses to different predator species in anuran larvae. Journal of Evolutionary Biology **14**:482-489.

Van Buskirk J., S. A. McCollum, and E. E. Werner. 1997. Natural selection for environmentally induced phenotypes in tadpoles. Evolution **51**:1983-1992.

Vitousek P. M., H. A. Mooney, J. Lubchenco, and J. M. Melillo. 1997. Human domination of Earth's ecosystems. Science **277**:494-499.

Voldseth R. A., W. C. Johnson, T. Gilmanov, G. R. Guntenspergen, and B. V. Millett. 2007. Model estimation of land-use effects on water levels of northern prairie wetlands. Ecological Applications **17**:527-540.

Vonesh J. R., O. De la Cruz. 2000. Modeling the effect of UV-B induced egg-stage mortality on the population dynamics of the common toad. American Zoologist **40**:1246-1247.

Vos C. C., P. W. Goedhart, D. R. Lammertsma, and A. M. Spitzen-Van der Sluijs. 2007. Matrix permeability of agricultural landscapes: an analysis of movements of the common frog (*Rana temporaria*). Herpetological Journal **17**:174-182.

Wassersug R. 1972. Mechanism of ultraplanktonic entrapment in anuran larvae. Journal of Morphology **137**:279-288.

Werner E. E., R. A. Relyea, K. L. Yurewicz, D. K. Skelly, and C. J. Davis. 2009. Comparative landscape dynamics of two anuran species: climate-driven interaction of local and regional processes. Ecological Monographs **79**:503-521.

Whitaker J. O. 1971. A study of the western chorus frog, *Pseudacris triseriata* in Vigo County, Indiana. Journal of Herpetology **5**:127-150.

White J. S., S. E. Bayley. 2001. Nutrient retention in a northern prairie marsh (Frank Lake, Alberta) receiving municipal and agro-industrial wastewater. Water Air and Soil Pollution **126**:63-81.

White J. S., S. E. Bayley, and P. J. Curtis. 2000. Sediment storage of phosphorus in a northern prairie wetland receiving municipal and agro-industrial wastewater. Ecological Engineering **14**:127-138.

Whiting A. V. Population ecology of the western chorus frog, *Pseudacris triseriata*. Montreal, Quebec: McGill University; 2004.

Wiens J. A. 1997. Metapopulation dynamics and landscape ecology. Pages 43-62 *In* I. A. Hanski and M. E. Gilpin, editors. Metapopulation biology: ecology, genetics and evolution, Academic Press, Toronto, Canada.

Wilbur H. M. 1997. Experimental ecology of food webs: complex systems in temporary ponds. Ecology **78**:2279-2302.

Wilbur H. M. 1987. Regulation of structure in complex-systems - experimental temporary pond communities. Ecology **68**:1437-1452.

Wilbur H. M., J. P. Collins. 1973. Ecological aspects of amphibian metamorphosis. Science **182**:1305-1314.

Williams B. K., K. Titus. 1988. Assessment of sampling stability in ecological applications of discriminant-analysis. Ecology **69**:1275-1285.

Wood S. L. R., J. S. Richardson. 2009. Impact of sediment and nutrient inputs on growth and survival of tadpoles of the Western Toad. Freshwater Biology **54**:1120-1134.

Woodward B. D. 1983. Predator-prey enteractions and breeding-pond use of temporary-pond species in a desert anuran community. Ecology **64**:1549-1555.

Young J. E., G. A. Sanchez-Azofeifa, S. J. Hannon, and R. Chapman. 2006. Trends in land cover change and isolation of protected areas at the interface of the southern boreal mixedwood and aspen parkland in Alberta, Canada. Forest Ecology and Management **230**:151-161.

Chapter 5: The effect of interference by wood frogs on larval performance

Introduction

Competition between species is important to the study of population and community ecology. Competitive interactions fall into three categories: 1) species have no effect on one another – species do not compete; 2) one species is disproportionately impacted by one or more species – asymmetric interspecific competition; or 3) both species are affected – symmetric competition. Competition is traditionally viewed to operate through two classes of mechanisms. Individuals in exploitative competition deplete shared resources, depriving others of their use. Interference competition is more direct whereby individuals harm one another by combat, releasing toxins or by preventing access to critical resources. Gurevitch et al. (1992) showed that competition within aquatic systems is at least as common as in other systems (proportion of cases examined with competition: 64.8% for freshwater vs. 67.9% in marine and 73.3% in terrestrial systems). However, the frequency and intensity of interference competition is generally unknown or untested (Schoener 1983).

The existence of resource exploitation as the main process can be investigated through behavioural observation, gut content analysis or analytical techniques such as stable isotopes (Chapter 2). Schoener (1983) showed that, among the studies he examined, exploitation and interference mechanisms occur equally often when interference is sought. Case and Gilpin (1974) demonstrated that interference competition is common and more important in determining the abundance and distribution of organisms than is exploitation. In general,

interference comes at a cost, but a profit is realized by the interfering species, or individual, given that more resources become available as a result of its actions (Case and Gilpin 1974). Growth rates, sizes and survival, or more generally, performance of the individual that interferes improves with interference creating asymmetries between the two competing individuals or species. Measuring interference in the field can be difficult as observed competitive asymmetries can also be produced incidentally if there are inherent large differences in body size, activity level or through exploitative competition (Wilbur 1997). Manipulations that exclude interactions or remove food limitations, can reveal mechanisms responsible for observed asymmetries.

Interactions between larvae of anuran species and other member of their aquatic food webs are commonly used as models to explore how competition structures communities. Interspecific competition is believed to be common among multispecies larval assemblages (Alford 1999) because species differences in feeding niches are perceived to be small and foods in aquatic environments are limited (DeBenedictis 1974, Wilbur 1977). However, uncertainty in larval diets (Altig et al. 2007) may overestimate the similarity in larval diets and may bias expectations of exploitative competition in favour of direct interactions. Evidence of competition within larval anuran communities includes changes in the rates of survival to metamorphosis or of growth. Other changes associated with competition include altered behaviour, differences in distribution among microhabitats within a pond, and/or changes in resource use. In general, growth and survival are expected to be higher in the absence of competition and their variation smaller as individuals should not be limited by resource availability. Competition produces greater variability in individual metrics as some individuals may escape competition or find

themselves in areas of lower competitor density or higher food density or quality. Documenting patterns of growth, survival, behaviour, distribution and resource use by tadpoles in natural environments may not reveal the mechanism of competition (competitive or interference). Measuring competition and interference is complicated in diverse food webs as predators may alter competitive interactions, depending on community composition, and thus manipulations of community membership such as occurs in mesocosm or laboratory experiments generally serve to examine interactions between competitors in the absence of predators and food limitations.

Although evidence of interference between tadpoles in natural ponds is rare (Petranka 1989, Biesterfeldt et al. 1993, Bardsley and Beebee 2001), evidence of interference between anuran larvae in the laboratory is more common (e.g. Richards 1958, Beebee 1991, Faragher and Jaeger 1998). Documented interference for amphibian larvae includes spatial displacement (Richter-Boix et al. 2004), aggression (Faragher and Jaeger 1998), chemical interference (Rose and Rose 1961, 1965, Akin 1966, Shvarts and Pyastolova 1970ab, Gromko et al. 1973, Runkova et al. 1974, Stepanova 1974, Steinwascher 1978a), or cellular-mediated interference where a single-celled organism is suspected as the cause Richards 1958, 1962, West 1960, Steinwascher 1979ab, Beebee 1991, Griffiths et al. 1991, Wong et al. 1994, Baker and Beebee 2000, Wong et al. 2000, Bardsley and Beebee 2001). Combinations of mechanisms have also been documented, e.g. behavioural and chemical interference (Faragher and Jaeger 1998); chemical and cellular-mediated interference (Akin 1966, Licht 1967, Biesterfeldt et al. 1993). Interference that reduces tadpole growth, survival and size at metamorphosis can have dramatic effects on population persistence for temperate anurans breeding in temporary

ponds. Recruitment of metamorphs depends upon tadpole survival, which can be limited by reduced growth rates that prolong tadpole exposure to predation or prolong development in drying ponds. Smaller size at metamorphosis reduces reproductive fitness and terrestrial survival, as both are positively correlated with size at metamorphosis (Smith 1987, Berven 1990).

Within the Beaver Hills region of Alberta, the boreal chorus frog (*Pseudacris maculata*, hereafter referred to as CF) and wood frog (*Lithobates sylvaticus*, hereafter referred to as WF) breed sympatrically in temporary ponds (Class II to IV wetlands as defined by Stewart and Kantrud 1971) where survival depends on rapid development. A number of authors have suggested that ranid tadpoles are competitively dominant to hylid tadpoles (Wilbur 1987, Morin and Johnson 1988, Alford 1989ab, Fauth and Resetarits 1991, Pehek 1995, Faragher and Jaeger 1998). My surveys support this claim, as I observed reduced CF larval growth rates in the presence of WF (Chapter 2). In Chapter 2 it was shown that reduced growth was not related to pond characteristics, or diet overlap (Chapter 2), suggesting that interference mechanisms may be occurring. Since agonistic interference was not observed between the larval species, I chose to use a series of laboratory experiments to directly test whether chemical interference might occur and be responsible for reduced CF growth rates. I hypothesize that reduced growth could be the result of inhibitory chemicals released by WF into shared water either directly or through feces. Therefore, my objective was to test whether CF tadpole growth, survival or size at metamorphosis could be reduced in the presence of 1) WF tadpoles and their feces, 2) WF tadpoles alone, or 3) WF feces alone. The first two cases assessed whether chemical interference occurs between these two species, while the third investigates the source of inhibition. These experiments are

a first step in determining if interference mediated through some inhibitory substance is responsible for the competitive patterns that I observed in both field surveys and mesocosm experiments.

Methods

Lab experiments were carried out at the University of Alberta's Biosciences Aquatics Facility. I stocked experiments with CF and WF tadpoles and/or eggs collected from ponds in Elk Island National Park (EINP, 53° 35′ N, 112° 52′ 15 W). Three experiments were conducted (Table 5-1), two in 2007 to confirm the existence of chemical inhibition, and a third in 2008 to confirm whether feces carried the agent that reduces CF performance. For the purposes of clarity I use italics to name *tank elements* and italicized small caps to identify *TREATMENT NAMES*.

Animal collection and tadpole housing

For the first two experiments conducted in 2007, I collected 76 CF egg masses from a pond known to have both CF and WF breeding in EINP on 26 April (egg masses contained 7-83 eggs, with a mean of 33, for a total of 2480 eggs collected). I collected masses from several locations within a pond to increase the diversity of the stocked tadpoles as egg masses found within 50 cm are likely from the same female. Around 480 eggs were used in the two experiments study; the remainder were used in the mesocosm experiment (Chapter 3). Eggs were held in plastic containers with pond water for four days, then placed into a holding tank (*CF holding tank*: 76 × 53 cm filled to a depth of 35 cm) with flow through de-chlorinated city water. All larvae were free-swimming within nine days of collection and were fed finely ground rabbit pellets (Hagen ART.# H-1153, 16% crude protein) *ad libitum* until larvae were assigned randomly to treatment aquaria on 14 May. The

remaining tadpoles were kept in the *CF holding tank* and used to replace tadpoles that died in the first week of trials. Tadpoles in the *CF holding tank* were fed at the same rate as those in the experiments (see below).

WF tadpoles collected for the experiments in 2007 came from another pond in EINP as few WF eggs were laid in the source pond for CF. As WF eggs had already hatched, I collected WF tadpole hatchlings from within the communal mass of deposited eggs (n ≈ 400) on 2 May, 2007 and housed them in a second holding tank (*WF holding tank*) until the start of the experiments. WF tadpoles in the holding tank were initially fed *ad libitum* and food was reduced in the holding tank as the experiment progressed to maintain water quality. Small nylon net fish breeder cages (Hagen Marina # 10934; 2L volume, 16.2 × 12.6 × 13.3 cm, mesh size = 300 μm) covered by black pantyhose (mesh size = 300 μm) leaving the top open were attached to the long side of all aquaria (hereafter referred to as *WF cages*). WF tadpoles were haphazardly selected and added to 16 of 32 *WF cages* on 14 May. WF tadpoles in treatments were replaced on day 8, as I believed that those grown at high densities in the *WF holding tank* were more likely to produce growth inhibitors than those in *WF cages*. However, I did not conduct further changes as WF tadpoles in the *WF holding tank* were significantly smaller than those in treatment cages after this date, and growth inhibitors are believed to be produced at higher rates in larger tadpoles (e.g. Wong et al. 2000).

For the third experiment in 2008, 17 CF eggs masses (945 eggs total, range 6-150 eggs per mass) were collected on 12 May, from a different pond in EINP from those in 2007. I placed eggs into plastic tubs (2-3 masses per tub until hatching) with a mix of pond and de-chlorinated water. As in 2007, masses were collected from several locations to increase genetic diversity of tadpoles. Tadpoles started

hatching on 13 May and continued until 21 May. Hatchlings were divided between this experiment and the isotope fractionation feeding trial described in Chapter 2. Tadpoles remaining after stocking both experiments were housed in the *CF holding tank* (as previously). Tadpoles that died in the first week of the experiment were replaced from the holding tank. CF tadpoles in the *holding tank* were fed the same rations as treatment tadpoles (see below). WF eggs for this experiment came from the same pond as CF eggs. Eggs were taken from 12 different locations within the WF communal mass to maximize genetic diversity (n = 484 eggs, $\overline{x}$ = 40.3±4.6 eggs from each mass). Tadpoles hatched on 21 May and 100 hatchlings were placed in a holding tank (*WF fecal holding tank*, 76 × 53 × 35 cm deep with 108 L of water) to generate feces for CF treatment tubs. The remaining WF tadpoles were used in the feeding trial described in Chapter 2. Mean WF mass in the holding tank was 19.2 ± 0.8 mg. I started fecal collections on 23 May. A number of tadpoles died within the first few days. I replaced the dead WF tadpoles in the *WF fecal holding tank* with new wild caught WF tadpoles on 27 May (n = 56). But most of those tadpoles also died (n = 20 remained by 12 June). Death may have been due to a fungal growth seen on many of the dead tadpoles or a result of a bacterial infection. On 12 June I placed 73 new WF tadpoles from a natural pond into a clean aquarium (*WF fecal tank*, 50 × 25 × 27 cm) filled with 25 L of water and these tadpoles were used to generate feces for the remainder of the experiment. Feeding rations are described in the experimental design.

Experimental design

Experiment #1: Influence of WF tadpoles and their feces on CF ("WF+F_W")

On 4 May 2007, I filled 20 glass aquaria (50 × 25 cm × 27 cm deep) with 28 L of de-chlorinated city water. The 20 aquaria (CF aquaria) were divided equally between two shallow water filled troughs with flow-through water around aquaria to maintain tank temperatures. Aquaria were kept in a temperature controlled room at 17°C with fluorescent lighting set at 16 hr L/ 8 hr D. On day 18, mean temperature across aquaria was 17.4±0.07°C ($\bar{x}$±SE, Standard error is used throughout unless otherwise noted) and on day 33, mean temperature was 16.4±0.05°C. Light-dark cycle and temperatures were similar to those observed in EINP during the tadpole growing season (unpublished data). Ten treatment and 10 control aquaria were assigned in alternating fashion. The experimental treatment aquaria had WF tadpoles in the *WF cage* and their feces were regularly moved into the corresponding main CF tadpole aquaria (see below). In the control aquaria *WF cages* were attached without tadpoles or feces. On 14 May, I released 15 CF hatchlings in each aquarium (mean body length = 3.16±0.04 mm, N = 20 tanks and did not differ between treatments). Body length is defined as the length from the snout to the point where the tail meets the body and hind limbs emerge. In treatment aquaria, I placed four WF tadpoles (mean body length of 5.09±0.08 mm N = 40 tadpoles) into each *WF cage*, a cage density of about 2 WF tadpoles/L. The experiment ran 73 days, 14 May – 25 July.

All tadpoles were fed ground rabbit pellets suspended in water. Initially I fed CF tadpoles at 20% of average tadpole mass based on the previous period's average mass across experiment #1 and #2. I chose 20% to prevent food limitations which

might discourage consumption of WF feces. Feeding usually occurred in the morning and food was dispersed throughout the aquarium. On day 29 CF feeding rate was reduced to 10% of average tadpole mass due to water fouling. Initially aquaria were not aerated, but I installed airstones in a corner away from the *WF cages* when oxygen levels reached 5% saturation on day 29. WF cages (both treatment and control) were provided 20% of average WF tadpole mass across experiments #1 and #2 from day 1-29, 10% day 30-54, and 5% until the end of the experiment. Every 2-3 days, uneaten food and feces were removed from all CF aquaria using aquarium nets. After the main tank was cleaned, feces and remaining food from treatment *WF cages* were moved with a turkey baster from the cage into the main tank. Food in control *WF cages* was discarded but water was transferred from the cage into the CF tank for consistency.

Responses used to assess whether CF were affected by interference chemicals produced by WF tadpoles or released from their feces were: change in mass; change in body length and total length; mass at emergence: date of emergence; mass at metamorphic climax; date of metamorphic climax; % survival at experiment end; and % emergence. I measured mass and body length of tadpoles and at emergence because these attributes correlate with post-metamorphic survival and reproduction (e.g. Wilbur 1977). In addition to the above responses, I calculated tadpole condition - Fulton's K = mass(g) / total length(cm)^3 × 10^3 – (Ricker 1975) for each seven day period. Measurement of changes in mass and length began on day 7, the last date when new CF tadpoles were added to replace mortalities, and continued to day 40. Tadpoles were weighed at least every seven days on a Mettler BB2440 Delta Range balance to 0.01 gram with an error of ±0.01 g. All CF tadpoles from within a treatment tank were poured into an aquarium net,

blotted dry with paper towel, and weighed as a group in a tared transparent water dish. I weighed all tadpoles from an aquarium together as individual tadpoles were initially too light. Tadpoles (dorsal or lateral surfaces) were photographed with a 1mm grid below the water dish with a Canon EOS digital Rebel XT with a 100mm macro lens. Individual tadpole body and total length were later measured to the nearest 0.01 mm based on the gridded images using either Photoshop 6.0 or GIMP 2.4.7. Emergence was defined as the eruption of both forelimbs, equivalent to Gosner stage 42 (Gosner 1960); metamorphic climax was when the tail was completely absorbed (Gosner stage 45). Tadpoles that emerged were weighed individually and then placed in labelled plastic terrariums with a small amount of water until tail resorption was complete when they were again weighed. Percent survival was the number of tadpoles remaining in the treatment aquaria at the end of the experiment plus all those that emerged divided by the number present on May 20 (last date of restocking).

Experiment #2: Influence of WF tadpoles only on CF ("WF")

On 4 May 2007, I filled 12 glass aquaria (40 × 20 cm × 23 cm deep) with 17 L of water. These aquaria were set up in the same room as experiment #1, and therefore had similar lighting conditions and air temperatures, but aquaria were in a different trough with flow through water around the aquaria. Due to unplanned differences in water sources, tank temperatures were about 2°C cooler than for experiment #1, making direct comparisons of responses between experiments unreliable. On day 18, mean temperatures across aquaria was 15.2±0.08°C (range 14.9-15.9°C) and on day 33, mean temperatures was 13.5±0.06°C (range 13.2-13.8°). Aquaria were set up as in experiment #1 with six treatment aquaria alternating with six control aquaria. Treatment aquaria had four WF tadpoles in

each *WF cage* and control aquaria had empty *WF cages*. On 14 May, I placed nine CF tadpole hatchlings into each aquarium with mean body length 3.23±0.04 mm. Mean body length was similar across the experiment. Density of CF tadpoles was consistent with experiment #1 (~0.53 tadpoles/L). Four WF tadpoles with mean body length of 4.90±0.13 mm (N = 48, weighed on 15 May) were added to each treatment *WF cage* also on 14 May (cage density ~2 WF tadpoles/L, consistent with experiment #1). Experiment #2 ran 73 days, from 14 May – 25 July.

Tadpole feeding and aeration regime were as in experiment #1. Feces and food from *WF cages* were removed and discarded from all *WF cages* every 2-3 days. CF aquaria were cleaned as in experiment #1.

To evaluate whether WF tadpoles alone influence CF, I measured tadpole change in mass, change in body length and total length, condition and % survival to experiment end. Tadpole mass and lengths were measured on the same dates and following the methods in experiment #1. I also measured mass at emergence and date of emergence, but due to low temperatures and slow development, I abandoned measures at metamorphic climax.

Experiment #3: Influence of wood frog feces only on CF ("F_W")

On 18 May 2008, I filled 24 plastic tubs (27 × 12 × 17 cm deep) with 4.7 L of de-chlorinated city water. The tubs were placed at the bottom of two large fibreglass tanks (12 tubs in each) and randomly assigned as treatment or control. The experiment was kept in a temperature controlled room at 20°C with 12 hr L/ 12 hr D. The temperature of tubs was recorded continuously using temperature loggers (Thermochron® iButton DS-1921G – Maxim Integrated Products). Water temperatures were 20±0.5°C and did not differ between treatments. On 22 May, I

randomly assigned five free-swimming CF tadpoles to each of the 24 plastic tubs (1.06 tadpoles/L). Mean body length of CF in tubs was 1.83±0.03 mm (N = 24 tubs) and did not differ between treatments. I increased the density from that used in experiments #1 and #2 to compensate for possible mortality. The 12 treatment tubs received WF feces daily generated by 50-100 WF tadpoles in the *WF fecal tank* (Table 5-1). The control tubs received an equal volume (see below) of de-chlorinated tap water to simulate the addition of water and feces.

CF tadpoles were fed ground rabbit pellets as in the other experiments. I fed tadpoles at 15% of average tadpole mass across treatments based on the previous week's average mass. For the first 14 days WF tadpoles in the *WF fecal holding tank* tadpoles were fed a mixed diet of rabbit pellets and spirulina (42% crude protein) to increase their size. For the remaining 43 days, I fed WF only rabbit pellets at 10% or less of the average mass to make feces collection easier; larger tadpole size, higher density and low food conditions should elicit greater inhibitor production (Rose and Rose 1961, Beebee and Wong 1992). WF feces were collected daily either by placing WF tadpoles in a plastic container with water, or collected directly from *WF holding tanks* with a turkey baster. Feces and food were separated and the WF feces was divided into 12 equal portions and applied to treatment tubs in about 5 ml of water each day. Five ml of water was put into control tubs to offset the disturbance caused by WF feces application. All CF treatment tubs were aerated with airstones daily for 10 minutes; WF *holding tanks* were aerated continuously. The experiment was terminated after 50% of all CF tadpoles in the experiment had emerged regardless of treatment; duration was 57 days, from 22 May – 17 July.

CF responses measured include change in tadpole mass, change in body and total length, mass at emergence, date of emergence, and % survival to experiment

end. Emergence was used as the experimental endpoint as mass at metamorphic climax was highly correlated to mass at emergence (Table 5-2) from CF in experiment #1. CF tadpoles were individually weighed weekly on a Mettler PJ300 to the nearest mg (± 0.001 g). As in the previous experiments, body length and total length were measured from photographs and tadpoles were removed at emergence (Gosner stage 42).

Data analysis

Analyses were similar among all three experiments. Mean values from within each aquarium/tub were used for analysis. For comparisons presented in the results, I report findings in the following format (control mean ± SE vs. WF treatment mean ± SE). I tested for normality of measures using Shapiro-Wilk's normality test, and tested for homogeneity of variances using F-tests. Percent survival and % emergence were each arcsine square root transformed before normality was assessed. Tadpoles raised together will compete for food and realize different growth rates producing a log-normal distribution of sizes (Wilbur 1977). Thus length and weight measures were log transformed log(x+1). Differences in growth (length and mass) trajectories between control and treatments were tested using Repeated Measures ANOVA. When differences were found, ANOVAs for individual dates were used to determine when differences in mass or length were significant.

As emergence and metamorphosis occurred over an extended period (33 days in experiment #1 and 21 days in experiment #3), I determined if date at emergence (and metamorphosis for experiment #1) influenced weight at emergence consistently and could be used as a covariate in an ANCOVA to help reduce variation

created by differences in emergence dates. To determine what impact WF tadpoles and their feces might have on CF tadpoles within natural ponds where eggs were collected, I compared the differences between control and WF treatments on July 6 when those ponds were usually dry. The variation of tadpole sizes (length and mass) in treatment tanks is expected to increase under interference competition (Steinwascher 1978b) as small tadpoles remain stunted whereas the large tadpoles continue to grow increasing the variation within cohorts within replicates. As such, I tested whether variation among CF tadpoles within aquaria averaged across a treatment were greater in WF treatments than in controls. All analyses were done using SYSTAT 12.02 (SYSTAT Software INC. 2007). Consistent with the other chapters I consider effects significant at alpha = 0.10.

Results

Experiment #1 Influence of WF tadpoles and their feces on CF ("WF+F_W")

CF tadpoles displayed reduced lengths and mass in response to the combined presence of WF tadpoles in cages and their feces added to the aquaria. Repeated measures of CF mass trajectories showed decreased mass gain in *WF+F_W treatment*s relative to controls (Figure 5-1, treatment × time interaction $F_{7,119}$=2.30, p=0.03 for mass; slope differences were linear until day 40 and thereafter quadratic). By day 40, CF tadpoles exposed to WF and their feces gained significantly less mass and significantly less length than when raised alone ($F_{1,17}$ = 4.17, p = 0.06 and $F_{1,17}$ = 3.73, p = 0.07 respectively, means given in Table 5-3). A one treatment aquarium was removed as a result of all CF tadpoles dying during the first week in one *WF+F_W* treatment even though WF tadpoles seemed unaffected. Further two more aquaria were removed on day 48, one treatment one control, as a result of a

mixup during weighing, resulting in only 17 aquaria being used in comparisons after day 48 compared to 19 aquaria for other comparisons. There was also a trend for greater variation (coefficient of variation) in lengths amongst tadpoles within an aquarium in *WF+F_W treatment*s (Figure 5-2) prior to emergence on day 40 ($F_{1,17}$ = 2.14, p = 0.16) and after peak emergence on day 54 ($F_{1,14}$ = 2.68, p = 0.12). Control tadpoles were significantly heavier than *WF+F_W treatment* tadpoles on day 47 ($F_{1,16}$ = 11.03, p = 0.004), but as individuals emerged, the difference in mean mass and length of CF tadpoles remaining in tanks decreased and differences were not significant in the subsequent periods (Figure 5-1). On day 40, despite differences between control and *WF+F_W treatment*s in tadpole mass and length, both CF tadpole condition (RM ANOVA $F_{1,16}$ = 0.001, p = 0.98) and CF tadpole tail:body length ratios were nearly equal between control and *WF+F_W treatment*s (Table 5-3), suggesting that *WF+F_W treatment* tadpoles were the same shape but smaller than control tadpoles.

Despite the smaller average mass and length in *WF+F_W* aquaria, mean date to forelimb emergence did not differ between treatments ($F_{1,15}$ = 0.41, p = 0.53, Table 5-3). The first tadpole emerged on day 38 and all aquaria had at least one tadpole emerge by day 58. With treatments and tanks pooled, mass of emerging tadpoles decreased linearly with experiment day (r^2 = 0.22, mass at emergence = -0.006×day + 0.676, $F_{1,164}$ = 47.07, p < 0.001). Mean mass for tadpoles emerging through the end of trials in *WF+F_W treatment* aquaria was lower than in controls ($F_{1,14}$ = 3.09, p = 0.10, Figure 5-3, mean day at emergence covariate $F_{1,14}$ = 15.70, p = 0.001). However, those that emerged prior to July 6 (when natural ponds dry = experiment day 54) were significantly heavier in control than in *WF+F_W treatment* aquaria ($F_{1,13}$ = 6.93, p = 0.021, Figure 5-3, mean day at emergence covariate $F_{1,13}$ = 11.81, p = 0.004, one

WF+F$_W$ aquaria was omitted due to no emergence by day 54). Of CF tadpoles emerging by day 54, there was significantly lower variation (CV) in mass at emergence in *WF+F$_W$ treatment* aquaria (0.21±0.03 vs. 0.13±0.02, $F_{1,14} = 5.34$, $p = 0.037$). Of those tadpoles emerging, 73% (131 out of 179) survived to metamorphic climax. Mean survival across treatments was similar (Table 5-3) with 80% surviving to experiment end. If all tadpoles reaching metamorphic climax were used, there was a trend for smaller mass at metamorphic climax for *WF+F$_W$ treatment* tadpoles when date of metamorphic climax was used as a covariate (ANCOVA $F_{1,14} = 2.37$, $p = 0.15$, day at metamorphic climax covariate $F_{1,14} = 8.03$, $p = 0.013$). However, the trend became significant when mass at metamorphic climax was adjusted for date of emergence ($F_{1,14} = 4.78$, $p = 0.046$, covariate - $F_{1,14} = 18.10$, $p = 0.001$) as mass at climax decreased with date. Similarly, a strong trend for lower mean mass at metamorphic climax was found for those tadpoles that emerged by July 6 in *WF+F$_W$ treatment* aquaria ($F_{1,13} = 4.13$, $p = 0.06$, Figure 5-4, mean emergence date as covariate $F_{1,13} = 6.51$, $p = 0.024$).

Experiment #2 – Influence of WF tadpoles only on CF ("WF")

On day 40 one *WF* treatment and one control replicate were removed, thus comparisons after day 40 were done using only five replicates of each treatment.

CF responses were inconsistent with respect to treatment and measurement period. By day 40, CF tadpoles in treatment aquaria were significantly longer (6.12±0.20 mm vs. 7.09±0.11 mm, $F_{1,8} = 15.02$, $p = 0.005$ change in mean body length) and had gained more mass (0.34±0.02 g vs. 0.40±0.01 g, $F_{1,8} = 4.97$, $p = 0.056$) than control tadpoles (Figure 5-5). However, just prior to emergence, day 54, differences in body length or mass were no longer evident and by day 61 CF

tadpoles in *WF* treatment aquaria tended to weigh less than those in control aquaria ($F_{1,8}$ = 2.57, p = 0.15, Figure 5-5). Growth rates (change in mass) in *WF* treatment aquaria were significantly lower than in control aquaria from day 40 to day 54 (0.10±0.01 vs. 0.03±0.02 g/day between day 40-54, $F_{1,8}$ = 8.14, p = 0.021, Figure 5-6B). Variation (CV) in tadpole length in *WF* treatments was significantly lower than in controls on day 40 (0.11 ±0.01 vs. 0.07±0.01; $F_{1,8}$ = 5.33, p = 0.05), but variation in tadpole length was similar among treatments as tadpoles approached metamorphosis ($F_{1,8}$ = 0.029, p = 0.87). Unlike experiment #1, condition in experiment #2 was significantly lower throughout in *WF* treatment aquaria (RM ANOVA $F_{1,8}$ = 9.02, p = 0.017, Figure 5-7) as tadpoles tended to be longer but weighed less.

Cooler temperatures led to fewer tadpoles emerging from aquaria by experiment end (28 %; 25 tadpoles emerged from 10 aquaria) in comparison to experiment #1 where 71% of tadpoles emerged (179 tadpoles from 17 aquaria). The first two tadpoles emerged from one control aquarium on day 58 and 61. Tadpoles in other aquaria started emerging by day 66 although one control aquarium had no tadpoles emerge by experiment end, day 73. I found a significant tendency for lower mean mass at emergence in *WF* treatment aquaria ($F_{1,7}$ = 4.36, p = 0.075, Table 5-3, for 18 total tadpoles emerging from 4 control and 5 treatment aquaria respectively).

Survival to experiment end was similar, but slightly lower in *WF* treatment aquaria (Table 5-3). I began aerating aquaria on day 26 as between day 22 and 33, 36% of all deaths occurred (8 out of 22 total), and may have been the result of low O_2 or high ammonia concentrations. Excluding these deaths resulted in no difference in % survival between treatments, 89±3.1% vs. 84±4.0% ($F_{1,8}$ = 0.23, p = 0.64).

Experiment #3 – Influence of wood frog feces only on CF ("F_W")

Growth and survival of CF tadpoles was reduced in the presence of WF feces. Growth rates of CF tadpoles were significantly lower in F_W treatment tubs (Figure 5-8, RM ANOVA on CF mass – $F_{1,22} = 8.15$, $p = 0.008$, treatment × day interaction – $F_{6,132} = 3.32$, $p = 0.004$). Slower growth rates in F_W treatment tubs (Figure 5-6C) resulted in significantly less mass gained ($F_{1,22} = 5.68$, $p = 0.03$, Figure 5-8B) and significantly less length gained ($F_{1,22} = 4.28$, $p = 0.05$, Figure 5-8A) by day 36. The smallest tadpoles in F_W treatment tubs did not gain mass from day 30 through day 43. The smallest tadpoles in each F_W treatment tub were not significantly different in mass than those in control tubs on day 30 (0.106±0.008 vs. 0.098±0.009 grams) but weighed significantly less than the smallest control tadpoles by day 43 (0.141±0.015 vs. 0.098±0.010 grams, $F_{1,22} = 5.11$, $p = 0.034$). There was no significant differences in the variation (CV) among the CF tadpoles within each tub when considering tadpole masses ($F_{1,22} = 1.65$, $p = 0.21$), or length measurements ($F_{1,22} = 0.58$, $p = 0.46$). Similarly, tadpoles were of similar condition and shape as no significant differences existed between control and treatment (condition: $F_{1,22} = 0.43$, $p = 0.52$ and tail:body ratio: $F_{1,22} = 0.14$, $p = 0.74$)

Decreased growth rates (mass - Figure 5-6C and length - Figure 5-8) in F_W treatment tubs before emergence did not translate into reduced developmental rates as the average date of emergence was similar between control and treatment aquaria (Table 5-3). The first tadpoles emerged on day 36 (in four separate tubs, of which three were from the F_W treatment) and in all but two of 24 tubs by day 46 (two F_W treatment tubs did not have tadpoles emerge until days 53 and 55). Mean mass of tadpoles emerging through the end of trials was slightly higher in F_W

treatment tubs, though differences were not significant ($F_{1,22}$ = 0.87, p = 0.36, Table 5-3). However, as in experiment #1, of those CF tadpoles that emerged before July 6 (when natural ponds dry, and 1 day past the median date of emergence in trial), those from F_W treatment tubs tended to weigh less than from control tubs ($F_{1,22}$ = 1.36, p = 0.26, Figure 5-4, two tubs in F_W treatment had none emerge and were assigned 0 g). Unlike experiment #1, where emergence mass decreased significantly over time (Table 5-2), there was only a slight negative relation in experiment #3 (pooling all 65 tadpoles r = − 0.15, p = 0.22) and considering date of emergence as a covariate did not significantly reduce variation within treatments unlike experiment #1. Mean total mass of emerging tadpoles from F_W treatment tubs at the end of trials was lower, although not significantly different than control tubs (0.648±0.056 vs. 0.578±0.060 grams, $F_{1,22}$ = 1.56, p = 0.22).

Control tubs had a slightly higher rate of emergence (2.9±0.3 vs. 2.5±0.3 out of 5 stocked tadpoles), and significantly better survival rates (tadpoles that emerged + tadpoles remaining) than the F_W treatment tubs (93±3.8 % versus 80±4.9%, $F_{1,22}$ = 4.91, p = 0.037).

Discussion

In the absence of either exploitative competition or direct behavioural interference, I found that the presence of WF tadpoles and/or their feces reduced CF growth rates, survival, and size at emergence. My data support the hypothesis that chemical interference exists under laboratory conditions. *S*haring water with WF tadpoles and exposure to their feces tended to cause a reduction in weight and length of CF tadpoles, and ultimately led to animals that were significantly smaller at emergence and smaller at metamorphosis. Experiment #3 further supports the

hypothesis that inhibitory substances are produced by WF tadpoles and that they are found in the feces. Chorus frog tadpoles exposed to WF feces alone were significantly smaller in length and weight prior to metamorphosis and suffered significantly higher mortality. In the absence of WF feces (experiment #2), CF tadpoles that were in visual contact and exposed to water inhabited by WF tadpoles displayed no differences in length or weight prior to emergence, but did have significantly reduced growth rates prior to emergence and tended to be lighter at emergence. In the absence of WF feces, a reduction in CF mass at emergence implies that WF tadpoles excrete interference chemicals (one or more) directly into the water. This study is novel as it demonstrates that chemical inhibitors produced by WF tadpole both in their feces and released into the water through skin, gills or urine reduce CF tadpole growth and ultimately lead to significantly smaller mass at emergence.

Results from these experiments were representative of larval performance in more natural venues (mesocosms and field). In comparison to my results on CF performance in natural ponds, larval periods in the lab trials were within the range of those observed in natural ponds (51-93 days, mean of 68 from 30 pond-years; versus 55 days in experiment #1 and #3, 78 days in experiment #2). Growth rates in lab trials were similar to those in mesocosms (0.20 mm/day versus 0.21-0.28 mm/day) and within the range observed in the field (0.14-0.41 mm/day, mean of 0.28 mm/day from 32 pond-years). Size at metamorphosis in experiments #1 and #2 were similar to those observed in the field (mean 13.33 mm, 10.4-17.2 mm n=265, versus 12.68 mm, 10.2-14.9 mm n=16). Performance of tadpoles in these experiments is representative of those in natural ponds, and therefore observed interference mechanisms may operate in natural ponds.

Surveys in natural ponds showed that CF tadpole body length decreased with increasing WF tadpole density (Chapter 2), even though there was little evidence for exploitative competition. My results combined with surveys from ponds suggest that if competition caused the differences in CF growth, chemical interference plays a role. The results of chemical interference in my trials are much weaker than those reported in other lab trials (Richards 1958, Licht 1967, Beebee 1991, Bardsley and Beebee 2001), and suggests that either *Anurofeca richardsi*, the agent involved in those studies, was rare in my trials, or another agent is responsible for the inhibition of chorus frog larval growth. *A. richardsi* (formerly *Prototheca richardsi* sensu Baker et al. 1999) is a unicellular parasitic organism that proliferates in the guts of anuran larvae where it can reduce feeding (appetite)-thereby prolonging food passage time, resulting in decreased growth and survival of affected tadpoles (Beebee 1991, Beebee and Wong 1992, Baker et al. 1999, Bardsley and Beebee 2001). *Anurofeca richardsi* has been found in the guts of wild wood frog tadpoles in North Carolina (Biesterfeldt et al. 1993) but the cells have never been reported in the guts of *P. maculata* and, as *A. richardsi* cells do not survive freezing or drying, it seems unlikely that they would be present at the temporary northern ponds from which eggs were collected for my trials. Whatever the agent(s), my trials suggest that chemical interference results in lower recruitment and lower fitness of surviving individuals (based on positive relationship between size at metamorphosis and fitness - Smith 1987, Berven 1990, Altwegg and Reyer 2003, Chelgren et al. 2006) which ultimately may lead to low population persistence for a short-lived species like boreal chorus frog.

Interference competition in anuran larvae

If interference competition has not been documented, then exploitative competition is the alternative mechanism to explain differences in growth rates or other performance metrics (i.e. size at metamorphosis, survivorship) that are caused by the presence of a competitor. Exploitative competition is often inferred in studies on anuran larvae despite a poor understanding of tadpole diets (Altig et al. 2007) due to an assumed overlap of diets combined with the perception that foods are in short supply (e.g. DeBenedictis 1974, Wilbur 1997). Competition for perceived limited resources, the pressure to develop before pond drying (as described by Wilbur and Collins 1973), and predation (e.g. Skelly 1996) within temporary breeding ponds are suspected as the main factors affecting recruitment from the larval environment. Unlike the threat of pond drying and predation, it is hard to imagine how one would test for exploitative competition without inadvertently including interference chemicals. Trials in mesocosms, aimed at determining if exploitative competition occurs, do not prevent the production of inhibitory agents; rather conditions stimulate their production by increasing tadpole densities and favouring food limitation (see Chapter 3).

Chemical interference effects on tadpole performance

Differences in light/dark cycles, temperatures, and stocking densities across the three experiments made direct comparisons of the actual results of the three experiments problematic. However, consistent reduction of CF growth across the three experiments and the tendency for CF tadpoles to emerge smaller when exposed to WF tadpoles suggest that chemical interference by WF may occur and I hypothesize this interference may explain the patterns of reduced CF performance

observed in natural ponds. I first discuss the results with respect to each measured response, and then I will discuss the mechanism and nature of the interference agent(s).

Reduction in growth rates

Only when WF feces were present (experiments # 1 and # 3), irrespective of WF tadpole presence, did CF tadpoles in WF treatments have consistently lower growth rates (Figure 5-6A,C) and smaller sizes before emergence. When feces were absent (experiment # 2), there was no difference between treatments in tadpole size before emergence (Figure 5-5). However, growth rates in WF treatements in experiment #2 were significantly lower in the 14 days prior to emergence in the absence of WF feces (Figure 5-6B, day 33-54) as they were in experiment #1 (Figure 5-6A, day 33-47). There is little likelihood that intact feces could have been transferred into the main aquarium holding CF in experiment #2, but perhaps leaving the feces in *WF cages* for two days prior to regular cleaning allowed chemicals to diffuse into the CF area. An alternative explanation is that WF tadpoles release inhibitors contained within their feces and directly into the water.

Greater variation in tadpole size

Inhibitory agents affect individuals differently depending on their susceptibility and contribute to varying growth of individuals. Intraspecific interactions can produce variation in tadpole growth (Wilbur and Collins 1973) and as larger tadpoles consume more food and realize greater growth than smaller tadpoles (Wassersug 1975) this can magnify observed difference. Chemical interference, like intraspecific interactions, increases variation among tadpoles as smaller tadpoles are more susceptible to interference (Richards 1958, Beebee and

Wong 1992, Wong et al. 2000). Interference is expected to produce a distribution of tadpole sizes with lower mean size, but rather than displaying proportionate deviation, variation tends to increase (Steinwascher 1978b). Tadpoles in my trials displayed variation in body length and mass within aquaria as expected from intraspecific interactions. However, CF tadpoles in WF treatments in experiments #1 (Figure 5-2) and #3 (Figure 5-8) had greater variation within aquaria and lower mean sizes than control replicates. This is consistent with the effects of chemical interference. My data also support the finding that smaller tadpoles are more susceptible to the effects of interference (Richards 1958, Beebee and Wong 1992, Wong et al. 2000). Shvarts and Pyastolova (1970b) suggested that some products of metabolism may inhibit growth of earlier cell lines and may explain why the smallest tadpoles in F_W treatments despite abundant food did not grow, or gain weight. The finding of greater variation in tadpole sizes and lower mean tadpole sizes in tanks exposed to WF in experiments #1 and #3, but not experiment #2, suggest chemical interference linked to WF feces.

Reduced survival

Results regarding survival and the influence of interference were inconclusive from the three trials. Significantly reduced survival was only observed in experiment #3 in the F_W treatment, but there were only trends of reduced survival in WF treatments from experiments #1 and #2 (Table 5-3). A number of tadpole mortalities were observed in experiments #1 and #2 during the period leading up to, and just after I began aeration, day 22 to 33, 18 tadpoles died in experiment #1 (11 control and 7 treatment - 37% of all deaths from day 7 to experiment end), while 8 tadpoles died in experiment #2 (3 control, 5 treatment – 36% of deaths). Survival clearly was related to reduced oxygen levels, but was

unrelated to treatment. Overall it is unclear from my results if interference chemicals produced by WF tadpoles affect survival of CF tadpoles.

Reduced size at emergence (metamorphosis)

My results support the hypothesis that interference by WF tadpoles results in significantly lower body mass for CF at emergence (Figure 5-4). When WF tadpoles were present in aquaria (experiments #1 and #2), CF emerged with lower mean mass. On the other hand, when only WF feces were present (experiment #3), I found no differences in size at emergence (Figure 5-8). The absence of differences in growth rate between WF and control treatments (Figure 5-6) early in experiments, when development rates are set (Wilbur and Collins 1973, Travis 1984), in experiment #3 may explain why I observed no difference at emergence. However, changes in growth rate later in development (experiments #1 and #2, Figure 5-6A,B) affected size at emergence and support the Wilbur and Collins model with respect to the timing and size at metamorphosis (Wilbur and Collins 1973, Hensley 1993). Chemical interference by WF tadpoles through waterborne secretion/excretion via skin or gills is therefore implicated in differences in CF mass at emergence.

Another reason for the similarity of weights at emergence between treatments in experiment #3 (F_W) could be higher temperatures and higher tadpole densities of CF in tubs. Conditions in experiment #3 led to smaller tadpole size throughout the trial and much smaller mass at emergence compared to experiments #1 and #2 (Table 5-4, Figure 5-4). The preferred body temperature of *Pseudacris triseriata* tadpoles (an allied species to CF) exposed to thermal gradients was 23°C but thermal preferences were weak as tadpoles chose temperature ranging from 12-

32°C (Dupre and Petranka 1985). Optimal temperature for growth of *P. ornata* tadpoles was between 20-25°C and increased up to 25°C, after which growth decreased (Harkey and Semlitsch 1988). The 20°C in experiment #3 is warmer than the 17.4°C and 13.5°C in experiments #1 and #2 respectively, but is within the optimal range for *P. ornata*. Intraspecific interactions, such as increased contact (Rot-Nikcevic et al. 2005) and competition in the small tubs, could be a reason for reduced growth and lower weights at metamorphosis and could have masked the effects of interference chemicals (as in Faragher and Jaeger 1998).

The mechanism of chemical interference

The existence of inhibitors in feces has been suggested in a number of studies on anuran larvae (Richards 1958, Licht 1967, Beebee 1991) and also in other taxa (e.g. snails Thomas et al. 1975, bony fish Tomasso 1994). If inhibitors are bound to tadpole feces, then results would suggest CF tadpoles consume feces as in Steinwascher (1978a). However, if inhibitors present in feces diffuse into the water and then are absorbed through a tadpole's skin or gills, this process might explain growth reduction in all three experiments. Steinwascher (1978b) showed inhibitors appear bound to feces of *Lithobates catesbeianus* and do not diffuse into the water as growth in treatment larvae was similar to controls when feces were present but inaccessible, whereas tadpole growth was reduced when feces were accessible. Therefore consumption of feces was required to inhibit tadpole growth.

I propose three patterns of feces consumption, and thereby inhibitor consumption, in my trials: 1) accidental consumption during ingestion of other foods, 2) selective consumption of feces which are easier to collect or digest than other foods, or 3) selective consumption of feces due to an attractant present in the

feces that causes tadpoles to switch to coprophagy. Accidental consumption of feces may result if tadpoles are indiscriminate feeders as food settled and mixed with feces of both WF and CF tadpoles in my trials (experiments #1 and #3). If feces particles are as easily suspended as food (Wassersug 1975), then feces may be consumed along with food as in Steinwascher (1978a, *L. catesbeianus*) and Gromko et al. (1973, *L. pipiens*). However, Kupferberg (1996) suggests that *Lithobates boylii* (family Ranidae) and *Hyla regilla* (family Hylidae) tadpoles are capable of actively selecting foods that optimize growth. If CF tadpoles (family Hylidae) in my trials were equally capable of selecting "optimal" foods, feces should not be consumed unless ground rabbit pellets are of lower nutritional value or of lower digestibility than feces. Steinwascher (1978a) showed that bacteria growing on *L. catesbeianus* feces did increase their energy value, but they were still of lower quality than rabbit pellets. To my knowledge there has been no evaluation of the digestibility of feces from tadpoles fed rabbit pellets compared to undigested rabbit pellets. With regards to selective consumption, *L. pipiens* tadpoles switched to consuming feces when energy required to graze on food was higher than energy gained by consuming inherently low energy feces (Steinwascher 1978a). As tadpoles are unlikely to be indiscriminate feeders, perhaps feces are easier to consume. The pellets I provided were finely ground and smaller than feces of either species, unlike the foods in the Gromko et al. and Steinwascher, and I suggest would be easier to ingest than feces. Therefore an attractant may be present within feces (Beebee and Wong 1992), and if WF feces are present, CF tadpoles may switch to coprophagy. In conclusion, whatever the reasons for their consumption, WF feces appear to possess an inhibitor that reduces tadpole growth rates, but if that inhibitor is bound to the

feces, then reduction of growth in their absence suggests that the inhibitor is also released directly into the water by WF tadpoles.

Chemical inhibition in the absence of feces has been suggested by a number of authors in systems where feces were removed (in *Lithobates sphenoephus utricularia* and *Hyla cinerea* Faragher and Jaeger 1998), inaccessible (Gromko et al. 1973, Steinwascher 1978b) or when water alone, conditioned by tadpoles raised at high densities (e.g. Rose and Rose 1961, Akin 1966, both using *L. pipiens*) was used as a treatment. Experiment #2, and to some extent experiment #1, support the production of chemical inhibitors released into the water and not exclusively found in tadpole feces. Chorus frog tadpoles in experiments #1 ($WF+F_W$) and #2 (WF) were exposed to interference associated with WF tadpoles with and without the presence of WF feces and growth reduced in the few weeks prior to emergence (Figure 5-6A,B) and ultimately led to smaller masses at emergence. Greater CF sizes in treatment (WF) aquaria early in the trial (Figure 5-5) might have resulted from greater tadpole densities (CF+WF) in those aquaria (West 1960), but production of inhibitors by WF released directly into the water midway through the trial significantly reduced growth of CF tadpoles. Evidence of a waterborne (not feces bound) interference chemical is suggested by greater growth in control treatments in the 14 days prior to emergence in experiments #1 ($WF+F_W$) and #2 (WF) but absent in experiment #3 (F_W). Waterborne interference is further supported as treatment differences in CF mass at emergence occurred only in experiments #1 and #2 where WF tadpoles were present (Figure 5-4). The timing of significant growth rate reduction in WF treatments in experiments #1 and #2 coincide with WF food reductions on day 33 (20% to 10%) and again on day 54 (10% to 5%). Reductions in food combined with greater WF tadpole sizes may have triggered, or increased,

inhibitor production. Alternatively, inhibitor concentrations may have reached sufficient concentrations to inhibit growth as production of inhibitors increases with tadpole size (Rose and Rose 1961, Beebee and Wong 1992) and was coincident with food reductions. D'Angleo et al. (1941) showed that starvation of WF late in development accelerated metamorphosis, and others (e.g. Wright et al. 1999 in *L. catesbeianus*, Glennemeier and Denver 2002 in *L. pipiens*) have shown that starvation also elicits stress hormone release (such as corticosteroids) which leads to production of thyroid hormones and the acceleration of development at the cost of growth (as reviewed by Denver 2009). My results support the growth retarding effects of corticosteroids (stress hormones) or thyroid hormones as they would reduce growth rates and accelerate metamorphosis which would result in reduced size at emergence in CF tadpoles.

Nature of the interference chemical

I have shown that chemicals produced by WF tadpoles in their feces and released directly into the water could be responsible for growth inhibition of CF tadpoles and reduced their mass at emergence. Chemical inhibition and communication is not unique to anurans as other aquatic vertebrates such as fish use chemical communication extensively (reviewed by Solomon 1977, Burnard et al. 2008). Intraspecific inhibitory substances in zebrafish, *Brachydanio rerio,* can be removed by activated charcoal (Roales 1981) or addition of methylchloroform (Yu and Perlmutter 1970). Similarly, chemical interference was believed to reduce growth in sea lamprey, *Petromyzon marinus* (Rodriguez-Munoz et al. 2003) as lampreys use chemical communication, and are therefore likely to respond to a chemical inhibitor. Anuran larvae (and their capacity for kin recognition reviewed

by Blaustein and Waldman 1992) and adults are capable of chemical communication (as demonstrated in *Leiopelma hamiltoni*, an archaic species evolutionarily basal to most living anurans, Waldman and Bishop 2004, and chemical communication is reviewed by Belanger and Corkum 2009) and it seems likely that species specific or heterospecific inhibitors are responsible for inhibition documented in this and other studies (i.e. Licht 1967). Within anuran larvae, a number of inhibitors have been suggested, but no inhibitor has been indisputably accepted. In Chapter 6 I discuss inhibitory agents suspected of affecting tadpole growth that are transported in tadpole feces and those that are transmitted in water.

Implications for natural populations

The literature on amphibian competition suggests that chemical interference operates, although it may be difficult to differentiate from resource based, exploitative competition. In the absence of exploitative competition as suspected in natural populations (Chapter 2), interference is left to explain the negative relation between CF tadpole growth rates and density of co-occurring WF tadpoles. Interspecific behavioural interference and food competition was prevented in laboratory trials and inhibition of CF tadpole growth was still recorded. Inhibition occurred in the presence of WF feces, but also occurred when feces were absent. Reduction in growth prior to metamorphosis may initiate endocrine cascades leading to metamorphosis (as reviewed by Denver 1997) and has the potential to produce smaller size at emergence (Wilbur and Collins 1973, Hensley 1993). Reduced size at emergence has the potential to reduce CF lifetime fitness (for chorus frogs Smith 1987, and for amphibians as reviewed by Wilbur 1997). If interference seen within my trials operates in natural ponds, those tadpoles exposed to higher

densities of WF are likely to have lower survival to first reproduction, breed a year later, and produce fewer offspring (Smith 1987).

Fecal cell-mediated interference, seen in previous laboratory studies, is stronger than inhibition observed in my experiments and I argue not evident in these trials as discussed above. My results support the presence of both waterborne and fecal mediated inhibitors. It is possible that there are multiple inhibitory agents or one agent that acts at different stages released by WF tadpoles, that act on CF tadpole growth early in development and others that reduces growth and accelerate development later. I argue that these agents are likely products of tadpole metabolism and those agents acting before emergence are either corticosteroids or thyroid hormones given the combined reduction in growth and the rapid development of affected tadpoles. Future efforts need to combine our ecological understanding of patterns associated with growth inhibition between species with the knowledge of endocrinologists to help determine what chemicals are present in water conditioned by crowded tadpoles and which of those chemicals are capable of affecting growth, survival and size at emergence at concentrations present at natural tadpole densities.

Table 5-1: Design for laboratory experiments testing for interference chemicals produced by wood frog tadpoles.

	Experiment 1		Experiment 2		Experiment 3	
Date (duration)	14 May - 25 July 2007 (73 days)		14 May - 25 July 2007 (73 days)		22 May – 17 July 2008 (58 days)	
Tank dimensions (L × W × D) cm	50 × 25 × 22.4		40 × 20 × 21.25		27 × 12 × 14.5	
Volume	28 L		17 L		4.7 L	
Treatment Name	*WF+F$_W$*	*CONTROL*	*WF*	*CONTROL*	*F$_W$*	*CONTROL*
# CF stocked	15	15	9	9	5	5
# WF in cage	4	0	4	0	n/a	n/a
# of replicates	10	10	6	6	12	12
Feeding rate	20 % of average mass*		20 % of average mass*		15 % of average mass	
Response variables	Growth Time to emergence Mass at emergence Mass at metamorphosis Survival Condition		Growth - Mass at emergence - Survival Condition		Growth Time to emergence Mass at emergence - Survival Condition	

* CF tadpoles were initially fed 20% of average mass, but decreased to 10% after day 29

Table 5-2: Regression analysis of mass at metamorphic climax on mass at emergence. All tadpoles regardless of treatment or aquaria of origin were combined in this analysis. $R = 0.83$, adjusted $R^2 = 0.68$, $n = 100$.

Effect	Coefficient	SE	*t*	*p*
Constant	0.047	0.013	3.64	< 0.001
Mass at emergence	0.510	0.035	14.52	< 0.001

Source	df	MSE	*F*	*p*
Regression	1	0.191	210.87	< 0.001
Residual	98	0.001		

Table 5-3: Summary of CF responses to WF (tadpoles) or feces (F_W) across the three experiments. Individual tadpole means from each replicate are used to calculate treatment means. Treatment means ± 1 SE are given for each response with the appropriate significance test below.

	Experiment #1 WF + F_W			Experiment #2 WF			Experiment #3 F_W		
	Control		Treatment	Control		Treatment	Control		Treatment
Before emergence									
Δ in mass (g)[a]	0.49 ± 0.01	†	0.43 ± 0.03	0.46 ± 0.03		0.45 ± 0.02	0.22 ± 0.01	*	0.20 ± 0.01
Δ body length (mm)[a]	8.00 ± 0.10	†	7.46 ± 0.27	11.14 ± 0.19		10.93 ± 0.20	7.51 ± 0.14	*	7.04 ± 0.17
Tail : body ratio	2.14 ± 0.03		2.14 ± 0.04	2.01 ± 0.09		2.15 ± 0.04	2.04 ± 0.03		2.03 ± 0.02
First emergence	42		38	58		66	36		36
Days to emergence	54.8±0.7		53.5 ± 1.9	Low emergence, not tested			45.9 ± 0.8		46.3 ± 1.4
At emergence									
Mass at emergence (g)									
By end	0.36 ± 0.01	†[b]	0.32 ± 0.01	0.43 ± 0.05	†	0.31 ± 0.03	0.22 ± 0.01		0.24 ± 0.01
By day 54	0.39 ± 0.01	*[b]	0.34 ± 0.01						
Mass at metamorphosis	0.24 ± 0.01	*[b]	0.21 ± 0.01	Not measured			Not measured		
Percent survival [c]	83 ± 4.1		77 ± 5.7	87 ± 3.7		73 ± 6.7	93 ± 3.8	*	80 ± 4.9

[a] change from initial mass to before emergence: day 40, 54 and 36 for experiments #1, #2 and #3 respectively
[b] means are adjusted for the effect of date at emergence covariate
[c] survival was measured until the end of the experiment; includes tadpoles remaining at experiment end

** $p \leq 0.01$, * $p \leq 0.05$, † $p \leq 0.10$

Table 5-4: Range of values of mass at emergence from all three experiments for all aquaria/tubs combined. Note precision of measurement was ±0.01 g in experiment #1 and #2, versus ±0.001 g in #3.

		Experiment		
		#1	#2	#3
Mass at emergence (g)	Mean	0.34	0.36	0.226
	Max	0.60	0.77	0.370
	Min	0.10	0.19	0.080
percentile	75th	0.41	0.40	0.261
	25th	0.28	0.31	0.190
Total # emerged	N	166	25	65
# less than 0.20 g		6	1	20

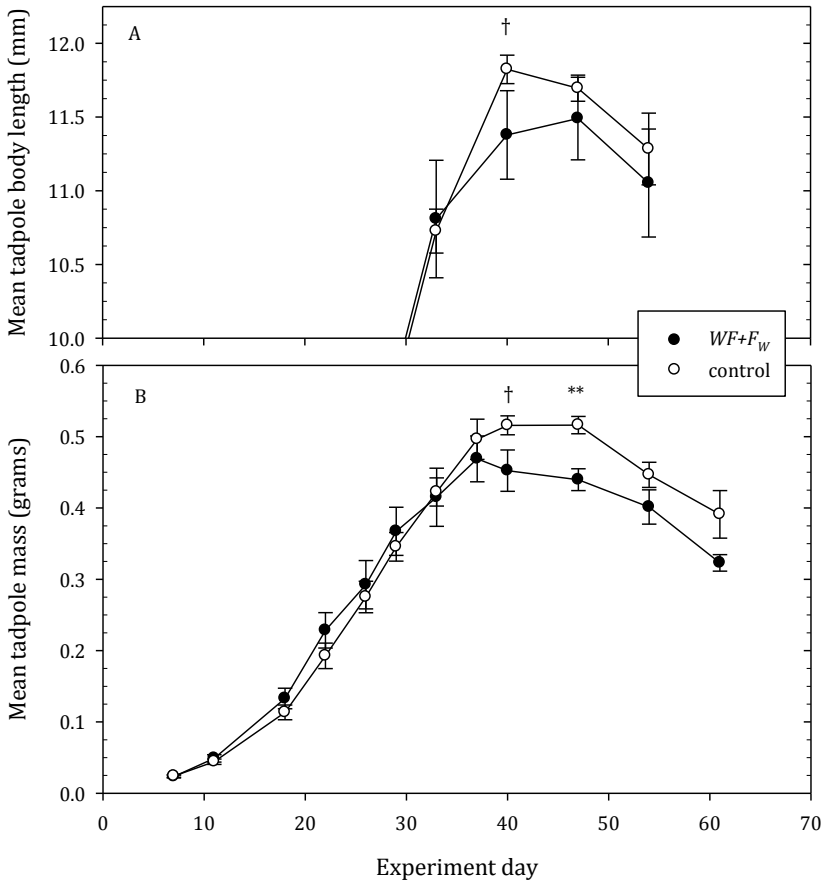


Figure 5-1: Effect of presence of WF tadpoles and WF feces on A) body length and B) mass of CF tadpoles in experiment #1. The individual tadpole treatment means ± 1 SE. The number of replicates include in the first 48 days were: control $n = 10$, $WF+F_W$ $n = 9$; and were reduced by one replicate each after that date. By day 40 the mean number of tadpoles in aquaria was 11.3±0.7 (range 7 to 15) for control aquaria and 11.1±0.9 (6 to 15) tadpoles in $WF+F_W$ aquaria.

** $p \leq 0.01$, * $p \leq 0.05$, † $p \leq 0.10$.

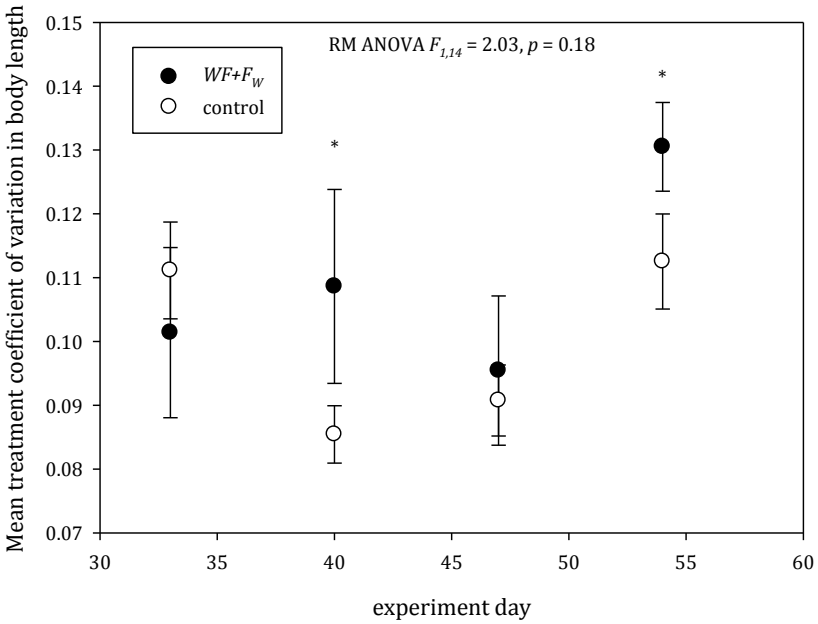


Figure 5-2: Mean coefficient of variation in body length ± 1 SE of chorus frog tadpoles remaining in aquaria in experiment #1. Tadpole stocking was 15 chorus frog tadpoles into each aquaria in both the control and wood frog and feces treatment aquaria (n = 10 for each treatment). Note, emergence began in both treatments after day 40. Significant differences are noted for comparisons between treatments on a given date.

** $p \leq 0.01$, * $p \leq 0.05$, † $p \leq 0.10$.

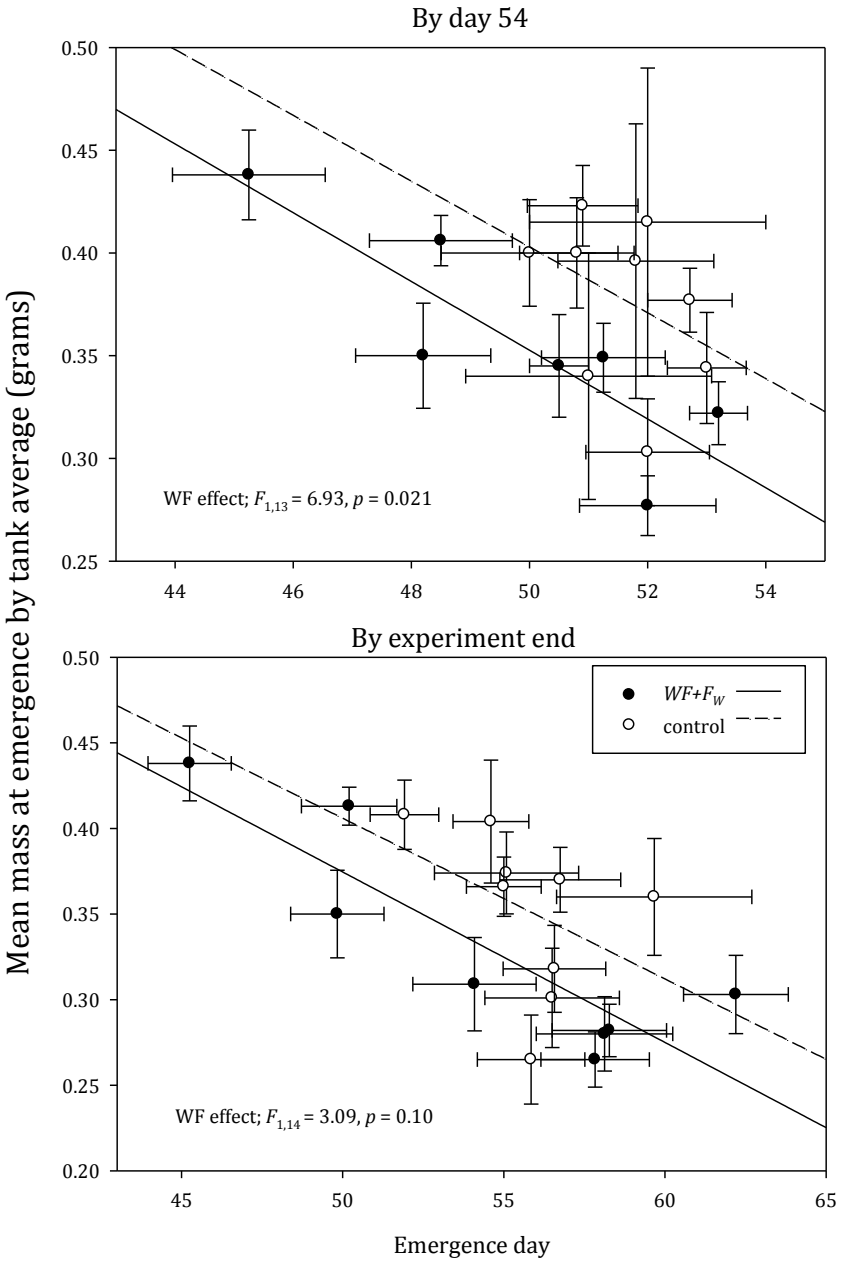


Figure 5-3: Relationship between chorus frog mean mass at emergence and experiment day for experiment #1. Shown are the individual tank means ± SE for both mass and emergence date and the regression lines of mass on emergence day. The upper panel shows the effect of WF and their feces on chorus frog mass at emergence for those tadpoles emerging before July 6 (day 54) from nine control with 2-11 individuals per aquaria and seven *WF+F*$_w$ aquaria with 4-12 individuals per aquaria. The lower panel displays the effect of WF tadpoles and their feces on all chorus frog tadpoles that emerged by trial end, day 73 from nine control aquaria with 6-14 individuals per aquaria and eight *WF+F*$_w$ aquaria with 5-12 individuals per aquaria.

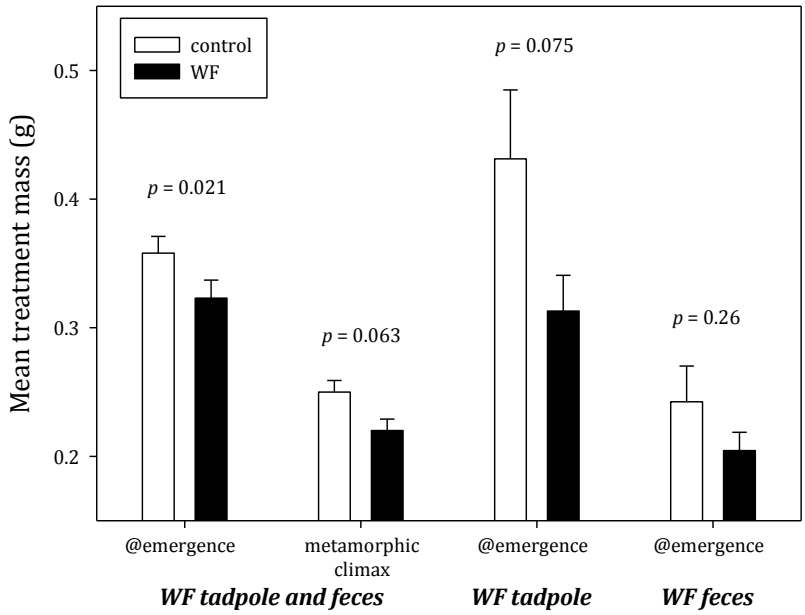


Figure 5-4: Comparison of the effect of WF tadpoles and/or their feces on chorus frog mass at emergence from the three experiments. Emergence for experiment #1 (*WF+F*$_W$) and #3 (*F*$_W$) are tadpoles that emerged by July 6. Means in experiment #2 (*WF*) are those that emerged by experiment end, as none emerged prior to July 6. Means for experiment #1 are adjusted for the day of emergence covariate. Emergence is the eruption of forelimbs, whereas metamorphic climax is the complete resorption of the tail. Sample sizes are: experiment #1, nine control (2-11 tadpoles/aquaria) and seven treatment (4-12 tadpoles/aquaria); experiment #2, four control (1-4 tadpoles/aquaria) and five treatment (1-4 tadpoles/aquaria); experiment #3, 12 control (1-3 tadpoles/tub) and 12 treatment (0-3 tadpoles/tub).

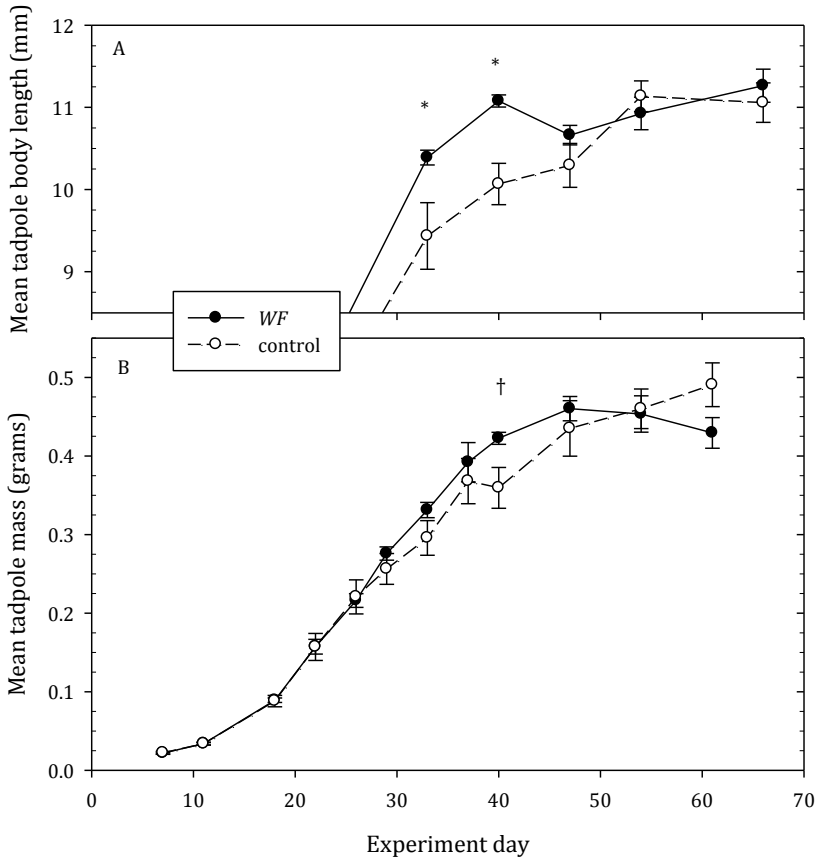


Figure 5-5: Effect of presence of four wood frog (WF) tadpoles suspended within cages on chorus frog tadpole A) body length and B) mass in experiment #2 where WF feces were discarded. Treatment mean body length and mass are shown ± 1 SE. There were initially six replicates in *WF* (filled circles, solid line) and control (open circles, dashed line) treatments for the first 32 days and five after that date. Initial stocking density of CF tadpoles was nine tadpoles in 17 L of water. By day 54 the mean number of tadpoles in aquaria was 8.0±0.4 (range 7 to 9) for *control* and 7.4±0.6 (6 to 9) tadpoles in *WF* treatment aquaria.

** $p \leq 0.01$, * $p \leq 0.05$, † $p \leq 0.10$.

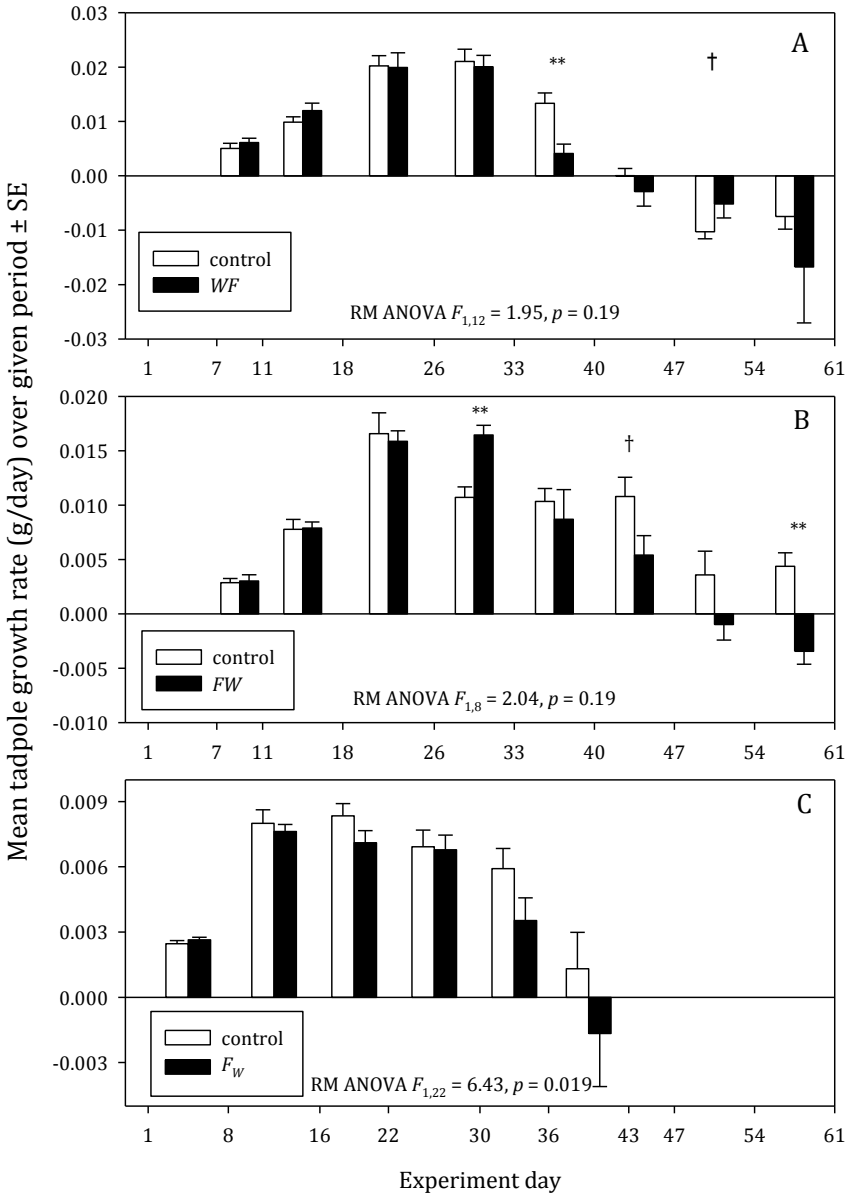


Figure 5-6: Mean tadpole growth rates over experimental period for each trial: A) experiment #1, where both WF and feces were present (*WF+Fw*); B) experiment #2, where only WF tadpoles were suspended in cages (*WF*); and C) experiment #3, where only WF feces were used in treatments (*Fw*). Growth rate is measured in change in mass from one measurement period to the next. Sample sizes are: experiment #1, ten control and nine treatment (6-15 tadpoles/aquaria) through to day 40 then decreased due to emergence of tadpoles; experiment #2, five control and five treatment (6-9 tadpoles/aquaria); experiment #3, 12 control and 12 treatment (4-5 tadpoles/tub).

** $p \leq 0.01$, * $p \leq 0.05$, † $p \leq 0.10$.

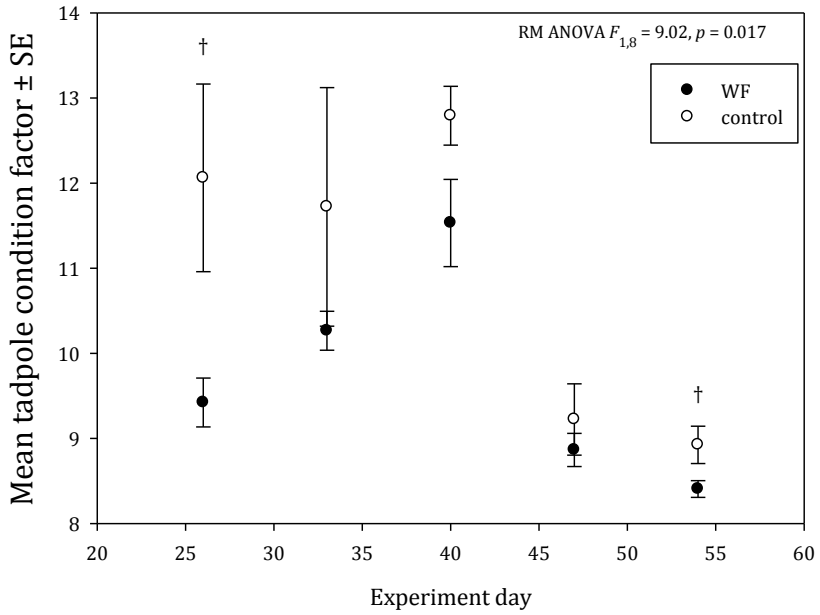


Figure 5-7: Patterns in condition of tadpoles in experiment #2 through time. Condition is calculated as average tadpole weight divided by mean tadpole total length cubed multiplied by 10^4. Treatment means ± 1 SE are shown from five control and five treatment (WF) aquaria with 6-9 tadpoles/aquaria.

** $p \leq 0.01$, * $p \leq 0.05$, † $p \leq 0.10$.

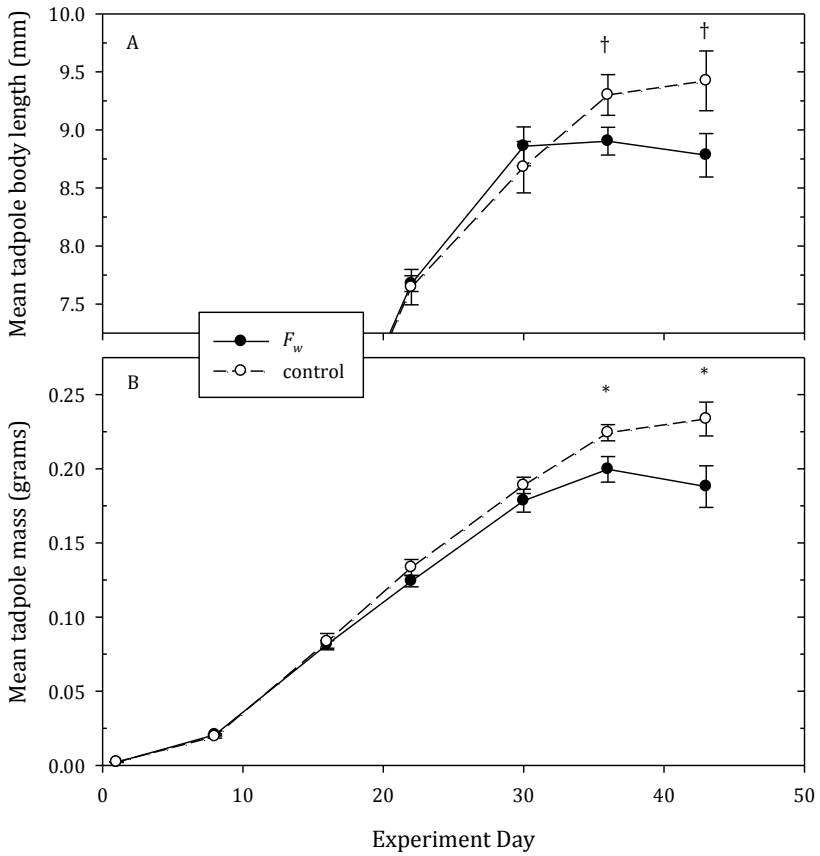


Figure 5-8: Effect of feces produced by crowded WF tadpoles on CF A) body length and B) mass in experiment #3. Treatment means for individual tadpole body length and mass are shown ± 1 SE. There are 12 replicates of F_W (closed circle, solid line) and control (open circle, dashed line) for all dates. Initial stocking density of CF tadpoles was five tadpoles in 4.5 L of water. By day 36 the mean number of tadpoles in aquaria was 4.8±0.1 (range 4 to 5) for both treatments.

** $p \leq 0.01$, * $p \leq 0.05$, † $p \leq 0.10$.

Literature Cited

Akin G. W. 1966. Self-inhibition of growth in *Rana pipiens* tadpoles. Physiological Zoology **39**:341-356.

Alford R. A. 1999. Ecology: resource use, competition, and predation. Pages 240-278 *In* R. W. McDiarmid, editor. Tadpoles: biology of anuran larvae, University of Chicago Press, Chicago.

Alford R. A. 1989a. Effects of parentage and competitor phenology on the growth of larval *Hyla chrysoscelis* . Oikos **54**:325-330.

Alford R. A. 1989b. Competition between larval *Rana palustris* and *Bufo americanus* is not affected by variation in reproductive phenology. Copeia **1989**:993-1000.

Altig R., M. R. Whiles, and C. L. Taylor. 2007. What do tadpoles really eat? assessing the trophic status of an understudied and imperiled group of consumers in freshwater habitats. Freshwater Biology **52**:386-395.

Altwegg R., H. U. Reyer. 2003. Patterns of natural selection on size at metamorphosis in water frogs. Evolution **57**:872-882.

Baker G. C., T. J. C. Beebee. 2000. Evidence for the induction of interference competition between anuran larvae in plastic pond cages. Amphibia-Reptilia **21**:25-37.

Baker G. C., T. J. Beebee, and M. A. Ragan. 1999. *Prototheca richardsi*, a pathogen of anuran larvae, is related to a clade of protistan parasites near the animal-fungal divergence. Microbiology (Reading, England) **145**:1777-1784.

Bardsley L., T. J. Beebee. 2001. Non-behavioural interference competition between anuran larvae under semi-natural conditions. Oecologia **128**:360-367.

Beebee T. J. C. 1991. Purification of an agent causing growth inhibition in anuran larvae and its identification as a unicellular unpigmented alga. Canadian Journal of Zoology-Revue Canadienne De Zoologie **69**:2146-2153.

Beebee T. J. C., A. L. C. Wong. 1992. Prototheca-mediated interference competition between anuran larvae operates by resource diversion. Physiological Zoology **65**:815-831.

Belanger R. M., L. D. Corkum. 2009. A review of aquatic sex pheromones and chemical communication in anurans. Journal of Herpetology **43**:184-191.

Berven K. A. 1990. Factors affecting population fluctuations in larval and adult stages of the wood frog (*Rana sylvatica*). Ecology **71**:1599-1608.

Biesterfeldt J. M., J. W. Petranka, and S. Sherbondy. 1993. Prevalence of chemical interference competition in natural populations of wood frogs, *Rana sylvatica*. Copeia **1993**:688-695.

Blaustein A. R., B. Waldman. 1992. Kin recognition in anuran amphibians. Animal Behaviour **44**:207-221.

Burnard D., R. E. Gozlan, and S. W. Griffiths. 2008. The role of pheromones in freshwater fishes. Journal of Fish Biology **73**:1-16.

Case T. J., M. E. Gilpin. 1974. Interference competition and niche theory. Proceedings of the National Academy of Sciences of the United States of America **71**:3073-3077.

Chelgren N. D., D. K. Rosenberg, S. S. Heppell, and A. I. Gitelman. 2006. Carryover aquatic effects on survival of metamorphic frogs during pond emigration. Ecological Applications **16**:250-261.

D'Angleo S. A., A. S. Gordon, and H. A. Charipper. 1941. The role of the thyroid and pituitary glands in the anomalous effect of inanition on amphibian metamorphosis. Journal of Experimental Zoology **87**:259-277.

DeBenedictis P. A. 1974. Interspecific competition between tadpoles of *Rana pipiens* and *Rana sylvatica*: an experimental field study. Ecological Monographs **44**:129-151.

Denver R. J. 2009. Stress hormones mediate environment-genotype interactions during amphibian development. General and Comparative Endocrinology **164**:20-31.

Denver R. J. 1997. Proximate mechanism of phenotypic plasticity in amphibian metamorphosis. American Zoologist **37**:172-184.

Dupre R. K., J. W. Petranka. 1985. Ontogeny of temperature selection in larval amphibians. Copeia **1985**:462-467.

Faragher S. G., R. G. Jaeger. 1998. Tadpole bullies: examining mechanisms of competition in a community of larval anurans. Canadian Journal of Zoology **76**:144-153.

Fauth J. E., W. J. Resetarits. 1991. Interactions between the salamander *Siren intermedia* and the keystone predator *Notophthalmus viridescens* . Ecology **72**:827-838.

Glennemeier K. A., R. J. Denver. 2002. Role for corticoids in mediating the response of Rana pipiens tadpoles to intraspecific competition. Journal of Experimental Zoology **292**:32-40.

Gosner K. L. 1960. A simplified table for staging anuran embryos and larvae with notes on identification. Herpetologea **16**:183-190.

Griffiths R. A., P. W. Edgar, and A. L. C. Wong. 1991. Interspecific competition in tadpoles - growth inhibition and growth retrieval in Natterjack Toads, *Bufo calamita*. Journal of Animal Ecology **60**:1065-1076.

Gromko M. H., F. S. Mason, and S. J. Smith-Gill. 1973. Analysis of crowding effect in *Rana pipiens* tadpoles. Journal of Experimental Zoology **186**:63-71.

Gurevitch J., L. L. Morrow, A. Wallace, and J. S. Walsh. 1992. A meta-analysis of competition in field experiments. American Naturalist **140**:539-572.

Harkey G. A., R. D. Semlitsch. 1988. Effects of temperature on growth, development, and color polymorphism in the ornate chorus frog *Pseudacris ornata*. Copeia **1988**:1001-1007.

Hensley F. R. 1993. Ontogenetic loss of phenotypic plasticity of age at metamorphosis in tadpoles. Ecology **74**:2405-2412.

Kupferberg SJ. The ecology of native tadpoles (*Rana boylii* and *Hyla regilla*) and the impact of invading bullfrogs (*Rana catesbeiana*) in a northern California river. California, USA: University of California at Berkeley; 1996. 289 p.

Licht L. E. 1967. Growth inhibition in crowded tadpoles - intraspecific and interspecific effects. Ecology **48**:736-750.

Morin P. J., E. A. Johnson. 1988. Experimental studies of asymmetric competition among anurans. Oikos **53**:398-407.

Pehek E. L. 1995. Competition, pH, and the ecology of larval *Hyla andersonii*. Ecology **76**:1786-1793.

Petranka J. W. 1989. Chemical interference competition in tadpoles - does it occur outside laboratory aquaria? Copeia **1989**:921-930.

Richards C. M. 1962. The control of tadpole growth by alga-like cells. Physiological Zoology **35**:285-296.

Richards C. M. 1958. The inhibition of growth in crowded *Rana pipiens* tadpoles. Physiological Zoology **31**:138-151.

Richter-Boix A., G. A. Llorente, and A. Montori. 2004. Responses to competition effects of two anuran tadpoles according to life-history traits. Oikos **106**:39-50.

Ricker W. E. 1975. Computation and interpretation of biological statistics of fish populations. Bulletin of the Fisheries Research Board of Canada **191**:1-382.

Roales R. R. 1981. The effect of growth inhibiting factors on the total lipid content of the Zebrafish, *Brachydanio rerio* (Hamilton-Buchanan). Journal of Fish Biology **18**:723-728.

Rodriguez-Munoz R., A. G. Nicieza, and F. Brana. 2003. Density-dependent growth of Sea Lamprey larvae: evidence for chemical interference. Functional Ecology **17**:403-408.

Rose S. M., F. C. Rose. 1965. The control of growth and reproduction in freshwater organisms by specific products. Mitteilungen Internationale Vereinigung Fur Theoretische und Angewandte Limnologie **13**:21-35.

Rose S. M., F. C. Rose. 1961. Growth-controlling exudates of tadpoles. Symposia of the Society for Experimental Biology **15**:207-218.

Rot-Nikcevic I., R. J. Denver, and R. J. Wassersug. 2005. The influence of visual and tactile stimulation on growth and metamorphosis in anuran larvae. Functional Ecology **19**:1008-1016.

Runkova G. G., Z. L. Stepanova, and L. A. Koval'chuk. 1974. Organ specificity in the action of metabolites of amphibian larvae on their endogenous metabolism under conditions of increased population density. Doklady Akademii Nauk SSSR **217**:328-329.

Schoener T. W. 1983. Field experiments on interspecific competition. American Naturalist **122**:240-285.

Shvarts S. S., O. A. Pyastolova. 1970a. Regulators of growth and development of amphibian larvae. I. specificity of effects. Soviet Journal of Ecology :58-62.

Shvarts S. S., O. A. Pyastolova. 1970b. Regulators of growth and development of amphibian larvae. II. Diversity of effects. Soviet Journal of Ecology **1**:122-134.

Skelly D. K. 1996. Pond drying, predators, and the distribution of Pseudacris tadpoles. Copeia **1996**:599-605.

Smith D. C. 1987. Adult recruitment in chorus frogs: effects of size and date at metamorphosis. Ecology **68**:344-350.

Solomon D. J. 1977. Review of chemical communication in freshwater fishes. Journal of Fish Biology **11**:363-376.

Steinwascher K. 1979a. Host-parasite interaction as a potential population-regulating mechanism. Ecology **60**:884-890.

Steinwascher K. 1979b. Competitive interactions among tadpoles - responses to resource level. Ecology **60**:1172-1183.

Steinwascher K. 1978a. The effect of coprophagy on the growth of *Rana catesbeiana* tadpoles. Copeia **1978**:130-134.

Steinwascher K. 1978b. Interference and exploitation competition among tadpoles of *Rana utricularia*. Ecology **59**:1039-1046.

Stepanova Z. L. 1974. The chemical nature of the products of metabolism of amphibian larvae, excreted into the water. Soviet Journal of Ecology (Ekologiya) **5**:148-149.

Stewart R. E., H. A. Kantrud. 1971. Classification of natural ponds and lakes in the glaciated prairie region. U.S. Fisheries and Wildlife Service Resource Publication **92**:57.

SYSTAT Software INC. 2007. Systat. 12.02. San Jose, California.

Thomas J. D., A. S. Lough, and R. W. Lodge. 1975. Chemical ecology of *Biomphalaria glabrata* (Say), Snail host of *Schistosoma mansoni sambon* - search for factors in media conditioned by snails which inhibit their growth and reproduction. Journal of Applied Ecology **12**:421-436.

Tomasso J. R. 1994. Toxicity of nitrogenous wastes to aquaculture animals. Reviews in Fisheries Science **2**:291-314.

Travis J. 1984. Anuran size at metamorphosis: experimental test of a model based on intraspecific competition. Ecology **65**:1155-1160.

Waldman B., P. J. Bishop. 2004. Chemical communication in an archaic anuran amphibian. Behavioral Ecology **15**:88-93.

Wassersug R. J. 1975. The adaptive significance of the tadpole stage with comments on the maintenance of complex life cycles in anurans. American Zoologist **15**:405-417.

West L. B. 1960. The nature of growth inhibitory material from crowded *Rana pipiens* tadpoles. Physiological Zoology **33**:232-239.

Wilbur H. M. 1997. Experimental ecology of food webs: complex systems in temporary ponds. Ecology **78**:2279-2302.

Wilbur H. M. 1987. Regulation of structure in complex-systems - experimental temporary pond communities. Ecology **68**:1437-1452.

Wilbur H. M. 1977. Interactions of food level and population density in *Rana sylvatica*. Ecology **58**:206-209.

Wilbur H. M., J. P. Collins. 1973. Ecological aspects of amphibian metamorphosis. Science **182**:1305-1314.

Wong A. L., R. A. Griffiths, and T. J. C. Beebee. 2000. The influence of tadpole size on cell-mediated competition between anuran larvae (*Bufo calamita* and *Rana temporaria*) in the laboratory. Amphibia-Reptilia **21**:431-438.

Wong A. L. C., T. J. C. Beebee, and R. A. Griffiths. 1994. Factors affecting the distribution and abundance of an unpigmented heterotrophic alga *Prototheca richardsi* . Freshwater Biology **32**:33-38.

Wright M. L., K. L. Proctor, and C. D. Alves. 1999. Hormonal profiles correlated with season, cold, and starvation in *Rana catesbeiana* (bullfrog) tadpoles. Comparative Biochemistry and Physiology C: Pharmacology Toxicology and Endocrinology **124**:109-116.

Yu M. L., A. Perlmutter. 1970. Growth inhibiting factors in zebrafish, *Brachydanio rerio* and blue gourami, *Trichogaster trichopterus*. Growth **34**:153-175.

Chapter 6: General conclusions and future directions

This final chapter of my dissertation reviews the evidence for interspecific competition between larvae of the boreal chorus frog and wood frog, and reviews the ecology of the boreal chorus frog as revealed through my projects. I then discuss possible future directions for research into interference competition between tadpoles, and on the use of stable isotopes to explore tadpole diets and their functional role in food webs.

The evidence for interspecific competition

Petren and Case's (1996) framework to demonstrate interspecific competition was used in the current work. They developed five steps that conclusively show competition occurs through resource exploitation in the absence of other mechanisms. I examined the case for exploitative competition, and although some of Petren and Case's steps are inferred rather than tested, I found that resource exploitation occurs. Nevertheless, exploitation does not account for all the differences in larval performance found as interference mechanisms also operate. I propose that interference mechanisms exist as my findings indicate that wood frog tadpoles release either chemicals or cells which reduce chorus frog tadpole growth rates and size at metamorphosis in the laboratory which may also occur in mesocosm and field venues. Below, I review my findings in light of Petren and Case's five steps.

Step 1) The two species must share a resource.

Using stable isotopes (Chapter 2) I showed that the two tadpole species occupy the same trophic position and, depending on venue or conditions, may share food resources. Within mesocosms, I found that both species' tadpoles shared the same food resources as isotope overlap was complete. In contrast, in natural ponds, wood frog tadpoles are generally more depleted in carbon^{13} relative to chorus frogs, which suggests the bulk of what wood frogs assimilate differs from chorus frogs. This resource partitioning in natural ponds may occur as a result of the greater diversity of resources available in comparison to the possibly limited diversity of foods available in mesocosms (Morin and Johnson 1988, Leibold and Wilbur 1992, Brown et al. 2006). The identity of foods consumed was unclear from stable isotope analysis (SIA), whereas quantitative fatty acid signature analysis (Iverson et al. 2004) may be required to identify tadpole foods. I did, however, find that the source of food was affected by temperature and hydroperiod of ponds.

Chorus and wood frog tadpoles are consistently part of the same trophic group (or guild), as demonstrated by their similarity in relative trophic positions and slight difference in carbon source (mean of 1.02 ‰, range 0-4‰) and is consistent with resource partitioning. Due to the limitations of SIA, if the two species compete for food resources, the identity of those shared foods may be obscured by consumption of other materials. However, the potential for competition is indicated by overlap in both carbon and trophic position in mesocosms and occasionally in natural ponds. Overall, results of stable isotope analyses were clear in that both tadpole species fed at a higher trophic position than co-occurring herbivorous snails

indicating that these tadpoles are omnivorous, and consume some animal material, as suggested by Schiesari et al. (2009) for wood frogs and other ranids.

Step 2) Growth, survivorship and/or reproduction of the competing species must be limited by the availability of this resource.

Slow growth in temporary ponds increases the risks of death from desiccation and size selective predation. Slow growth combined with rapid development in drying environments also results in smaller size at metamorphosis. Reduced size at metamorphosis may lead to delayed sexual maturity (Smith 1987), lower survival in the first year (Smith 1987, Altwegg and Reyer 2003, Chelgren et al. 2006), delayed age at first breeding (Smith 1987, Berven 1990), and reduced egg production by females (Smith 1987, Chelgren et al. 2006). Smaller sizes at metamorphosis without growth compensation in later, terrestrial stages may lead to reduced lifetime fitness of an individual. In mesocosms, I found that addition of fertilizers improved growth and survival of tadpoles and generally led to larger size at metamorphosis. Greater survival and growth are consistent with increased availability of food resources. In field surveys, I found that higher tadpoles living at higher densities displayed reduced growth rates and size at metamorphosis an observation that is also consistent with reduced availability of resources. Together these results suggest that lower levels of food resources can lead to reduced growth and size at metamorphosis for both CF and WF which may result in reduced reproductive potential and lifetime fitness for individuals.

Attempts to identify the limited resource using (SIA) were not conclusive because tadpole diet is a mix of materials, not all of them assimilated. Several studies infer that periphyton, common in tadpole guts (e.g. Farlowe 1928, Seale and Beckvar 1980), is the principal resource for which tadpoles compete (Dickman

1968, Kupferberg et al. 1994, Schiesari et al. 2009). Isotope ratios of tadpole tissues did not match those for periphyton, which may suggest that periphyton is not the resource for which tadpoles compete. However, periphyton signatures may fluctuate throughout the tadpole growing season (Post 2002) and obscure their importance to tadpole diets based on isotope analysis. Wetzel (2006) found that periphyton is a complex community of active, inactive and senescent heterotrophic and autotrophic microorganisms plus inorganic and dead organic particles, and extracellular mucopolysaccharides. Of the autotrophs, algae and diatoms may pass through tadpole guts unchanged (Kupferberg et al. 1994) suggesting that tadpoles may consume the periphyton to access other resources, such as bacteria and other micro-organisms that live within the periphyton film. As algae and diatoms may constitute a sizeable fraction of material which are not assimilated, the isotopic carbon ratio of tadpoles may differ significantly from periphyton samples. Although the specific food source of the tadpoles was not identified, I was able to demonstrate in Chapter 3 that in mesocosms an increase in nutrients, such as fertilizer, will increases chorus frog tadpole growth, survival and size at metamorphosis. Leibold and Wilbur (1992) also used fertilizers to increase the availability of tadpole foods. It stands to reason that fertilizers increase food abundance or quality and chorus frog tadpoles respond with greater survival, and growth. Wood frog tadpoles in the same mesocosm trials also responded to elevated nutrient levels, however, unlike chorus frog tadpoles, trends for increased larval growth, survival and size at metamorphosis in fertilized treatments were not significant.

Reduced abundance of foods also appears to be responsible for the reduced growth of tadpoles and size at metamorphosis in both agricultural and natural protected ponds (Chapter 2 and 4). I found both chorus and wood frog tadpole

growth rates decreased significantly with trophic position suggesting that growth was better for tadpoles functioning as herbivores/omnivores than tadpoles consuming a more carnivorous diet as indicated by their relative trophic position. Density increased both chorus frog and wood frog trophic levels, more so for wood frogs, and was accompanied by greater similarity of trophic level between species with increased larval density. A change in trophic position with larval density suggests not only are tadpoles capable of adapting to changing food conditions, but that the abundance of algal foods can be insufficient to sustain growth of tadpoles when larval densities are elevated.

Step 3) One species must negatively affect the acquisition of this resource by the other.

I have demonstrated that the presence of wood frog tadpoles reduced the growth and size at metamorphosis of chorus frog tadpoles in natural ponds (Chapter 2 and 4), and in mesocosms (Chapter 3) even though the specific component(s) of tadpole diets was not identified. I also found that the simple presence of wood frog tadpoles in mesh cages or their feces added to aquaria reduces chorus frog performance in replicated laboratory trials (Chapter 5). I infer that the availability or utility of resources to tadpoles is affected by the presence of wood frog tadpoles.

As discussed above, greater interspecific densities resulted in greater similarity in trophic position. From this, I infer that resources become rarer at higher interspecific densities and reduced the performance of both larvae. Chorus frog tadpoles have simpler mouth parts so they may be less efficient grazers on periphyton. On the other hand, their smaller size and finer gill filter used for ultraplanktonic entrapment (Wassersug 1972) may enhance their ability to filter small particles (< 10 μm) that are dislodged during grazing (Wassersug 1972, Seale

and Wassersug 1979). Wood frog tadpoles have two more tooth rows than chorus frogs, and their oral apparatus has greater emargination which confers greater ability to grasp and graze from a surface (Wassersug and Yamashita 2001, Wassersug pers. comm.). If the source of competition in natural and mesocosm settings is periphyton, grazing by wood frogs may alter or reduce periphyton in those systems such that it limits the ability of chorus frog tadpoles to graze effectively or efficiently.

In Chapter 5 the two species did not share food resources, only surrounding water, as the species were separated by mesh netting. Reduced growth and size at metamorphosis of chorus frog tadpoles suggests that the mere presence of wood frog tadpoles affects chorus frog resource acquisition or efficiency of resource use. Among the mechanisms that explain this phenomenon are behavioural or chemical interference and resource diversion. Although consumption of wood frog feces was possible in two of the experiments, feces were not the sole food consumed. The simplest and most plausible mechanism is interference, suggesting that resource exploitation does not occur in the absence of other competitive mechanisms.

Step 4) This reduced resource acquisition must translate into reduced demographic parameters such as survivorship or fecundity of the inferior competing species, or other long-term evolutionary change.

Although food intake was not measured in any venue, I am able to infer from the observation that patterns of reduced performance are caused by greater interspecific larval densities (Chapters 2, 3, 4), which provides reasonable evidence that acquisition of resources reduced future survival and reproduction. Additional support comes from the higher trophic position and species' similarities at higher tadpole densities, which decreased chorus frog growth rates and resulted in reduced

size at metamorphosis which may limit survival and fitness (Smith 1987, Berven 1990, Altwegg and Reyer 2003, Chelgren et al. 2006). As shown in the laboratory, the mere presence of wood frog larvae affects the chorus frog metamorphs that emerge. Chorus frog larvae grow slower and are smaller at metamorphosis in the presence and at higher densities of wood frog larvae.

Step 5) Interference mechanism and other processes must be ruled out.

Three possible mechanisms for the observed effects on chorus frog larval growth and size at metamorphosis are exploitive, interference and apparent competition. Exploitative competition, although inferred above, is ruled out as the only mechanism operating as in laboratory trials (Chapter 5) chemical interference was implicated in the reduction of chorus frog growth and size at metamorphosis. Chorus frog tadpoles sharing water with wood frog tadpoles (and/or addition of wood frog feces), where physical contact was prevented by suspending wood frog tadpoles in mesh cages, responded to wood frog presence with reduced growth. When only wood frog tadpoles were suspended but their feces were removed, I found a tendency for chorus frog size at emergence and growth prior to metamorphosis to be reduced. When both feces and wood frog tadpoles were present, I similarly found reduced size at emergence. Other researcher have shown that a unicellular organism, *Anurofeca richardsi,* inhibits tadpole growth (Richards 1958, Beebee 1991, Baker et al. 1999, Bardsley and Beebee 2001), although its impact on natural populations is absent or negligible (Biesterfeldt et al. 1993, Griffiths et al. 1993). I did not test for the presence of *A. richardsi* in these trials, but I am sceptical of its involvement especially when in the absence of feces I found reduced growth and size at emergence. In the absence of feces there can be no *A.*

richardsi cells, thus I am suggesting that other agents are causing growth inhibition in chorus frogs.

Apparent competition, due to differential predation or susceptibility to pathogens between the two species, can create patterns similar to those observed. Though apparent competition was not implicitly tested in the field study, the density of predators at natural ponds did not explain changes in chorus frog performance (Chapter 4); I did not assess pathogens but also saw no clear symptoms related to diseases. In addition, in the absence of predatory invertebrates in replicated mesocosms and indoor aquaria, I still saw reduced performance in chorus frogs. This also suggests that reduced tadpole densities, as a result of predation, are not responsible for the reduction of tadpole growth rates and size of chorus frogs at emergence seen in natural ponds. Rather, similar effects on chorus frog growth in mesocosms and lab trials suggest that interference competition is the mechanism responsible for the reduced growth and size at metamorphosis of boreal chorus frogs as a result of chemical agents released by wood frog tadpoles.

Conclusions

The boreal chorus frog and wood frog display similar egg laying dates, a tendency to use the same temporary ponds, and are often the only amphibian members of pond communities in the Beaver Hills of Alberta. These similarities may cause their larvae to depend on the same foods and as foods in aquatic systems are often limited, the potential for interference or exploitative competition is therefore likely. Synchrony of breeding may reduce intra-guild predation by wood frog tadpoles (Sours and Petranka 2007) on both chorus and wood frog eggs and hatchlings. However, synchrony and early breeding likely serves to ensure larvae a

greater probability of developing before pond drying. What drives the coexistence of these two species? Perhaps, as suggested by Schoener (1983), conditions exist where interference competition offsets the effects of exploitative competition permitting the two species to coexist even under high resource overlap. However, I have demonstrated that under certain conditions the two species differ in their resource use; the evidence is the differences in their isotope signatures that suggest that tadpoles are partitioning the available foods within the same trophic level. I have also demonstrated that interference operates in the laboratory, and therefore may also operate in the field. New methods are needed to measure the impact of both resource exploitation and interference simultaneously without affecting the nature of the interaction so that we can determine the relative importance of each mechanism.

Ecology of the boreal chorus frog

One of the goals of this dissertation was to examine the larval ecology of the boreal chorus frog. Throughout the dissertation I have detailed many aspects of the ecology and environment in which chorus frog larvae develop and factors that affect their growth, survival and size at metamorphosis. I will briefly review some of the aspects of the breeding ecology and larval ecology of the boreal chorus frog, *Pseudacris maculata*.

Breeding and egg deposition occurs shortly after snow melt has occurred. Adult chorus frogs converge on breeding ponds and start calling as early as 12 April in the Beaver Hills Region. The mean lengths (snout-to-urostyle) are males 22.6 mm (range 19.8 – 28, n = 88), females 22.3 mm (range 19.3 – 28, n = 55) and immature

frogs 18.6 mm (15-21.7, n = 94) based on data combined from 2005-2007. The ponds used by chorus frogs in the aspen parkland generally have open canopies with downed emergent vegetation composed mainly of *Carex* spp. Males call from within patches of last year's emergent vegetation. Some males call from within dead vegetation, which may be raised above the water's surface providing additional cover from predation. Although, I observed others calling from areas where the vegetation was at the water's surface. Calling males were heard from 12 April until early June. June 3rd was the last date choruses were heard in the aspen parkland.

Eggs are laid over a period of seven weeks resulting in several larval cohorts developing within one pond (eggs were seen laid as late as 3 June 2005). Eggs are laid mainly on the underside of *Carex* leaves and stems in small masses of between 7 and 146 eggs (mean of 36 based on 95 masses counted during the 2007 and 2008 seasons). I also found several egg masses deposited on submerged vegetation (*Potamogeton* spp.). Within *Carex* beds, I found that eggs appeared to be laid in communal areas (also mentioned by Smith 2002), as numerous masses were deposited along the length of *Carex* vegetation. Several of those masses may have been from the same female, as females have between 134 and 515 mature eggs within their ovaries (158 to 747 ova from five females), similar to the 384 found by Ouellet et al. (2009). The eggs were exposed to greater variation of temperatures than the pond water because of their location on vegetation and near the surface. Daily average water temperatures near eggs were on average 1.06±0.48°C higher compared to pond temperatures (based on three egg masses from three separate ponds in 2007, but only 0.02°C higher in 2006 from five masses). Eggs hatched between seven and 14 days after being laid. Chorus frog eggs were frequently affected by a fungal infection, *Saprolegnia*, and suffered high mortality. Most egg

masses found in 2006 were infected and few hatchlings emerged from those ponds, whereas in 2007, infection rates appeared lower and larval densities were much higher. Chorus frog eggs seemed more susceptible to the infection than wood frog eggs, as only a few wood frog masses showed signs of fungal infection.

Once hatched, the chorus frog tadpoles tend to stay around the submerged *Carex* detritus, where they appear to feed from the epiphyton that grows on the surface. The common interactions with other species are predation by invertebrates and competition with wood frog larvae. I observed that larval dytiscid beetles are the main predators in these ponds. Although larval Anisoptera were plentiful in ponds, I did not observe any predation events by anisopteran larvae. The hatchlings seemed to be more susceptible to predation, because they tended to be in the same downed vegetation where larval dytiscids were abundant. As demonstrated in each of the previous chapters, wood frogs and chorus frog larvae are commonly found in the same ponds, and within the same microhabitats (based on observation and dipnet captures). Though I never observed any agonistic behaviour between the two anuran larvae, I did observe both species feeding from epiphyton on *Carex* detritus. Growth rates of chorus frog larvae were reduced by intraspecific density, but both growth and size at metamorphosis decreased with wood frog larval densities. Future work on the ecology of chorus frogs should try to determine the actual diet of larvae and whether it differs over time; possibly through the use of lipid fatty acid signatures (Iverson et al. 2004). Knowledge on the diet through ontogeny is particularly important if later cohorts show significant overlap with earlier ones, as it may impact their ability to grow and develop prior to pond drying. I examined the ovaries of a number of female chorus frog, and found a number of immature ova. If females are capable of having several spawning opportunities that produce

metamorphs, this adaptation would allow females to breed in ponds that fill, or refill, later in the spring and may explain the prolonged calling of the males.

As metamorphosis approached (end of June), chorus frog larvae were often found in the shallows near the shore. Whether this behaviour increased the rate of tail resorption is unclear, but it likely was important to allow the froglets to emerge from the water to maintain osmotic balance. Larval periods in the ponds surveyed ranged from 51 to 93 days from egg-laying date to the first appearance of metamorphs (mean of 68 days from 30 pond-years, from 15 different ponds). These development rates are similar to those reported for *P. maculata* from Montreal, Quebec (Whiting 2004), formerly *P. triseriata* sensu (Lemmon et al. 2007). Size at metamorphosis was 13.33 ± 0.25 mm (10.4 – 17.2 from 265 metamorphs 2005-2007), somewhat larger than the 10.88 mm (± 0.67 SD) near Montreal. After tail resorption, froglets were found along the edge of the drying ponds, and even in ponds that had already dried.

Although, I did not track metamorphs into August, sizes were approaching those of adults. Chorus frogs are known to reach sexual maturity (up to 63% of individuals) by the end of their first summer (Whiting 2004). A stage-structured model created for a population of *P. maculata* from Montreal suggested that population persistence was most dependent upon larval survival within the breeding pond, whereas survival in the first year was the next most critical stage to ensuring population persistence. Reduced survival of metamorphs within crop lands is suspected in Chapter 4, as metamorphs emerged larger from crop than from protected ponds, but larval densities, a reflection of adult density, were lowest in those crop ponds. Research into the terrestrial dispersal of metamorphs and adults

would answer whether survival is indeed lower in crop lands, and reveal information about the interconnectivity of pond populations as they relate to the ability of chorus frog populations to recover from drought or reappear after fish have disappeared from locations (Werner et al. 2007). This apparent local extinction and recolonization is consistent with the operation of metapopulations, and may reveal why models for chorus frogs show a greater dependence on landscape features than on pond features (Gibbs et al. 2005, Price et al. 2005, Browne et al. 2009).

Future directions/ideas on interference and stable isotopes

Interference mechanisms in anuran larvae; where to from here?

Identifying the causative agent(s) and the method of transmission acting in this interference is of importance to understanding the interactions between boreal chorus frog and wood frog tadpoles. I showed that a substance released by wood frog tadpoles reduces both the growth and size at metamorphosis of boreal chorus frog tadpoles. I now review interference competition in anurans and then outline possible avenues for future research to identify the agent(s).

Inhibition of crowded tadpoles: Anurofeca richardsi

Probably the most studied and invoked inhibitory agent in interference competition involving anuran larvae is *Anurofeca richardsi*. First identified by Richards (1958), *A. richardsi* (formerly *Prototheca richardsi* sensu Baker et al. 1999) is a unicellular parasitic organism that proliferates in the guts of anuran larvae where it can reduce feeding (appetite)- thereby prolonging food passage time, resulting in decreased growth and survival of affected tadpoles (Beebee 1991,

Beebee and Wong 1992, Baker et al. 1999, Bardsley and Beebee 2001). The feces of affected tadpoles contain *A. richardsi* cells which are transferred to other tadpoles through coprophagy. An attractant produced by *A. richardsi* may encourage tadpoles to consume infected feces (Beebee and Wong 1992). The inhibitory effects of *Anurofeca* cells are greater on smaller tadpoles, whereas larger tadpoles seem less susceptible and continue to grow while producing more cells (Richards 1962, Steinwascher 1979, Beebee 1991); this difference in susceptibility appears to be the mechanism of interference observed and potentially would cause asymmetric competition. *A. richardsi* has been reported in the guts and feces of wild caught *Lithobates sylvaticus* tadpoles (Biesterfeldt et al. 1993), and are reported to reduce growth and survival of *Pseudacris streckeri* tadpoles as well as for other species of *Ranidae* and *Hylidae* raised in water conditioned by crowded tadpoles (Richards 1958, Licht 1967).

Despite the effect of *A. richardsi* on lab raised tadpoles, its role in nature has long been questioned (Petranka 1989). Wong et al. (1994), Baker and Beebee (1997, 2000), Bardsley and Beebee (2001), and Biesterfeldt et al. (1993) have documented the presence of *Anurofeca* cells in wild anuran larval populations (Biesterfeldt et al. in North Carolina whereas others were in the United Kingdom), but Petranka (1989) and Lawrence Licht (pers. comm.) both found the cells to be absent from the ponds they surveyed in North Carolina and Texas respectively. In two of these studies, *Anurofeca* and interference only occurred when tadpoles were raised in plastic cages (Baker and Beebee 1997, 2000). In mesh cages reduced tadpole growth was more strongly related to food availability than to *Anurofeca* abundance (Bardsley and Beebee 2001). The inhibitory effect of *Anurofeca* in nature is further questioned, as reduced growth of wood frog tadpoles in North Carolina ponds (Biesterfeldt et al.

1993) occurred even though *Anurofeca* abundance was 100 times lower than that reported to inhibit larval growth from lab raised tadpoles (Richards 1962). The effects of *A. richardsi* on larvae in natural ponds are absent to weak and could be sufficiently explained by pure exploitative competition hence the reason for the lack of interest in this organism since 2001 (TJC Beebee and J Petranka personal communications). Unfortunately many authors have inferred that growth inhibitors may be operating without experimental evidence or trust exploitative competition to explain observed competitive patterns. Though no one has confirmed *Anurofeca* occurs in Canadian wetlands, its presence is somewhat unlikely. *Anurofeca* cells do not survive freezing or tolerate low temperatures, and cells can be killed by exposure to UV (Richards 1958, West 1960). Though *A. richardsi* may use snails as intermediate or temporary hosts (Wong et al. 1994, Hertel et al. 2004), the abundance of *Anurofeca* is reduced by competition with microorganisms and predation by bacterial pathogens (Wong et al. 1994, Baker and Beebee 1997, 2000).

We would need to confirm the presence of *A. richardsi* in the lab and in my survey ponds in order to implicate *A. richardsi* in the inhibition of tadpole growth. However, because of its rarity in the wild, and its inability to survive outside of tadpole guts, *A. richardsi* is not likely to be responsible for patterns of interference between tadpoles in natural ponds. My results also provide little support for *A. richardsi* as the causative agent, as significant reduction of growth took longer to develop (≥ 36 days versus 7-14 days in (Richards 1958, Licht 1967, Beebee 1991), was of lesser magnitude (10-15% reduction in weight, versus 75% reduction in Richards (1958), and did not consistently alter survival by experiment end, whereas with *Anurofeca* significant greater mortality occurs in 7-12 days (Richards 1958, 1962, West 1960). I believe that other inhibitory agents are responsible for the

reduced growth and size at metamorphosis seen in experiments #1 and #2 (Chapter 5) and patterns of reduced chorus frog tadpole growth as interference occurred in the absence of obvious competition for food in natural ponds (Chapters 2 and 4).

Inhibition of crowded tadpoles: the usual suspects

I believe that we need to consider the role of chemicals used in signalling and in developmental control if we are to discover the identity of the agent(s) responsible for the observed interference of wood frog tadpoles on chorus frog tadpoles. Rose and Rose (1965) suggest there are both short and long lasting inhibitory agents. Long lasting agents could be associated with *A. richardsi*, whereas short lived agents might be secreted by tadpoles in their feces or into the water and may inhibit tadpole growth in ponds in the absence of *A. richardsi*. Chemicals known to be products of metabolism and excreted by tadpoles are amino acids, proteins, hormones, and phytohormones (Shvarts and Pyastolova 1970). Many of these chemicals are known to be used as signalling agents (Waldman and Bishop 2004, Belanger and Corkum 2009), but none have equivocally shown to inhibit tadpole growth. Amino acids and simple proteins have been isolated from water conditioned by crowded *Rana* tadpoles that inhibited growth in conspecifics and accelerated the growth in heterospecifics (Runkova et al. 1974). The addition of trypsin, a proteinase, prevented inhibition in Runkova et al.'s trials and implicates proteins as the cause of inhibition. Proteins have also been proposed as the inhibition agent by Rose and Rose (1961), Stepanova (1974) and Biesterfeldt et al. (1993), but they did not publish any follow-up studies.

Hormones, such as thyroid hormones and corticosteroids, have also been considered likely to regulate growth and metamorphosis in anuran larvae (reviewed

by Furlow and Neff 2006, Brown and Cai 2007, Denver 2009). The notion that hormones of one species might be the cause of inhibition of tadpoles of another species was first mentioned by Akin (1966) and by Runkova et al. (1974). Unfortunately these hormones have not been explicitly tested for their roles in interference competition. Gromko et al. (1973) suspected that stress hormones might be responsible for the inhibition observed in trials, but did not publish any results of corticosteroid analyses suggested in their future directions. Corticosterone, a glucocorticoid hormone, is released from the adrenal cortex in response to activation of the hypothalamic-pituitary axis, and is important to vertebrates responding to environmental variation (McEwen and Wingfield 2003). Glucocorticoids are important to the survival of organisms as they increase the energy available to an organism during stressful periods by mobilizing energy stores (Romero 2002). However, if the stress continues, the loss of energy stores may result in reduced growth and ultimately death. More than 30 years later, revisiting Gromko et al.'s research seems relevant to identifying the agent(s) responsible for the effect of tadpole crowding and interspecific competition.

Corticosterone has been implicated in growth reduction during tadpole crowding and it may also be the agent involved for interference that created the competitive patterns observed in my field surveys and the observed reduced growth and size at metamorphosis of chorus frog tadpoles in lab trials when wood frog tadpoles were caged in aquaria with chorus frog tadpoles or when their feces were added to chorus frog tubs. A number of authors have reported that corticosterone levels are elevated in crowded tadpoles (Glennemeier and Denver 2002a, Belden et al. 2005, Rot-Nikcevic et al. 2005, Belden et al. 2007) and those exposed to food limitations, starvation or novel diets (Glennemeier and Denver 2002a, Crespi and

Denver 2005, Hu et al. 2008, Ledon-Rettig et al. 2009). The correlation between elevated whole-body corticosterone and reduced growth further implicates corticosterone in the inhibition of growth (Glennemeier and Denver 2002b, Belden et al. 2007, Lorenz et al. 2009). Treating tadpoles with exogenous corticosterone added to water in which tadpoles are growing has been shown to increase whole-body corticosterone levels and decrease tadpole growth rates and/or size at metamorphosis (in North American species like *Pseudacris regilla* - Belden et al. 2005, *Lithobates pipiens* - Glennemeier and Denver 2002ab, *Anaxyrus boreas* - Hayes et al. 1993, *Scaphiopodidae* species - Ledon-Rettig et al. 2009, and in exotic species like *Xenopus laevis* - Hu et al. 2008, Lorenz et al. 2009). The magnitude and even the appearance of these reductions are sometimes dependent on the temperature, dose and timing of exposure (Hayes et al. 1993), but clearly suggest that corticosterone has a role in inhibiting tadpole growth. Additional evidence of corticosterone's role in growth inhibition is that the addition of metyrapone, (a corticoid synthesis inhibitor), or other glucocorticoid signal blockers reverses growth suppression caused by density but not food deprivation (Glennemeier and Denver 2002ab, Ledon-Rettig et al. 2009). Conclusive evidence of corticosterone being released by one species and affecting another is still missing. Enzyme linked immunosorbent assay (ELISA) may help in identifying the agent involved. A series of experiments to produce clear evidence that corticosterone is the main agent in interference would require verification that corticosterone was produced by crowded tadpoles and released into their holding water, and that this water could inhibits the growth, survival and/or size at metamorphosis of tadpoles of a second species. However, if corticosterone could not be isolated, but another hormone related to activating corticosterone release, such as corticotrophin-releasing factor (reviewed by Denver

2009) was demonstrated to be present, this might offer another mechanism for the operation of interference through corticosterone. The series of experiments proposed on corticosterone mirrors those I performed in trying to determine the source of growth inhibition in chorus frog tadpoles. The proposed experiments differ as a known agent is being tested versus my experiments that inferred the presence and action of inhibitory substances.

I propose that the effects on mass at emergence for chorus frog tadpoles in experiments #1 and #2 are the result of either corticosteroids or another thyroid regulating hormone released by wood frog tadpoles. Wood frog tadpoles have been shown to respond to both increased density and food deprivation by increasing whole-body corticosterone levels (Rot-Nikcevic et al. 2005, Belden et al. 2007). Further, the growth and size at metamorphosis of *Pseudacris regilla* tadpoles decreased with the addition of corticosterone to their rearing tanks (Belden et al. 2005). If *P. maculata* respond in a similar fashion to *P. regilla*, I reason that corticosterone released by wood frog tadpoles could reduce growth rates, as seen in Figure 5-6, and accelerate development through to metamorphosis (as in Hayes et al. 1993). It is plausible that corticosteroid production, in response to crowding and changing conditions, occurred as a result of food reductions in experiments #1 and #2, or that conditions prior to food reductions had already created differences in the corticosterone levels between treatments and the reduction of foods increased the production of corticosterone in wood frogs which triggered a response in adjacent chorus frog tadpoles. Whether concentrations of corticosterone used in lab trials are relevant to natural populations has been questioned (Belden et al. 2005, Ledon-Rettig et al. 2009), but if tadpole densities are sufficiently high in small patches, a

spike in corticosterone levels may lead to an endocrine cascade such that growth suppression may be observed in response to interspecific density.

Tadpoles and stable isotopes; thoughts, pitfalls and suggestions

I gained some insights while trying to determine what tadpoles eat and their role in the temporary ponds on how to best employ stable isotopes to examine the functional relationships of tadpoles. I believe the most important step to determining tadpole diets is quantitative measurement of fractionation (the difference between food source and consumer). Without this basic knowledge, the elucidation of tadpole foods will remain elusive as the carbon ratios of assimilated and consumed foods do not match when tadpole feces contain numerous undigested biota. The analysis of feeding trials of known foods enables comparison of tissue signatures with consumed foods. If the difference between the food and the consumer's tissue is consistent across different foods, then that fractionation can be used to examine the relationship of tadpoles to the natural foods available. I have confirmed that both wood frog and chorus frog tadpoles have similar fractionation rates when fed either rabbit pellets or spirulina pellets. The transferability of these fractionation rates to natural foods are unknown, and requires validation as both rabbit pellets and spirulina pellets are produced to maximize digestibility and absorption, two qualities that natural foods may be lacking. If the same fractionation trials were completed using a range of natural foods this information would allow us to examine food webs and try to place tadpoles within the food web and further clarify the diets of these and other tadpole species.

My inability to identify specific components of complex tadpole foods and uncertainty in species specific assimilation capabilities left some doubt as to

whether or not tadpoles share food resources. The best information I have is that chorus frog and wood frog tadpoles occupy similar trophic positions within ponds, and that the source of carbon is somewhat different. How different is difficult to assess, because a statistical difference of 1.02‰ can occur by several different mechanisms. First, the tadpoles may consume slightly different foods but from the same trophic level. This might occur as a result of different morphology as discussed earlier, whereby wood frog tadpoles may be better at scrapping foods from surfaces, whereas chorus frog tadpoles are less effective at scrapping and may only remove the top layer. Differential consumption, combined with, or in the absence of, species specific digestibility of consumed resources may further increase species differences with respect to assimilated carbon. Second, as discussed in Chapter 2, the slight differences in fractionation may have resulted in statistically significant differences in the carbon source for each species. If this is the case, then the two species may have greater overlap in resources, as identified by isotopic analysis, and if this resource was limited it would result in competition between the two species and may be partly responsible for observed patterns of chorus frog growth reduction at higher wood frog densities. Whatever the reason for species differences in $\delta^{13}C$, greater resolution of tadpole diets and isotopic fractionation combined with knowledge of the species specific ability to graze from surfaces may provide insight into how the two species share resources and how this process might affect interspecific interactions.

If stable isotope analysis continues to be used to elucidate tadpole diets, I believe that sampling is of the utmost importance. Subsampling, either by filtration or mechanical separation, of potential foods both in time and within a possible food source may reveal the source of tadpole foods. Resources that are continually

changing or being renewed, such as periphyton or other algal resources, may reflect tadpole signatures at one instant but may change by the time sampling occurs. Perhaps cross referencing stable isotope analysis with gut content validation may clarify how assimilation and consumption differ and why it might be difficult to identify food sources from stable isotopes. These challenges may deter researchers from using stable isotopes to identify tadpole foods and species interactions.

Where stable isotopes promises to be the most useful is in identifying the trophic status of tadpoles and in exploring resource partitioning in diverse anuran tadpole assemblages. Determining the true status of tadpoles is of importance to understanding their ecology and understanding how ecosystems may function if those species are lost (e.g. Connelly et al. 2008). Already it is becoming clear that tadpoles consume a greater amount of animal material than commonly assumed (this study and Schiesari et al. 2009). A study of the ecology of tadpole function would not be complete without knowledge of which foods are being consumed. Stable isotopes provide the ability to examine the degree of resource partitioning or resource overlap that occurs in tadpole assemblages. Systems where more than two species exist at any one time will provide a unique opportunity to examine the commonly held belief that tadpoles compete for resources. If it could be shown that tadpoles that occur together partition resources as they do in the wood frog / chorus frog system it would call into question much of what we believe about competition between anuran larvae. A major weakness of stable isotope analysis is that if the resources tadpoles compete for are not a large part of their diet or are of similar isotopic values then the analyses will not reveal how or if competition occurs.

Literature Cited

Akin G. W. 1966. Self-inhibition of growth in *Rana pipiens* tadpoles. Physiological Zoology **39**:341-356.

Altwegg R., H. U. Reyer. 2003. Patterns of natural selection on size at metamorphosis in water frogs. Evolution **57**:872-882.

Baker G. C., T. J. C. Beebee. 2000. Evidence for the induction of interference competition between anuran larvae in plastic pond cages. Amphibia-Reptilia **21**:25-37.

Baker G. C., T. J. C. Beebee. 1997. Microenvironmental effects on competition between Rana and Bufo larvae, and on the abundance of Prototheca richardsi, in small fish-ponds. Herpetological Journal **7**:149-154.

Baker G. C., T. J. Beebee, and M. A. Ragan. 1999. *Prototheca richardsi*, a pathogen of anuran larvae, is related to a clade of protistan parasites near the animal-fungal divergence. Microbiology (Reading, England) **145**:1777-1784.

Bardsley L., T. J. Beebee. 2001. Non-behavioural interference competition between anuran larvae under semi-natural conditions. Oecologia **128**:360-367.

Beebee T. J. C. 1991. Purification of an agent causing growth inhibition in anuran larvae and its identification as a unicellular unpigmented alga. Canadian Journal of Zoology-Revue Canadienne De Zoologie **69**:2146-2153.

Beebee T. J. C., A. L. C. Wong. 1992. Prototheca-mediated interference competition between anuran larvae operates by resource diversion. Physiological Zoology **65**:815-831.

Belanger R. M., L. D. Corkum. 2009. A review of aquatic sex pheromones and chemical communication in anurans. Journal of Herpetology **43**:184-191.

Belden L. K., M. J. Rubbo, J. C. Wingfield, and J. M. Kiesecker. 2007. Searching for the physiological mechanism of density dependence: Does corticosterone regulate tadpole responses to density? Physiological and Biochemical Zoology **80**:444-451.

Belden L. K., I. T. Moore, J. C. Wingfield, and A. R. Blaustein. 2005. Corticosterone and growth in Pacific Treefrog (*Hyla regilla*) tadpoles. Copeia **2005**:424-430.

Berven K. A. 1990. Factors affecting population fluctuations in larval and adult stages of the wood frog (*Rana sylvatica*). Ecology **71**:1599-1608.

Biesterfeldt J. M., J. W. Petranka, and S. Sherbondy. 1993. Prevalence of chemical interference competition in natural populations of wood frogs, *Rana sylvatica*. Copeia **1993**:688-695.

Brown C. J., B. Blossey, J. C. Maerz, and S. J. Joule. 2006. Invasive plant and experimental venue affect tadpole performance. Biological Invasions **8**:327-338.

Brown D. D., L. Cai. 2007. Amphibian metamorphosis. Developmental Biology **306**:20-33.

Browne C. L., C. A. Paszkowski, A. L. Foote, A. Moenting, and S. M. Boss. 2009. The relationship of amphibian abundance to habitat features across spatial scales in the Boreal Plains. Ecoscience **16**:209-223.

Chelgren N. D., D. K. Rosenberg, S. S. Heppell, and A. I. Gitelman. 2006. Carryover aquatic effects on survival of metamorphic frogs during pond emigration. Ecological Applications **16**:250-261.

Connelly S., C. M. Pringle, R. J. Bixby, R. Brenes, M. R. Whiles, K. R. Lips, S. Kilham, and A. D. Huryn. 2008. Changes in stream primary producer communities resulting from large-scale catastrophic amphibian declines: can small-scale experiments predict effects of tadpole loss? Ecosystems **11**:1262-1276.

Crespi E. J., R. J. Denver. 2005. Roles of stress hormones in food intake regulation in anuran amphibians throughout the life cycle. Comparative Biochemistry and Physiology A-Molecular & Integrative Physiology **141**:381-390.

Denver R. J. 2009. Stress hormones mediate environment-genotype interactions during amphibian development. General and Comparative Endocrinology **164**:20-31.

Dickman M. 1968. Effect of grazing by tadpoles on structure of a periphyton community. Ecology **49**:1188-1193.

Farlowe V. 1928. Algae of ponds as determined by an examination of the intestinal contents of tadpoles. Biological Bulletin **55**:443-448.

Furlow J. D., E. S. Neff. 2006. A developmental switch induced by thyroid hormone: *Xenopus laevis* metamorphosis. Trends in Endocrinology and Metabolism **17**:38-45.

Gibbs J. P., K. K. Whiteleather, and F. W. Schueler. 2005. Changes in frog and, toad populations over 30 years in New York State. Ecological Applications **15**:1148-1157.

Glennemeier K. A., R. J. Denver. 2002a. Role for corticoids in mediating the response of *Rana pipiens* tadpoles to intraspecific competition. Journal of Experimental Zoology **292**:32-40.

Glennemeier K. A., R. J. Denver. 2002b. Small changes in whole-body corticosterone content affect larval *Rana pipiens* fitness components General and comparative endocrinology **127**:16-25.

Griffiths R. A., J. Denton, and A. L. C. Wong. 1993. The effect of food level on competition in tadpoles - interference mediated by Protothecan algae. Journal of Animal Ecology **62**:274-279.

Gromko M. H., F. S. Mason, and S. J. Smith-Gill. 1973. Analysis of crowding effect in *Rana pipiens* tadpoles. Journal of Experimental Zoology **186**:63-71.

Hayes T., R. Chan, and P. Licht. 1993. Interactions of temperature and steroids on larval growth, development, and metamorphosis in a toad (*Bufo boreas*). Journal of Experimental Zoology **266**:206-215.

Hertel L. A., C. S. Barbosa, R. A. A. L. Santos, and E. S. Loker. 2004. Molecular identification of symbionts from the pulmonate snail *Biomphalaria glabrata* in Brazil. Journal of Parasitology **90**:759-763.

Hu F., E. J. Crespi, and R. J. Denver. 2008. Programming neuroendocrine stress axis activity by exposure to glucocorticoids during postembryonic development of the frog, *Xenopus laevis*. Endocrinology **149**:5470-5481.

Iverson, S. J., C. Field, W. D. Bowen, and W. Blanchard. 2004. Quantitative fatty acid signature analysis: a new method of estimating predator diets. Ecological Monographs **74**: 211-235.

Kupferberg S. J., J. C. Marks, and M. E. Power. 1994. Effects of variation in natural algal and detrital diets on larval anuran (*Hyla regilla*) life-history traits. Copeia **1994**:446-457.

Ledon-Rettig C. C., D. W. Pfennig, and E. J. Crespi. 2009. Stress hormones and the fitness consequences associated with the transition to a novel diet in larval amphibians. Journal of Experimental Biology **212**:3743-3750.

Leibold M. A., H. M. Wilbur. 1992. Interactions between food-web structure and nutrients on pond organisms. Nature **360**:341-343.

Lemmon E. M., A. R. Lemmon, J. T. Collins, J. A. Lee-Yaw, and D. C. Cannatella. 2007. Phylogeny-based delimitation of species boundaries and contact zones in the trilling chorus frogs (*Pseudacris*). Molecular Phylogenetics and Evolution **44**:1068-1082.

Licht L. E. 1967. Growth inhibition in crowded tadpoles - intraspecific and interspecific effects. Ecology **48**:736-750.

Lorenz C., R. Opitz, I. Lutz, and W. Kloas. 2009. Corticosteroids disrupt amphibian metamorphosis by complex modes of action including increased prolactin expression. Comparative Biochemistry and Physiology C-Toxicology & Pharmacology **150**:314-321.

McEwen B. S., J. C. Wingfield. 2003. The concept of allostasis in biology and biomedicine. Hormones and Behavior **43**:2-15.

Morin P. J., E. A. Johnson. 1988. Experimental studies of asymmetric competition among anurans. Oikos **53**:398-407.

Ouellet M., C. Fortin, and M. Grimard. 2009. Distribution and habitat use of the boreal chorus frog (*Pseudacris maculata*) at its extreme northeastern range limit. Herpetological Conservation and Biology **4**:277-284.

Petranka J. W. 1989. Chemical interference competition in tadpoles - does it occur outside laboratory aquaria? Copeia **1989**:921-930.

Petren K., T. J. Case. 1996. An experimental demonstration of exploitation competition in an ongoing invasion. Ecology **77**:118-132.

Post D. M. 2002. Using stable isotopes to estimate trophic position: models, methods, and assumptions. Ecology **83**:703-718.

Price S. J., D. R. Marks, R. W. Howe, J. M. Hanowski, and G. J. Niemi. 2005. The importance of spatial scale for conservation and assessment of anuran populations in coastal wetlands of the western Great Lakes, USA. Landscape Ecology **20**:441-454.

Richards C. M. 1962. The control of tadpole growth by alga-like cells. Physiological Zoology **35**:285-296.

Richards C. M. 1958. The inhibition of growth in crowded *Rana pipiens* tadpoles. Physiological Zoology **31**:138-151.

Romero L. M. 2002. Seasonal changes in plasma glucocorticoid concentrations in free-living vertebrates. General and Comparative Endocrinology **128**:1-24.

Rose S. M., F. C. Rose. 1965. The control of growth and reproduction in freshwater organisms by specific products. Mitteilungen Internationale Vereinigung Fur Theoretische und Angewandte Limnologie **13**:21-35.

Rose S. M., F. C. Rose. 1961. Growth-controlling exudates of tadpoles. Symposia of the Society for Experimental Biology **15**:207-218.

Rot-Nikcevic I., R. J. Denver, and R. J. Wassersug. 2005. The influence of visual and tactile stimulation on growth and metamorphosis in anuran larvae. Functional Ecology **19**:1008-1016.

Runkova G. G., Z. L. Stepanova, and L. A. Koval'chuk. 1974. Organ specificity in the action of metabolites of amphibian larvae on their endogenous metabolism under conditions of increased population density. Doklady Akademii Nauk SSSR **217**:328-329.

Schiesari L., E. E. Werner, and G. W. Kling. 2009. Carnivory and resource-based niche differentiation in anuran larvae: implications for food web and experimental ecology. Freshwater Biology **54**:572-586.

Schoener T. W. 1983. Field experiments on interspecific competition. American Naturalist **122**:240-285.

Seale D. B., N. Beckvar. 1980. The comparative ability of anuran larvae (genera, *Hyla*, *Bufo* and *Rana*) to ingest suspended blue-green-algae. Copeia **1980**:495-503.

Seale D. B., R. J. Wassersug. 1979. Suspension feeding dynamics of anuran larvae related to their functional morphology. Oecologia **39**:259-272.

Shvarts S. S., O. A. Pyastolova. 1970. Regulators of growth and development of amphibian larvae. II. Diversity of effects. Soviet Journal of Ecology **1**:122-134.

Smith M. A. 2002. *Pseudacris triseriata triseriata*: reproduction. Herpetological Review **33**:125.

Smith D. C. 1987. Adult recruitment in chorus frogs: effects of size and date at metamorphosis. Ecology **68**:344-350.

Sours G. N., J. W. Petranka. 2007. Intraguild predation and competition mediate stage-structured interactions between wood frog (*Rana sylvatica*) and upland chorus frog (*Pseudacris feriarum*) larvae. Copeia **2007**:131-139.

Steinwascher K. 1979. Competitive interactions among tadpoles - responses to resource level. Ecology **60**:1172-1183.

Stepanova Z. L. 1974. The chemical nature of the products of metabolism of amphibian larvae, excreted into the water. Soviet Journal of Ecology (Ekologiya) **5**:148-149.

Waldman B., P. J. Bishop. 2004. Chemical communication in an archaic anuran amphibian. Behavioral Ecology **15**:88-93.

Wassersug R. 1972. Mechanism of ultraplanktonic entrapment in anuran larvae. Journal of Morphology **137**:279-288.

Wassersug R. J., M. Yamashita. 2001. Plasticity and constraints on feeding kinematics in anuran larvae. Comparative Biochemistry and Physiology A-Molecular and Integrative Physiology **131**:183-195.

Werner E. E., K. L. Yurewicz, D. K. Skelly, and R. A. Relyea. 2007. Turnover in an amphibian metacommunity: the role of local and regional factors. Oikos **116**:1713-1725.

West L. B. 1960. The nature of growth inhibitory material from crowded *Rana pipiens* tadpoles. Physiological Zoology **33**:232-239.

Wetzel R. G. 2006. Wetland ecosystem processes. Pages 285-312 *In* D. P. Batzer and R. R. Sharitz, editors. Ecology of freshwater and estuarine wetlands, University of California Press, Berkeley, California.

Whiting A.V. Population ecology of the western chorus frog, *Pseudacris triseriata*. Montreal, Quebec: McGill University; 2004.

Wong A. L. C., T. J. C. Beebee, and R. A. Griffiths. 1994. Factors affecting the distribution and abundance of an unpigmented heterotrophic alga *Prototheca richardsi* . Freshwater Biology **32**:33-38.

Appendix 1: Ponds used

Appendix Table 1-1: List of ponds used during study within the different landscape types, giving UTM coordinates, area of pond in early spring and at time of tadpole density sampling, hydroperiod as measured from first egg deposition in that year. Missing values indicate that ponds were not measured in that given year.

Pond	UTM (zone 12)		Spring Area (at density sampling) (m^2)			Hydroperiod (days)		
	Easting	Northing	2005	2006	2007	2005	2006	2007
Protected park ponds								
N16	380619	5952276	682 (310)	682 (112)	682 (109)	95	74	91
N17	380541	5952129	2166 (1434)	-	-	138	120	-
N28	378008	5940646	2378 (1186)	-	-	138	-	-
N233	377181	5942295	523 (492)	454 (187)	454 (187)	95	83	83
N233-S			975 (302)	-	-	138	-	-
N235	377386	5941654	(174)	-	-	95	-	-
N238	378302	5939599	456 (444)	456 (67)	456 (67)	137	74	90
N301	376797	5942087	635 (559)	635 (384)	635 (384)	138	83	102
N302	377064	5941954	497 (230)	497 (194)	497 (194)	138	89	102
N303	377143	5941976	1769 (1068)	-	-	138	-	-
N325	376113	5952106	1426 (832)	-	-	138	87	-
N332	377655	5953548	442 (342)	530 (264)	530 (264)	112	87	81
N4	377219	5943152	494 (420)	-	-	138	-	-
N6	377521	5941298	-	-	55	77	55	66
N25	377720	5941561	920	-	-	99	77	-

AGRICULTURE PONDS	UTM (zone 12)		Upland land-use	Spring Area (at density sampling) (m^2)		Hydroperiod (days)	
	Easting	Northing		2006	2007	2006	2007
Pasture ponds							
P257	374503	5953998	Cattle	295	295	87	87
P279	381048	5953950	Cattle	1604 (598)	1604 (598)	95	102
P406	372290	5952304	Cattle	806 (371)	806 (361)	83	90
P7	374537	5954954	Sheep	2303	-	107	-
Crop ponds							
C12	374499	5953863	Hay	2479	2479	107	102
C295	367737	5944616	Hay		-	83	-
C13			Hay	-	467	-	87
C17	387423	5953148	Row crop	424 (381)	424 (381)	107	102
C25	374493	5953364	Row crop	609 (247)	609 (247)	93	88
C18	387380	5951375	Row crop	458	-	107	-

Appendix 2: List of invertebrates

Appendix Table 2-1: List of invertebrate species found at study ponds within ponds in the Beaver Hills region of Alberta. Stage of animals was adult unless otherwise noted: larvae (L), adult (A).

Invertebrate class	Taxonomic group	Common name or as referred to in text
Zooplankton and other invertebrates	**Insecta**	
	Diptera	
	Chaoboridae (L)	Chaoborus – phantom midge
	Chironomidae (L)	Chironomids
	Culicidae (L)	Mosquito larvae
	Tipulidae (L)	Crane fly larvae
	Hemiptera	
	Corixidae (A)	Boatmen
	Notonectidae (A)	Backswimmer *(tadpole predator)*
	Gerridae (A)	Water strider
	Tricoptera	Caddisfly larvae
	Crustacea	
	Amphipoda	
	Hyalella azteca	Scud
	Anostraca	Fairy shrimp
	Cladocera	Daphnia
	Copepoda	
	Cyclopoida	Cyclops
	Calanoida	Calanoids
	Ostracoda	Seed shrimp
Predatory Beetles and larvae	Coleoptera	
	Dytiscidae (L, A)	Tigers and diving water beetles
	Acilius sp.	
	Hydaticus sp.	
	Graphoderus sp.	
	Dytiscus sp.	
	Coptotomus sp.	
	Gyrinidae	Whirligig beetle
Predatory Odonate larvae	Odonata	
	Zygoptera (L)	Damselfly larvae
	Anisoptera (L)	Dragonfly larvae
Snails	Gastropoda	
	Physidae	
	Physa sp.	Common snail
	Planorbidae	Small red snail
Clams	Pelecypoda	
	Sphaeriidae	Fingernail clam
Leeches	Hirudinea	Leech
Spiders	Arachnida	
	Hydracarina	Water mites
	Araneae	
	Pisauridae	
	Dolomedes triton	Nursery-web spider

Appendix Table 2-2: List of plant species found at study ponds in the Beaver Hills region of Alberta.

Vegetation type	Species name	Common name or as referred to in text
Aquatic vegetation		
	Aulacomnium palustre	Ribbed bog moss
	Ceratophyllum demersum	Rigid hornwort
	Hippuris vulgaris	Common Mare's tail
	Juncus spp.	Rushes
	Lemna minor	Common duckweed
	Polygonum sp.	Polygonum
	Potamogeton sp.	Pondweed
	Potentilla palustris	Marsh cinquefoil
	Ranunculus aquatilis	Water crowfoot
	Utricularia vulgaris	Common bladderwort
	Sagittaria cuneata	Arumleaf arrowhead
	Sphagnum angustifolium	Peat moss
Emergent vegetation		
Sedges	Cyperaceae	
	Carex aquatilis	Water sedge
	Carex atherodes	Wheat sedge
	Carex bebbii	Bebb's sedge
	Carex diandra	Lesser panicled sedge
	Carex lasiocarpa	Slender sedge
	Carex utriculata	Northwest territory sedge
	Eleocharis acicularis	Needle spikerush
	Eleocharis palustris	Common spikerush
	Scirpus hudsonianus	
	Scirpus lacustris	Bullrush
Grasses	Poaceae	
	Agrostideae	
	Alopecurus aequalis	Short-awned foxtail
	Calamagrostis canadensis	Bluejoint reedgrass
	Calamagrostis hyperborea	Northern reedgrass
	Deschampia sp.	Hairgrass
	Phleum pratense	Timothy
	Muhlenbergia	
	Aveneae	
	Koeleria cristata	June grass
	Chloridae (chloris)	
	Beckmannia syzigachne	Slough grass
	Hordeae	
	Hordeum jubatum	Foxtail barley
	Festuceae (fescue)	
	Poa pratensis	Kentucky bluegrass
	Poa palustris	Fowl bluegrass
	Glyceria grandis	Tall manna grass
	Bromus kalmii	Prairie brome
	Festuca pratensis	Meadow fescue
	Phragmites australis	Common reed
	Phalarideae (canary grass)	
	Phalaris arundinacea	Reed canary grass
Cattails	*Typha latifolia*	Cattail
Horsetails	*Equisetum* spp.	
Shrubs and Trees	*Alnus rugosa*	Alder
	Amelanchier alnifolia	Saskatoon
	Betula papyrifera	Paper birch
	Cornus stolonifera	Red-osier dogwood
	Corylus cornuta	Beaked hazelnut
	Lonicera dioica	Honeysuckle
	Picea glauca	White spruce
	Populus balsamifera	Balsam poplar
	Populus tremuloides	Trembling aspen
	Ribes glandulosum	White currant
	Rosa acicularis	Prickly wild rose
	Rubus idaeus	European raspberry
	Viburnum edule	Cranberry

Appendix Table 2-2: continued

Vegetation type	Species name	Common name or as referred to in text
Emergent vegetation		
Herbaceous vegetation	*Achillea millefolium*	Yarrow
	Agastache foeniculum	Blue giant hyssop
	Anemone Canadensis	Agrimony
	Artemisia frigida	Sage
	Aster borealis	Wild sarsaparilla
	Aster conspicuous	Bog aster
	Aster puniceus	
	Cicuta bulbifera	Hemlock
	Cirsium arvense	Canada thistle
	Elymus trachycaulus	Slender wheatgrass
	Epilobium angustifolium	Fireweed
	Geum aleppicum	Yellow avens
	Heracleum maximum	Cow parsnip
	Medicago sativa	Alfalfa
	Mentha arvensis	Wild mint
	Potentilla gracilis	Graceful cinquefoil
	Potentilla norvegica	Rough cinquefoil
	Ranunculus macounii	Macoun's buttercup
	Rumex crispus	Curled dock
	Rumex occidentalis	Western dock
	Scutellaria galericulata	Marsh skullcap
	Senecio congestus	Marsh fleabane
	Sium suave	Water parsnip
	Smilacina stellata	False Solomon's Seal
	Solidago canadensis	Goldenrod
	Sonchus arvensis	Corn Sow Thistle
	Sonchus oleraceus	Common sow thistle
	Trifolium hybridum	Clover
	Urtica dioica	Common nettle

Appendix 3: Trends in $\delta^{13}C$ and $\delta^{15}N$ for tadpoles through development

Serial collections of tadpoles were made at six ponds in Elk Island National Park during spring and summer 2005 for the purpose of analyzing the stable isotopes of carbon and nitrogen from tadpole tissues. The goal was to determine if tadpole diets changed with ontogeny, as measured by $\delta^{15}N$ and $\delta^{13}C$, and how ratios compared between the boreal chorus frog and wood frog. Tadpoles were collected weekly after eggs had hatched (as early as May 3), and at some ponds collections continued with tadpoles or metamorphs until July 27. Between one and 11 tadpoles were analyzed for stable isotopes on a given date per each species. Below I present the graphical results from several ponds to show the temporal trends in $\delta^{13}C$ and $\delta^{15}N$. The trend for the trophic level of tadpoles is identical to that of $\delta^{15}N$, and so I show the snail value to indicate the level of primary herbivores (which is used to calculate the trophic level as in Chapter 2).

Temporal trends were analyzed first by pond and then across ponds. First I compared serial data within each pond comparing mean tadpole species' value for each date across dates using a paired t-test. I also used a repeated measure ANOVA using five of the ponds for which I had at least six repeated measures. Ponds acted like subjects with species as the main fixed effect.

In the separate paired t-tests, four of the six ponds showed significant separation of $\delta^{13}C$ between species (mean difference 0.59‰, range –0.18 to 1.28‰, Appendix Table 3-1), while only three ponds displayed significant separation in tissue $\delta^{15}N$, or tadpole trophic level (mean difference 0.12‰, range –1.23 to 0.93‰, Appendix Table 3-1). Comparing species as subjects across ponds using the repeated measures resulted in no significant difference in either carbon or nitrogen (Appendix Table 3-1). There was a significant effect of date for either $\delta^{13}C$ and $\delta^{15}N$, due to depletion of both $\delta^{13}C$ and $\delta^{15}N$ through the summer, however, after metamorphosis both isotopes became more enriched (Appendix Figure 3-1). For $\delta^{13}C$ there was a significant date×species effect owing to a greater depletion over time for chorus frog tadpoles as confirmed by a significant date×species effect in the linear polynomial test ($F_{1,8} = 5.34$, $p = 0.06$). Chorus frog $\delta^{13}C$ was initially much

more enriched than that of wood frogs, but by July 6 chorus frog $\delta^{13}C$ had decreased much more than those of wood frogs across ponds such that the direction of difference had changed.

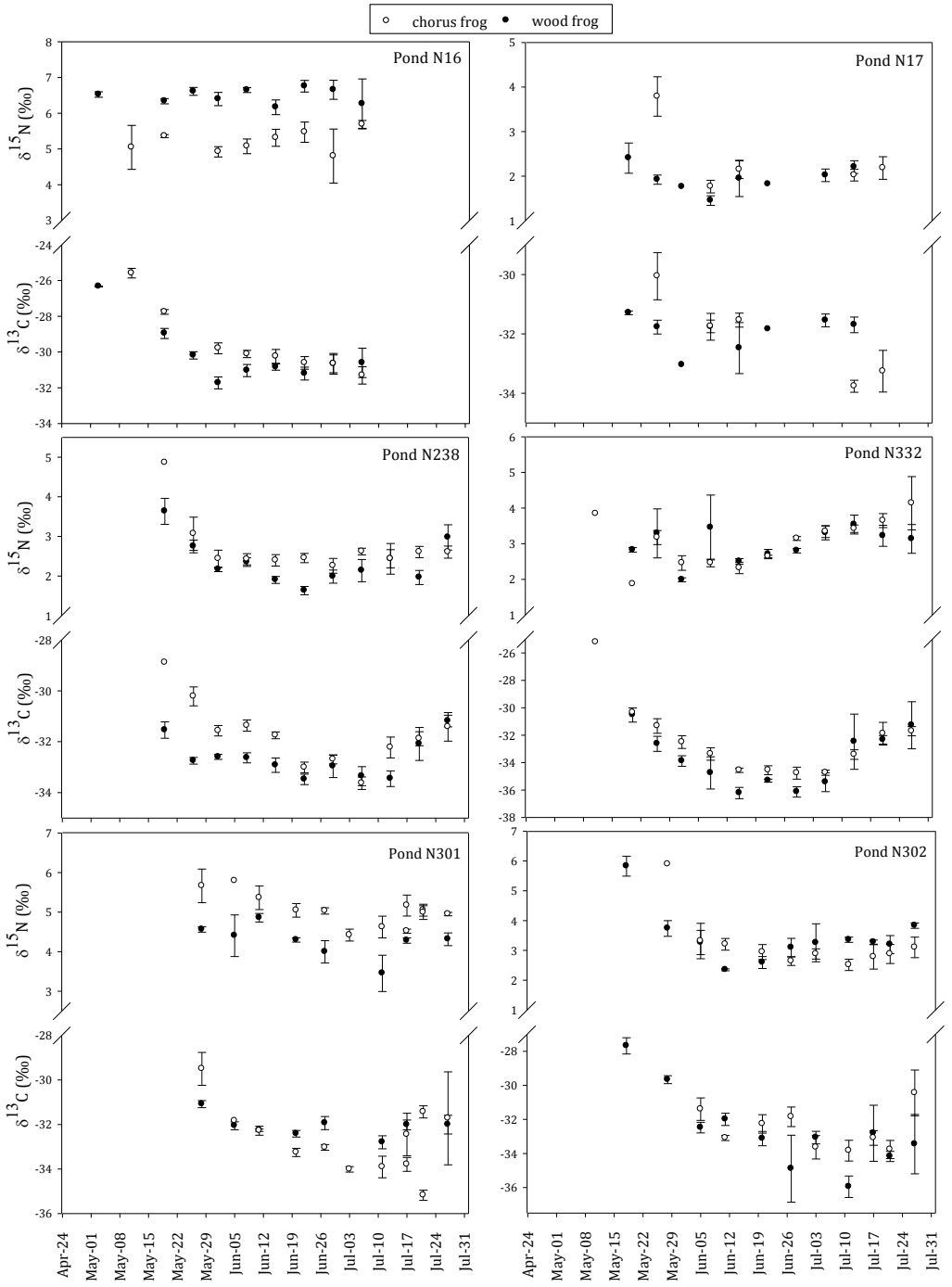
The results of the serial collections support conclusions in Chapter 2 that tadpole diets were likely similar, and the two species appear to follow the same temporal signature. Maternal investment in eggs was found such that eggs found were similar to adult male sand females collected during breeding (enriched in $\delta^{13}C$ and $\delta^{15}N$ compared to tadpoles, for example points on May 11 in pond N16 (n=2) and N332 (n=1) are from adult chorus frogs). Tadpole stable isotopes then become depleted through consumption of sources with a depleted carbon signal (e.g. methanotrophic bacteria and fungal hyphae in pond sediments which are strongly depleted in $\delta^{13}C$ – Grey et al. 2004). Consumption of terrestrial material (likely invertebrates) after metamorphosis is inferred due to enrichment in $\delta^{13}C$ and an increase in trophic level ($\delta^{15}N$). Enrichment in both $\delta^{13}C$ and $\delta^{15}N$ with metamorphosis may also result from metabolizing tail tissue which should result in enrichment of both isotopes.

The U-shaped temporal trends for $\delta^{13}C$ in both species differ from those reported for two other North American ranids (Schiesari et al. 2009), where carbon was more depleted at smaller sizes and was more enriched in larger tadpoles. Reasons for the difference may reflect tadpole diets, or seasonal change in abundance of resources; the two ranids in Schiesari et al. were summer breeding species. However, from the description of research samples in Schiesari represent those collected from different sized tadpoles on a given date and not throughout the season as in my study. Carbon values reported by Schiesari are similar to those in my study (less than -30‰), as was the relative trophic position of wood frogs; wood frogs fed at the same level of known aquatic predators (salamanders in Schiesari, anisopteran larvae and dytiscids in my survey ponds).

Appendix Table 3-1: Results of comparisons between boreal chorus frog (CF) and wood frog (WF) $\delta^{13}C$ and $\delta^{15}N$ for samples taken throughout tadpole development (n = 1-11 tadpoles per species per date) within ponds between May 3 and July 27, 2005 from six natural ponds in Elk Island National Park, Alberta. Results of repeated measures ANOVA for effect of species (chorus frog versus wood frog) treating ponds (n = 5) as subjects and mean species' δ as replicated from May 26 to July 6.

	$\delta^{13}C$				$\delta^{15}N$			
Pond	Difference (CF – WF)	df	Paired *t*	*p*	Difference (CF – WF)	df	Paired *t*	*p*
N16	0.65	6	2.04	0.09	-1.23	6	-7.24	< 0.001
N17	0.15	3	0.19	0.86	0.55	3	1.22	0.31
N238	0.94	10	3.15	0.01	0.39	10	2.98	0.01
N301	-0.18	7	-0.55	0.60	0.93	7	8.94	< 0.001
N302	1.28	8	2.54	0.04	0.08	8	0.24	0.81
N332	0.71	10	2.82	0.02	-0.01	10	-0.08	0.94
Average	0.59				-0.12			

Repeated Measures ANOVA								
Between subjects	MSE	df	*F*	p	MSE	df	*F*	p
Species	7.34	1	0.32	0.59	0.05	1	0.01	0.92
Error	22.95	8			4.55	8	4.55	
Within subjects								
Date	11.57	5	19.87	<0.001	0.79	5	3.13	0.02
Date×Species	1.52	5	2.60	0.04	0.12	5	0.48	0.79
Error	0.58	40			0.25	40		



Appendix Figure 3-1: Temporal trends in mean individual whole tadpole and metamorph tissue $\delta^{13}C$ and $\delta^{15}N$ for both chorus frog and wood frog tadpoles from six ponds in Elk Island National Park in 2005. Note in N16 and N332 chorus frog samples on May 11 represent values from breeding adults. Species means ± SE represent between 1 and 11 tadpoles on a given date. The date when samples represent only metamophs with tail partly to completely resorbed in each pond was: N16 - July 6; N17 - July 13; N238 – July 20; N332 – July 13; N301 – July 21; N302 – July 21. Note pond pairs N16 and N17, N301 and N302 were within 300 m of each other and are shown next to eachother.

Literature Cited

Grey, J., A. Kelly, S. Ward, N. Sommerwerk, and R. I. Jones. 2004. Seasonal changes in stable isotope values of lake-dwelling chironomid larvae in relation to feeding and life cycle variability. Freshwater Biology **49**: 381-389.

Schiesari L., E. E. Werner, and G. W. Kling. 2009. Carnivory and resource-based niche differentiation in anuran larvae: implications for food web and experimental ecology. Freshwater Biology **54**:572-586.

Appendix 4: Data used for correlations

Appendix Table 4-1: Summary of tadpole metrics and environmental parameters recorded in a) 2005, b) 2006, and c) 2007. Abbreviations used are META = mean size at metamorphosis, CF LP = chorus frog larval period, CF GR = CF growth rate, WF GR = wood frog growth rate, MDT = mean daily average temperature, HYDRO = hydroperiod, TN = total nitrogen, TP = total phosphorous, NH_4 = ammonium.

a) 2005

Pond	META	CF LP	CF GR	WF GR	Tadpole density (# tadpole/L)			MDT	HYDRO	Depth	TN	TP	NH_4
	mm	days	mm/d	mm/d	WF	CF	Total	°C	days	cm	µg/L	µg/L	µg/L
N4	-	-	-	-	0.007	0.024	0.031	13.3	138	25	-	-	-
N6	-	68	-	n/a	0.000	-	-	-	-	24	-	-	-
N16	14.72	61	0.277	0.429	0.038	0.261	0.298	15.0	95	39	-	-	-
N17	13.78	68	0.273	0.307	0.013	0.041	0.054	15.1	138	25	-	-	-
N25	-	73	-	-	0.009	0.286	0.294	16.4	99	20	-	-	-
N28	14.91	-	-	-	0.012	0.067	0.079	-	138	43	-	-	-
N233	15.76	68	0.390	n/a	0.000	0.150	0.150	14.4	95	30	-	-	-
N233-s	15.67	-	-	-	0.001	0.060	0.061	13.4	138	33.5	-	-	-
N235	-	62	-	-	0.003	0.086	0.090	-	95	32	-	-	-
N238	12.69	89	0.357	0.397	0.005	0.056	0.062	13.8	137	28	-	-	-
N301	11.52	93	0.167	0.456	0.008	0.069	0.077	14.1	138	28	-	-	-
N302	12.89	79	0.228	0.368	0.005	0.033	0.033	12.4	138	20	-	-	-
N303	-	-	-	-	0.007	0.070	0.077	14.2	138	29	-	-	-
N325	-	-	-	-	0.014	0.021	0.035	15.3	138	47	-	-	-
N332	14.26	68	0.303	0.353	0.011	0.074	0.085	17.4	112	23	-	-	-

b) 2006

Pond	META	CF LP	CF GR	WF GR	Tadpole density (# tadpole/L)			MDT	HYDRO	Depth	TN	TP	NH_4
	mm	days	mm/d	mm/d	WF	CF	Total	°C	days	cm	µg/L	µg/L	µg/L
N16	12.40	63	0.321	0.349	0.110	0.012	0.122	16.5	74	23	15000	1291	78.9
N233	13.00	69	0.319	0.345	0.000	0.036	0.036	15.9	83	30.5	2730	836	42.7
N238	-	-	0.252	0.280	-	-	-	15.5	74	8	2620	1194	21.4
N301	-	-	0.210	0.210	0.033	0.008	0.042	12.9	83	30.5	3350	1161	11.4
N302	-	-	0.314	0.311	0.081	0.014	0.095	15.3	89	23	1410	275	12.3
N332	-	-	0.304	0.276	0.007	0.013	0.020	15.8	87	19	3520	125	26.7
P7	-	-	0.210	0.288	0.011	0.001	0.012	16.6	107	37	4100	1194	57.6
P257	13.00	76	0.245	0.400	-	-	-	-	87	37.3	2070	345	13.4
P279	12.75	76	0.247	0.433	0.033	0.035	0.068	19.3	95	60	2630	257	187
P406	11.32	67	0.231	0.320	0.187	0.198	0.385	16.7	83	23	4110	2746	38.7
C12	-	76	0.238	0.503	-	-	-	-	107	-	7330	3527	473
C17	-	-	0.302	0.419	0.028	0.004	0.064	16.5	107	63	1720	172	34.2
C18	n/a	n/a	n/a	0.370	0.018	0.000	0.018	15.5	107	54.5	1620	100	21.5
C25	-	69	0.411	0.513	0.004	0.005	0.009	16.4	93	26	5500	4975	293

Appendix Table 4-1: continued

c) 2007

Pond	META	CF LP	CF GR	WF GR	Tadpole density (# tadpole/L)			MDT	HYDRO	Depth	TN	TP	NH_4
	mm	days	mm/d	mm/d	WF	CF	Total	°C	days	cm	µg/L	µg/L	µg/L
N16	13.83	57	0.338	0.508	0.110	0.036	0.146	16.8	91	35	7250	2890	349
N233	14.19	64	0.327	0.468	0.113	0.116	0.230	14.6	83	32	2550	685	35
N238	11.57	64	0.218	0.371	0.152	0.053	0.205	16.3	90	37	2400	1195	30
N301	13.96	78	0.253	0.464	0.135	0.066	0.201	13.4	102	43	2640	655	20
N302	-	78	-	0.411	0.161	0.014	0.175	13.4	102	32	1760	550	27
N332	12.36	64	0.213	0.336	0.127	0.309	0.435	17.3	81	22	4170	1080	34
N6	-	-	-	0.655	0.064	0.184	0.247	-	66	9	6920	9855	-
P257	13.07	58	0.327	0.531	0.183	0.090	0.273	16.5	87	35	2130	306	54
P279	-	58	0.296	0.553	0.022	0.052	0.074	19.8	102	37	2210	350	64
P406	12.45	51	0.139	0.186	0.308	0.508	0.816	17.2	90	31	3770	2055	65
C12	14.17	58	0.236	0.364	-	-	-	-	102	-	2790	1635	216
C13	12.67	58	0.383	0.282	0.020	0.060	0.080	16.7	87	22	8390	8720	106.5
C17	-	72	0.312	0.529	0.157	0.026	0.182	17.6	102	37	2920	267	67
C25	12.95	58	0.295	0.336	0.049	0.026	0.075	16.7	88	29	3660	2865	55

Appendix Table 4-2: Correlations between landscape variables from 15 ponds in the Beaver Hills region of Alberta. Variable names and descriptions are given in Table 4-1.

	Road	Dist. Forest	Dist. Water	Forested	Non-forested	Crop	Pasture	Constructed
Water	−0.10	−0.30	0.14	0.72**	0.62**	−0.88***	−0.68**	−0.74**
Road		0.12	0.19	−0.14	0.24	0.05	−0.25	0.00
Dist. Forest			0.04	−0.08	−0.31	0.19	0.37	0.58*
Dist. Water				−0.15	0.45†	−0.06	−0.31	−0.11
Forested					0.34	−0.90***	−0.29	−0.30
Non–forested						−0.67**	−0.66**	−0.66**
Crop							0.47†	0.56*
Pasture								0.76***

*** $p \leq 0.001$, ** $p \leq 0.01$, * $p \leq 0.05$, † $p \leq 0.10$

Appendix Table 4-3: Correlations between abiotic variables measured at ponds in the Beaver Hills region of Alberta. The number of ponds in the 2005 matrix was 14, in 2006 was 14, and in 2007 was 13. Variable names and descriptions are given in Table 4-1.

2005	Temperature	Depth	Area
Hydroperiod	−0.54†	0.07	0.46†
Temperature		0.02	0.14
Depth			0.38

2006	NIONS	Hydroperiod	Temperature	TP	NH_4	Depth	Area
TN	−0.37	−0.26	0.04	0.73**	0.55*	−0.51†	0.52†
NIONS		0.10	0.28	0.02	0.30	0.34	0.14
Hydroperiod			0.20	−0.24	0.34	0.71**	0.37
Temperature				−0.10	0.18	0.41	0.36
TP					0.53*	−0.50†	0.38
NH_4						0.01	0.72**
Depth							0.11

2007	NIONS	Hydroperiod	Temperature	TP	NH_4	Depth	Area	pH
TN	−0.30	−0.42	0.25	0.81***	0.59*	−0.53†	−0.01	0.38
NIONS		0.29	−0.26	−0.13	0.30	0.45	0.30	−0.75**
Hydroperiod			−0.08	−0.40	0.05	0.59*	0.49†	−0.41
Temperature				0.02	0.43	−0.18	0.31	0.22
TP					0.46	−0.59*	0.15	0.28
NH4						−0.19	0.44	−0.12
Depth							0.04	−0.59*
Area								−0.02

*** $p \leq 0.001$, ** $p \leq 0.01$, * $p \leq 0.05$, † $p \leq 0.10$

Appendix Table 4-4: Correlations between biotic variables measured at ponds in the Beaver Hills region of Alberta. The number of ponds in the 2006 matrix was 14, and in 2007 was 13. Variable names and descriptions are given in Table 4-1.

2006	Dytiscidae	Anisoptera	Zygoptera	Emergent veg.	*Physa* sp.	*Chl a*
Cattails	0.23	−0.56*	0.23	−0.33	−0.50†	−0.42
Dytiscidae		0.15	0.32	0.31	−0.11	0.35
Anisoptera			−0.35	0.69**	0.35	0.41
Zygoptera				−0.14	0.10	−0.20
Emergent vegetation					0.63*	0.40
Physa						0.05

2007	Dytiscidae	Anisoptera	Zygoptera	Emergent veg.
Cattails	−0.45	0.07	0.46	−0.43
Dytiscidae		0.07	−0.49†	0.55*
Anisoptera			0.05	−0.12
Zygoptera				−0.39

*** $p \leq 0.001$, ** $p \leq 0.01$, * $p \leq 0.05$, † $p \leq 0.10$

Appendix 5: Effect of timing on tadpole density measures

Rationale for conducting analyses using pond-years

Total tadpole densities at all locations surveyed in both years were higher in 2007 than in 2006 (paired $t_8 = 3.246$, $p = 0.012$ – Wilcoxon signed rank test $Z = 2.67$, $p = 0.008$). Tadpole densities in 2007 samples were on average three times higher than in 2006 (0.259 tadpole/L versus 0.090 tadpole/L respectively). Sampling took place 15-21 days later in 2006 (days from wood frog hatching date). The differences in density could be produced given a daily survival probability of 0.932-0.951 (a daily survival probability of 0.951 over a 60 day period produces the typical survival estimate of 5% for tadpoles survival to metamorphosis). I believe tadpole densities were similar between years in park ponds based on egg mass counts, given wood frog egg production was slightly higher in 2006 than in 2007 (60.6 ± 14.5 versus 49.0 ± 10.8 egg masses ± SE in 2006 and 2007 respectively; the Wilcoxon test showed that 4 out of 5 ponds had more eggs in 2006 than 2007, $Z = 1.826$, $p = 0.068$). Chorus frog egg masses could not be counted accurately due to difficulty in locating masses and insuring all masses would be counted but also because of the prolonged breeding periods of chorus frogs (> 30 days). Despite possible similarities in tadpole densities, correcting for date of sampling, I chose to analyze each year separately.