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Distribution Characteristics of the Eggs, Tadpoles, and Metamorphs of the Northern Leopard Frog (*Rana pipiens*) and their Relation to Conservation Strategies

by

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Abstract

Anuran population persistence often hinges on successful metamorph dispersal, but information on this life-stage and its movement characteristics is scarce. Two years of monitoring the emigration patterns of metamorph Northern Leopard Frogs (*Rana pipiens*), a “Threatened” species in Alberta, revealed that they orient non-randomly upon emigration from their breeding pond. Emigration direction was similar between years and the short-term capture and handling of metamorphs does not appear to disrupt their natural emigration behaviour. Metamorph emigration patterns were not related to the non-random distributions of earlier life-stages (egg clusters and tadpoles), suggesting that metamorph *R. pipiens* orient on land using cues other than those detected during their larval period. Natural metamorph dispersal was compared to that of a reintroduced population and results suggest that reintroduced animals do not exhibit natural behaviour. My findings provide insight into the behaviour of metamorph anurans and have implications for *R. pipiens* conservation in Alberta.

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Table of Contents

List of Tables

List of Figures

CHAPTER 1: GENERAL INTRODUCTION

The vertebrate taxon Amphibia comprises over 6,000 species (Roelants et al. 2007). It consists of three Orders – the frogs and toads (Anura, comprising 87% of all amphibians), salamanders and newts (Urodela; 9%), and caecilians (Gymnophiona, 4%; Duellman and Trueb 1994). Amphibians are morphologically, behaviourally and ecologically diverse (Blaustein and Wake 1995) and have long been model organisms for examining the relationships of competition and predation on survival (e.g. Jaeger 1971; Keen 1982; Laurila 2000), population and community structure (e.g. Beard et al. 2003), and the evolution of life history strategies (e.g. Uller et al. 2006). In the last two decades, the interest in amphibian biology has surged because of the recognition that species across this Class have disappeared in many areas of the world (Mendelson et al. 2006).

The World Conservation Union (IUCN) Global Amphibian Assessment (GAA) recently determined that 32% of amphibians are threatened with extinction (Stuart et al. 2004). By comparison, only 12% of birds and 22% of mammals are threatened (IUCN 2007). The current extinction rate that is at least 105 times that of background amphibian extinction rates from the fossil record reveals that current amphibian declines are most likely not a natural phenomenon (McCallum 2007). Although the primary factor driving amphibian declines is habitat loss and fragmentation (e.g. clearcutting, draining of wetlands; Alford and Richards 1999; Belden 2006), recent evidence suggests many other factors, such as disease and pathogens (Pounds et al. 2006), global climate change (Blaustein et al. 1998; Piha et al. 2007), invasive species (Collins and Storfer 2003), chemical contamination (Boone et al. 2007), and overexploitation (Beebee and Griffiths

2005), are also threatening amphibian populations. In many instances these factors work synergistically, whereby subtle effects of any single factor are exacerbated by their interaction with one or more other factors (Blaustein and Kiesecker 2002). For example, global warming is accelerating the prevalence of infectious disease outbreaks, which in turn seem to be causing widespread declines and several extinctions (Pounds et al. 2006). Amphibian losses are deserving of attention not only because they manifest themselves as a global phenomenon, but also because amphibians may serve as indicators of the overall state of environmental conditions (Blaustein and Wake 1995).

Several features of amphibians make them particularly vulnerable to changes in their environment. First, they have a moist, permeable skin that is thin enough to permit gas exchange. This permeability also allows absorption of toxic chemicals from the environment (Pough et al. 2001). Secondly, the most common expression of the life cycle of amphibians incorporates three distinct stages: jelly-covered eggs are laid in an aquatic habitat, which then hatch into larvae that subsequently metamorphose into the more terrestrial adult form. Because of this bi-phasic life-style, they are exposed to both aquatic and terrestrial stressors (Blaustein and Kiesecker 2002). For example, in aquatic habitats the unshelled eggs of amphibians are often directly exposed to soil, water and sunlight (Blaustein and Kiesecker 2002), and are also potentially exposed to increased UV-B radiation (especially when water levels fall), which can lower hatching success (Blaustein et al. 1995). Toxins, such as herbicides and industrial chemicals permeating both land and water, can be harmful to amphibians either directly by causing mortality (Alford and Richards 1999), or indirectly by impairing their reproduction (Blaustein et al. 1995). Because of their sensitivity to environmental perturbation, amphibians have been advocated as excellent 'biological indicators' (e.g. Collins and Storfer 2003). This assertion, however, has not been well documented (Beebee and Griffiths 2005).

Because amphibians are critical components of many ecosystems, amphibian declines are likely to have considerable impacts on the potential success of other organisms (Ranvestel et al. 2004). Amphibians account for a considerable portion of energy flow in many ecosystems (Regester et al. 2006). For example, Burton and Likens (1975) estimated that in New Hampshire, salamander biomass is equal to that of small mammals and more than twice that of birds at certain times of the year. Aquatic breeding amphibians import energy from terrestrial to aquatic habitats through egg deposition (Whiles et al. 2006). Their larvae have considerable impacts on nutrient cycling and primary production in aquatic habitats (Seale 1980; Flecker et al. 1999; Pryor 2003; Ranvestel et al. 2004) and some larvae have even been considered to be keystone species (e.g. *Ambystoma tigrinum*; Wissinger et al. 1999). After metamorphosis, amphibians export energy back to the terrestrial environment as they leave the aquatic habitat (Whiles et al. 2006). Also, as ectotherms, amphibians derive nearly all of the energy they use for thermoregulation from an external source (e.g. solar radiation), thus efficiently transferring energy to other trophic levels (Pough 1980). Amphibians are important prey taxa in some ecosystems. For example, the occurrence of western terrestrial garter snakes (*Thamnophis elegans*) in the Sierra Nevada is strongly associated with the presence of amphibians (Jennings et al. 1992). Without doubt, declines will have cascading effects throughout aquatic and terrestrial food-webs (Ranvestel et al. 2004).

At present, there is no unifying strategy for conserving amphibians (Semlitsch and Rothermel 2003), but protecting and restoring their habitat to allow for natural recolonization seems to be the most effective method (Calhoun and Hunter 2003). However, habitat protection has focused primarily on protecting wetlands > 4 ha in size (Kaiser 1998), yet smaller, isolated wetlands also act as crucial breeding sites (Semlitsch et al. 1996). Also, many amphibians require access to multiple aquatic and terrestrial habitats, but terrestrial habitat requirements away from breeding sites are poorly understood (Marsh and Trenham 2001) rendering the identification and protection of critical terrestrial habitat challenging. In addition, many amphibian populations exist as metapopulations (many connected local populations that each has a substantial probability of extinction; Levins 1970). Therefore, if dispersal routes between local populations are disturbed or destroyed (e.g. by road construction), remaining populations can become increasingly isolated from each other, which increases their extinction probability (Marsh and Trenham 2001). Lastly, some amphibians are declining even in protected, natural areas (Collins and Storfer 2003). Clearly, protecting habitat alone may not be sufficient to conserve amphibians (Gibbons 2005) and more manipulative conservation options in the form of translocations or reintroductions are sometimes used, and are often necessary as local extirpation occurs.

Conservation translocations aim to establish new populations of animals in order to enhance their local, regional or national status (Edgar et al. 2005). Reintroductions are a form of translocation and are defined as the attempt to re-establish a species in an area in which it naturally existed, but from which it has become extirpated (IUCN 2004). Amphibians are often considered to be ideal candidates for reintroduction programs because they have the capacity for rapid build-up of population numbers both in the wild and in captivity, and are considered to have low levels of both parental care and behavioural complexity (Bloxam and Tonge 1995; Marsh and Trenham 2001). As a result, amphibian reintroduction programs are popular (see Table 1.1). However, while some amphibian reintroduction programs have been successful (i.e. second generation adults breeding; Semlitsch 2002), the majority have not. This latter category includes metamorph reintroductions of the Northern Leopard Frog (*Rana pipiens*) in Alberta. Other attempts have had unknown outcomes (Dodd and Seigel 1991; Dodd 2005). Even though releasing early life stages (egg clusters and tadpoles) is recommended for most amphibians (Semlitsch 2002), this technique has not proved successful for some species (e.g. *Bufo houstonensis*; Dodd and Seigel 1991). Despite their popularity as reintroduction subjects, amphibians present distinct challenges because of their complicated ecological, genetic and demographic characteristics. For example, they often exhibit strong site fidelity and have low dispersal rates, which can lead to local adaptations that strongly influence genetic structure (Storfer 1999). The suitability of a species for reintroductions needs to be determined on a case-by-case basis (Dodd 2005) and reintroductions should only be considered when several theoretical considerations have been addressed.

The following criteria should be addressed prior to undertaking reintroduction of any amphibian species: 1) establish the cause of decline; 2) know the population genetics and social structure of the species; 3) understand the potential for disease transmission when moving animals; 4) have a good understanding of the species' life history; and 5) implement a long-term monitoring program to evaluate the reintroduction's success (Dodd and Seigel 1991). Of these five criteria, failed amphibian reintroductions are

Table 1.1. Amphibian reintroduction projects grouped by Order. C = captive population, W = wild population, SC = Semi-captive population, U = unknown, N = not successful, I = initial success has been observed, S = successful.

Species	**Location**	**Life-history stage released**	**Approximate number of animals / year**	**Total number of sites**	**Source of animals**	**Status**	**Reference**
Anura – Frogs / Toads							
Mallorcan Midwife Toad (*Alytes muletensis*)	Spain	Adult	15–195	3	C	U	Buley and Garcia 1997, Dodd 2005
Puerto Rican Crested Toad (*Peltophryne lemur*)	Puerto Rico	Tadpoles	12-15,000	U	C	I	Bloxam and Tonge 1995
						N	Dodd 2005
			90,000 since 1986	3	C	U	Barber 2007
Natterjack Toad (*Bufo calamita*)	Britain	Eggs	5,000–6,000	20	W	I / S	Denton et al. 1997
		Metamorphs	Unknown				
Romer's Frog (*Philautus romeri*)	Hong Kong	Tadpoles	1,600	3 - 8	C	I	Dudgeon and Lau 1999
		Metamorphs	1,200	3 – 8	C		
Wyoming toad (*Bufo baxteri*)	USA	Metamorphs / Adults	9,500 across 3 years	1	C	U	Dreitz 2006
European Tree Frog (*Hyla arborea)*	Latvia	Metamorphs	4,000 across 4 years	1	C	S	Zvirgds 1998
Houston Toad (*Bufo houstonensis*)	USA	Tadpoles / Metamorphs/ Adults	0.5 million across 3 years	10	U	N	Dodd and Seigel 1991

Table 1.1 continued

Wood Frog (*Rana sylvatica*)	USA	Eggs	U	1	U	S	Guttman et al. 1991
Northern Leopard Frog (*Rana pipiens*)	British Columbia, Canada	Metamorphs	14,400 across 4 years	4	C	U	Adama and Beaucher 2007
		Tadpoles	10,000 across 4 years				
Northern Leopard Frog (*Rana pipiens*)	Alberta, Canada	Metamorphs	13,000	3	SC	U	Alberta Northern Leopard Frog Recovery Team 2005
		Eggs	U (8,500 tadpoles in total across 3 years)	1	W	I	Romanchuk and Quinlan 2006
Urodela – salamanders							
Tiger Salamander (*Ambystoma tigrinum*)	USA	Eggs	1,000	1	W	S	Reinert 1991
Spotted Salamander (*Ambystoma maculatum*)	USA	Eggs / Larvae	Unknown	1	Unknown	S	Sexton and Phillips 1986

criticised most often for having a poor understanding of the species life history (Dodd 2005).

In Alberta, *Rana pipiens* populations have undergone significant declines since the mid to late 1970's (Roberts 1992) and different reintroduction strategies have been adopted in order to attempt to re-establish populations in their historical range. The most intensive of these programs has involved reintroducing recently-metamorphosed individuals (metamorphs), but this program has not shown many signs of success. One factor that may have contributed to the apparent low success of this program is that metamorphs represent the least understood life stage in not only *R. pipiens*, but in anuran ecology in general (Rothermel and Semlitsch 2002); yet this was the life-stage used for release. Therefore, ensuring reintroduced animals survive in a new habitat would be challenging without knowing some of their basic ecological characteristics. Despite these knowledge gaps in the life-history of *R. pipiens*, reintroductions remain a conservation priority for this species in Alberta (Alberta Northern Leopard Frog Recovery Team 2005). Clearly, assembling critical life-history data for this species is an urgent requirement. For this reason, the primary goal of this thesis focuses on gathering missing life-history information for *R. pipiens*.

Natural history of the Northern Leopard Frog (*Rana pipiens*)

Of the approximately 250 extant species of true frogs (*Ranidae*) found throughout the world, about one-quarter are found in the Americas, with the largest concentrations in the southern United States and Mexico (Hillis and Wilcox 2005). North American leopard frogs of the *Rana pipiens* species complex are among the most recognizable and widely studied frogs (Hillis 1988). The group is comprised of 27 species, ranging from Canada to Costa Rica, which differ in characteristics such as dorsal patterns and colour, head shape and position of the male vocal sacs (Hillis 1988; Minton 2005). Of these species, the Northern Leopard Frog (*Rana pipiens*, Schreber 1782) is the most widely distributed and the only one found in Canada (represented by *R. p. pipiens*; Hillis 1988). Populations extend into all provinces and the Northwest Territories. Recently, Frost et al. (2006) proposed major taxonomic changes to the genus *Rana*, including reassigning *Rana pipiens* to the genus *Lithobates*. However, because the name *Rana pipiens* has legal status in Canada and adopting the new name could hinder the dissemination of information among biologists (Green 2007), the traditional name is maintained for this project.

General characteristics

Rana pipiens is a semi-terrestrial frog identified by conspicuous dark spots, bordered by light coloured rings scattered on its back and sides (Seburn and Seburn 1998). It has a whitish belly and prominent, light-coloured cutaneous ridges of skin that run the length of the back along each side. The background colour of *R. pipiens* is commonly green, but may be brown or golden. Adults are 50 to 100 mm long, and females are generally larger than males (Dole 1965; Merrell 1977).

Rana pipiens uses a variety of habitats during its life cycle. Breeding is generally restricted to a period between April and June, depending on the temperature (Hine et al. 1981; Corn and Livo 1989). Typical breeding habitats are associated with non-forested areas (DeBenedictis 1974; Werner and Glennemeier 1999; Guerry and Hunter 2002) and consist of temporary or permanent fishless ponds (Merrell 1977; Anholt et al. 2000), beaver ponds (Hine et al. 1981), wet meadows (Gilbert et al. 1994) and the slow-moving water of streams and rivers. Females mate only once per year (Corn and Livo 1989; Gilbert et al. 1994) and tight oval clusters of 500 to 6,500 eggs are deposited (Merrell 1968; Corn and Livo 1989). Only one egg cluster is deposited each year (Corn and Livo 1989). These eggs take 5–20 days to hatch (Hine et al. 1981; Gilbert et al. 1994), at which point herbivorous tadpoles often exploit the opportunity for rapid growth in the breeding habitat. Tadpoles metamorphose 60–90 days after hatching (Merrell 1977). Newly metamorphosed *R. pipiens* disperse from their natal pond into meadows or other wetlands (Dole 1971).

Typical summer habitat for *R. pipiens* consists of grassy areas, meadows, and fields. Although heavily wooded and cultivated areas are generally avoided (Werner and Glennemeier 1999; Guerry and Hunter 2002), forests do not pose a barrier to the movement of *R. pipiens* (Dole 1971). Whereas metamorphs may move more than 5 km from their natal site in their first season (Dole 1971), adults often show affinity for certain spots, moving less than 10 m in a day (Dole 1965).

In the fall, *R. pipiens* moves toward ponds and lakes where it hibernates for the winter, usually by resting on the bottom under rocks or by burying itself in shallow pits in silty substrates (Cunjak 1986). When water temperatures reach 7–10°C (Licht 1991) in the spring, sub-adults and adults emerge and reproductive individuals return to breeding sites. Sexual maturity is reached after 1–3 years (Merrell 1977; Hine et al. 1981).

Distribution and limiting factors

Rana pipiens occurred historically from Newfoundland and southern Québec west, across the Canadian provinces and northern and central portions of the United States, to British Columbia (Minton 2005). Although they presently occur throughout most of their historical range, declines across the western 2/3 of their range began 20–30 years ago (Seburn and Seburn 1998). This species has always had a limited distribution in British Columbia, but currently only one extant population is known to exist (Adama and Beaucher 2007) and this is listed as Endangered by the Committee on the Status of Endangered Wildlife in Canada (COSEWIC; Adama et al. 2004). In Alberta, *R. pipiens* ranged widely south of 55°, but populations have almost completely disappeared from central Alberta and are greatly reduced in southern Alberta (Roberts 1981; Figure 1.1). A remnant population exists in the northeastern corner of the province (Kendell 2002). Range contractions have occurred since the 1970's in both Saskatchewan and Manitoba (Seburn and Seburn 1998). COSEWIC has listed *R. pipiens* as a Species of Special Concern in Alberta, Saskatchewan and Manitoba (Saskatchewan Environment, 2006), and in Alberta it is currently listed as Threatened under Alberta's *Wildlife Act* (Kendell 2003).

The leading hypothesis for causes of the decline of *R. pipiens* is the alteration of habitat (e.g. land-clearing, wetland draining, livestock activity; Kendell 2002). *Rana pipiens* requires three, often spatially disjunct, habitats to complete its life cycle (temporary or permanent ponds for breeding, terrestrial summer foraging habitat, and aquatic overwintering sites that do not freeze solid or become anoxic in the winter). The unavailability of any of these can not only result in the elimination of a population

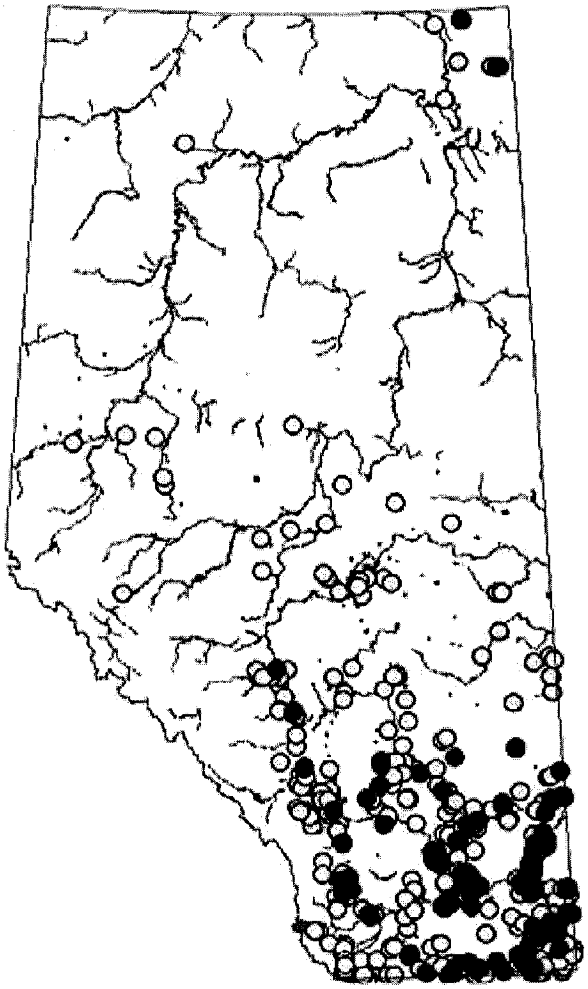


Figure 1.1. Historical distribution (open circles) and distribution since 2000 (dark circles) of *Rana pipiens* in Alberta (adapted from the Alberta Northern Leopard Frog Recovery Team 2005).

(Seburn and Seburn 1998), but can reduce the chances that natural recolonization of formerly occupied habitat will occur (Roberts 1991).

Other factors thought to be implicated in the decline of this species include overharvesting, introduced predators, contaminants and disease. During the early 1970's, *R. pipiens* was commercially harvested (over one million frogs per year in Manitoba) for teaching and research needs, bait and even food (Seburn and Seburn 1998). Across their western range, the introduction and spread of bullfrogs (*Rana catesbeiana*) from eastern to western North America has been suggested as a cause of decline because they prey on *R. pipiens* tadpoles, metamorphs and even adults, but this remains uncertain (Hayes and Jennings 1986). Instead, introduced fish are more likely to be a causal factor through predation on unprotected *R. pipiens* embryos (Hayes and Jennings 1986); however, *R. pipiens* generally breeds in fishless ponds (Merrell 1977). Waterbodies exposed to high levels of herbicides, including the widely used atrazine, have been shown to lead to hermaphroditism in *R. pipiens*, thereby impairing the reproductive abilities of populations (Hayes et al. 2003). Although its effects remain poorly understood (Pearl et al. 2007), the fungal disease, chytridiomycosis (*Batrachochytrium dendrobatidis*) has been implicated as a causal agent in the declines of *R. pipiens* in B.C (Adama and Beaucher 2006). The fungus has recently been detected in three of four *R. pipiens* sites in Alberta (D. Whiteside, personal communication, October 16, 2007), but its effects on population dynamics remain unclear. The most common disease of *R. pipiens* is 'red leg', caused by a bacterium (*Aeromonas hydrophila*), but is not generally considered to be lethal unless individuals are already under stress (Seburn and Seburn 1998). The overall cause(s) of decline remain elusive, but is likely due to a multitude of these factors.

Reintroduction efforts

To help the recovery of *R. pipiens* populations in western Canada, several reintroductions have taken place. In B.C., a captive rearing and reintroduction program began in 2001, but the population there remains extremely vulnerable and recovery is confounded by the presence of *Batrachochytrium dendrobatidis* (Adama and Beaucher 2006). In Alberta, an experimental reintroduction program involved moving adult frogs from the lower Red Deer River drainage to two sites in central Alberta (Roberts 1991; 1992). Although some individuals persisted throughout several winters (Fisher 1999), the overall outcome of these efforts has not been well documented. An experimental reintroduction project occurred near Magrath, Alberta in order to evaluate the effectiveness of on-site egg rearing and tadpole release as a reintroduction method (Romanchuk and Quinlan 2006). This project has shown initial signs of success, as young frogs have overwintered two years in a row (Romanchuk and Quinlan 2006).

The most formal and publicized reintroduction effort for *R. pipiens* in Alberta, and also the one that led to the development of the project that constitutes this thesis, took place between 1999 and 2004. The goal of this program was to re-establish *R. pipiens* into areas of its formerly occupied range in the upper Red Deer River and North Saskatchewan River basins (Kendell 2002). During each year of the program, egg clusters were collected and transported from natural sites in southern Alberta to two man-made outdoor ponds in central Alberta where they were captively reared from the egg stage to metamorphosed frog. Over the six years of the program, in excess of 13,000 metamorphs were reintroduced collectively at three sites in central Alberta (Alberta Northern Leopard Frog Recovery Team 2005). Although ongoing monitoring occurred at the release sites during the years of reintroduction (Kendell 2004), there has been no documentation suggesting self-sustaining populations have been established at any of the release sites since the program ended.

Knowledge gaps

The majority of studies on *R. pipiens*, and on pond-breeding anurans in general, have been conducted on aggregations of adults, eggs and tadpoles at reproductive sites (Alford and Richards 1999). Relatively little is known about anuran movements away from breeding sites, even though this is where most anurans spend the majority of their time (Madison 1997). Interpond dispersal is one such movement, and is the means by which declining populations may be naturally rescued or recolonized following extirpation (Rothermel and Semlitsch 2002). Because adult anurans rarely switch breeding sites, metamorphs are responsible for most dispersal events (Berven and Grudzien 1990). High levels of metamorph survival may therefore be necessary to maintain gene flow and to rescue local populations from extirpation (Trenham et al. 2000; Rothermel and Semlitsch 2002).

The basic details of successful metamorph dispersal are unknown, but one important factor may be the orientation of their initial dispersal movements (emigration) when leaving the natal pond (Madison 1997). Apart from two observational studies (Dole 1965; 1971), and one study that examined dispersal movements along streams (Seburn et al. 1997), the terrestrial emigration abilities of *R. pipiens* are poorly studied. Metamorph orientation ability may have consequences for population dynamics. For example, if metamorphs orient non-randomly from their breeding pond, their likelihood of encountering neighbouring wetlands (and potentially colonizing sites) may either increase or decrease compared to a more uniform emigration pattern (Timm et al. 2007). Another unexplored aspect of movement behaviour relates to whether these patterns are fixed or are subject to variation in young, naïve animals. A flexible emigration strategy has the advantage of being able to respond to variation in available dispersal cues. To understand whether emigration behaviour is consistent, however, requires knowledge of whether metamorphs use cues for orienting and if so, when they begin to attain information necessary for effective orienting.

If metamorphs use cues for emigration, it is not yet clear when they begin to perceive them. Most anuran emigration studies examine the metamorph life-stage only and often involve transferring tadpoles to experimental ponds where emigration is subsequently examined (e.g. Rothermel and Semlitsch 2002; Rothermel 2004; Patrick et al. 2007). Such studies rarely consider whether metamorphs perceive orientation cues just before or after metamorphosis (DeMaynadier and Hunter 1999) or whether they perceive terrestrial cues throughout the entire tadpole stage (Rothermel 2004). Whichever the case is, the interpretations of most experimental studies could be missing a key aspect of the development of orientation behaviour.

Exploring these knowledge gaps for *R. pipiens* will not only improve our general knowledge of this anuran species, but could also provide insight into why previous metamorph reintroductions may have failed, and potentially enhance future conservation strategies. For example, emigration studies have been used to identify terrestrial habitat around breeding ponds in need of protection (e.g. Dodd and Cade 1998). They are also used to determine the impact of habitat fragmentation on metamorph behaviour and, therefore, on dispersal and colonization rates, which are important for population persistence (e.g. Rothermel 2004; Mazerolle and Vos 2006). Further, if metamorph *R. pipiens* are unable to detect suitable habitat upon emigration, they may need to be 'guided' towards safe sites to maximize survival following reintroduction. Lastly, a multiple life-stage study can help address whether reintroduction procedures might disrupt the development of metamorph orientation strategies. A natural history and field ecology study is very pertinent to this issue and will provide important biological data in order to address these topics (Bury 2006).

Research objectives

The primary goal of this thesis is to examine the natural emigration behaviour of newly metamorphosed *R. pipiens*. In approaching this I also collected supplementary data about the location and environmental conditions of egg clusters and similar data for tadpoles to help interpret my main thesis topic. Thus, in this thesis I address four main objectives: 1) to document egg cluster distribution within naturally occurring ponds, 2) to determine the distribution of resulting tadpoles in the same ponds, 3) to determine the pattern of metamorph distribution prior to and during emigration, and 4) to determine metamorph dispersal movements in a natural population compared to those of a reintroduced population.

In Chapter 2, I describe the methods by which my study sites were chosen, and I investigate the spatial distribution of the egg clusters of *R. pipiens* and their hatching rates, in three naturally occurring breeding ponds. Because oviposition site selection studies generally focus on adult selection of breeding ponds only, information from this chapter provides a unique perspective on breeding site selection within ponds.

In Chapter 3, I investigate factors influencing the spatial distribution of *R. pipiens* tadpoles in the same ponds, providing rare insight into tadpole spatial distribution within natural ponds.

In Chapter 4, I assess metamorph spatial distribution prior to emigration in relation to several physical variables, as well as their orientation when leaving the breeding pond. I also experimentally examine an aspect of emigration behaviour that questions whether orientation is consistent at the same site by returning metamorphs to their natal pond after their initial emigration attempt. Using data from previous chapters, I investigate whether emigration direction is related to the spatial distribution of earlier life-stages.

In Chapter 5, I use information from the previous chapter to retrospectively interpret the outcomes of the attempted reintroductions that triggered the current study. Despite the limitations involved with comparing samples from different field sites, this chapter provides important insight into the behaviour of reintroduced animals.

In Chapter 6, I summarise the findings of Chapters 2 through 5 and discuss how they contribute to our understanding of the Anura. I conclude with the management implications that stem from my work, and areas where future research should be directed.

Whereas the knowledge gained from this study could fill some knowledge gaps in anuran ecology, it can also be used to enhance conservation initiatives that focus on *R. pipiens* and, more generally, those of other species. Lastly, I hope the results of my study catalyze the interests of conservationists to challenge underlying assumptions of reintroduction programs and to determine how natural history data can be used to help secure the future of amphibians.

CHAPTER 2: OVIPOSITION SITE SELECTION AND HATCHING RATES IN *RANA PIPIENS*

Introduction

Oviposition site selection plays an important role in the reproductive success of amphibians (Marsh and Borell 2001). It can influence not only the physical and biotic conditions under which eggs develop (Crump 1991), but also the distribution of later aquatic and terrestrial life-stages. As a result, there should be strong selective pressure for adult amphibians to choose to oviposit in high-quality sites over low-quality sites. Whereas many studies have examined how pond-breeding anurans discriminate among breeding ponds (Hopey and Petranka 1994; Rudolf and Rödel 2005), few have explored selection of oviposition sites within ponds. In this chapter I address this knowledge gap by examining the distribution of northern leopard frog (*Rana pipiens*) egg clusters and their hatching rates within natural breeding ponds. Later chapters will compare these findings to ecological information related to subsequent life stages.

The choice of where to lay eggs within waterbodies may be influenced by abiotic (e.g. water temperature and depth, degree of shading, substrate type; Caldwell 1986; Crump 1991) and biotic conditions. Predation is an important mortality factor for larval anurans (tadpoles; Lane and Mahony 2002). Species such as treefrogs (*Hyla* spp) are able to detect and avoid ovipositing where predators are present (Resetarits and Wilbur 1989; Rieger et al. 2004). Other *Hyla* species also discriminate among oviposition sites based on the potential for parasitic infection (Kiesecker and Skelly 2000). Many anurans select oviposition sites depending on where conspecifics have already laid eggs (Marsh and Borrell 2001) because their presence or absence may indicate areas of high, or low, habitat quality.

Reproductive adults do not always make ovipositing decisions that maximize the survival of egg clusters (Heard 1994); therefore it may also be important for eggs to respond to environmental cues. Tadpoles are well known for their ability to alter their behaviour (Beiswenger 1977), morphology (Relyea and Werner 2000), and rate of development (Tejedo and Reques 1994; Denver 1998) in response to a variety of factors. Less is known about whether eggs exhibit any type of flexibility, specifically in the timing of egg hatching.

In order to understand site selection behaviour, egg cluster deposition patterns within breeding ponds must be determined. In this chapter I report on whether natural *R. pipiens* oviposition site-selection is spatially random. To minimize time spent at breeding sites, where congregations of adults may render themselves easy targets for predators, it may be advantageous for adults to spend little or no time selecting deposition locations. This could result in a random pattern of egg clusters within ponds. However, since adults could reduce the risk of egg mortality as a consequence of predation or competition if they actively select deposition locations (Goldberg et al. 2006), I expect breeding adults to deposit eggs in a non-random distribution within and among ponds. Because abiotic and biotic features of ponds vary from pond to pond, I predict the pattern of egg cluster deposition to be pond-specific.

Rana pipiens eggs take 5–20 days to hatch (Hine et al 1981; Gilbert et al. 1994), but whether the day of the season egg clusters were laid correlates with time until hatching is unknown. Offspring from egg clusters laid late in the breeding season may face higher competition and predation risks compared to those from earlier egg clusters. Similarly, if air temperatures increase as the breeding season progresses, egg clusters laid later in the season should take less time to hatch because warm temperatures are known to speed up the rate of development (Hassinger 1970). Therefore, I predict that egg clusters laid earlier in the spring will take longer to hatch compared to egg clusters laid later in the season.

Methods

Study sites

Some of the few remaining populations of *R. pipiens* in Alberta are located in or near Cypress Hills Interprovincial Park (49° 39' N and 110° 01' W) in southeastern Alberta. The park occupies approximately 2500 km^2 and spans the Alberta–Saskatchewan border. The Cypress Hills attain an elevation of 1200–1300 m and are covered by endemic montane and grassland floras. Typical *R. pipiens* breeding habitat consists of wet meadows, temporary and permanent beaver ponds, or slow-moving backwaters of streams and rivers (Wright 1914; Merrell 1968; Corn and Livo 1989; Gilbert et al. 1994).

RANA (Researching Amphibian Numbers in Alberta) has been intermittently monitoring a successful leopard frog breeding pond in the Alberta side of the park since

2000. This pond formed my primary study site (hereafter referred to as the "RANA" pond; 49° 39' 43.8" N; 110° 03' 33.6" W). To maximize the effectiveness of surveying and trap checks I selected other breeding ponds that were as close to the RANA pond as possible. Although this is a non-random sample of ponds, these study locations comprised all known *R. pipiens* breeding ponds within a 25 km radius, enabling baseline data to be acquired as efficiently as possible in the time available. Breeding ponds were also chosen based on accessibility, presence of adult *R. pipiens*, and evidence of breeding activity (adults calling and presence of egg clusters).

To help identify *R. pipiens* breeding ponds, in 2006 I surveyed ponds visually for egg clusters and adult *R. pipiens*, and conducted call surveys (arriving at a potential breeding pond approximately 0.5 hr after sunset and listening for up to 20 min for adult male *R. pipiens*) in the evenings to detect breeding activity. If at least one adult was heard, indicating that *R. pipiens* was attempting to breed in the pond, I returned to the pond the following day to confirm egg cluster deposition. One successful breeding pond was found 1.6 km south-east of the RANA pond (49° 39' 34.8" N and 110° 02' 12.6" W; Figure 2.1), located next to Battle Creek and is hereafter referred to as the "BC" pond. Another pond was located approximately 0.5 km east of the BC pond; referred to as the "Gap" pond (49° 39' 21.5" N and 110° 01' 43.6" W). The fourth study site was located approximately 25 km southwest of the RANA pond, on the south slopes of the Cypress Hills and is referred to as the "ENR" pond (as it is located on Eagles Nest Ranch Road) (49° 34' 48.0" N and 110° 20' 55.5" W).

The RANA pond is surrounded by aspen woodlands to the east, west, and south

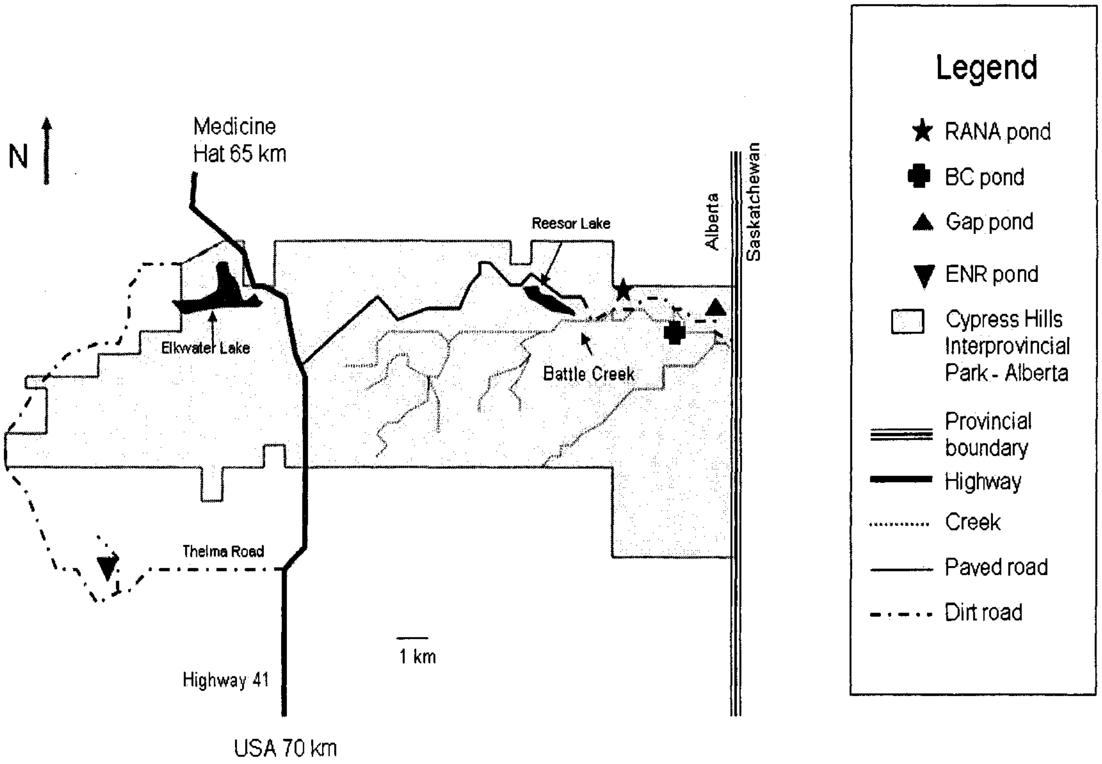


Figure 2.1. Map of the Alberta side of Cypress Hills Interprovincial Park. Study ponds are indicated by symbols (see Legend) and are not drawn to scale.

sides, whereas the north side has a relatively steeper slope of mixed grass prairie. The surface area is 0.1 ha, and a gravel road is located approximately 10 m south of the pond. The BC and Gap ponds have a surface area of 0.17 ha and 0.04 ha respectively, and are also surrounded by mixed grass prairie and aspen woodland, but the Gap pond has less woodland than the other sites. The ENR pond has a surface area of 0.24 ha and is surrounded by mixed forest, mainly white spruce (*Picea glauca*) on the west and north sides and deadfall on the south and east sides (Yaremko 1994). A gravel road borders the pond 10–30 m to the east and south. Predatory fish were absent from all study ponds, but other predators were present. In particular, aquatic insects were observed at all sites, and painted turtles (*Chrysemys picta*) were seen at the ENR pond. Beavers (*Castor canadensis*) and muskrats (*Ondatra zibethicus*) have been seen at both the RANA and ENR ponds.

Survey technique

Egg cluster surveys were undertaken over a period of approximately 50 days from April to June 2006, encompassing the entire breeding season of *R. pipiens* in the area (as determined by the initiation and cessation of calling). Shoreline surveys are considered appropriate for detecting *R. pipiens* egg clusters (Merrell 1977; Yaremko 1994); therefore each pond was surveyed at least once every two days along its entire shoreline in an effort to locate all egg clusters deposited. When a new *R. pipiens* egg cluster was identified, the closest location on the shoreline was marked with a flag to minimize the risk of accidentally counting the same cluster twice (Gilbert et al. 1994; Egan and Paton

2004; Dougherty et al. 2005). It was possible to distinguish individual egg clusters even when they were touching each other because of differences either in the shapes of the clusters or in the stage of egg development (Merrell 1977). During each survey period, egg clusters that had previously been located were observed and their status recorded as un-hatched (egg cluster intact), partly hatched (both eggs and tadpoles seen) or hatched (no egg cluster seen). Hatching was evident when the newly hatched tadpoles were seen aggregating around their old egg cluster, feeding on any remaining jelly. Techniques described in this paragraph were successfully used by Yaremko (1994) to document breeding behaviour and deposition sites of *R. pipiens* in areas similar to those in this study.

General observations were recorded during each survey. For each egg cluster, its depth (cm) below the water surface and distance (cm) from the shore were measured. This was done by standing on the shoreline and, without touching the cluster, placing a metre stick next to it in order to measure the depth of the cluster, and then placing the metre stick on the closest point on the shoreline to the cluster and measuring the distance between the shore and the egg cluster. The distance (in cm) to the closest adjacent egg cluster was also measured using a metre stick, or if this distance was greater than 1 m the distance was estimated (in m). The water pH (Hanna instruments; model HI 98127) and temperature (degrees Celsius) within 10 cm of each cluster were measured at least twice during its development. Weather conditions (precipitation level, air temperature and barometric pressure) during the breeding season were documented using data from the closest Environment Canada weather station, located in Cypress Hills, Saskatchewan, at 49° 39' N and 109° 31' W at an elevation of 1270 m. The breeding season was considered to be over when calling surveys failed to reveal any sign of activity, and no new egg clusters had been located for more than two weeks.

To determine whether egg clusters were deposited uniformly around the pond, the shoreline of each pond was divided into four sections (quadrants). This was initially done in July of 2005, at which time the RANA pond was the only pond being studied and pond levels had likely decreased since the beginning of the breeding season. The location of the first quadrant was randomly selected at the RANA pond, and the shoreline was subsequently divided into four sectors of equal length. Because of the methodology required for a later part of this study at the RANA pond, quadrants were marked out using fencing material that was installed perpendicular to the shoreline. The exact location of each fence (and therefore each quadrant) was constrained by shoreline topography and vegetation surrounding the RANA pond. An additional constraint on the design was that the perimeter of the pond was mapped to the closest metre at the beginning of the breeding season in 2006, when the RANA pond was probably at its highest water level of the summer. As a result, because the quadrants were designated when the pond had a lower level of water than when they were mapped the following year, the shorelines did not measure equal lengths at the time of mapping (see Results). However, I could not alter the location of the quadrants at this point as I needed to retain an identical design to the one used for data collection in 2005. For the other study ponds, I used the RANA pond as a reference, such that the angles (i.e. general cardinal directions of the compass) of each quadrant were identical across ponds. This methodology allowed me to compare general cardinal directions of egg cluster distribution within and across ponds, although not all sites were absolutely identical (an unavoidable outcome of using natural rather than artificially constructed breeding sites).

Statistical analyses

I used qualitative descriptions to determine whether egg clusters were distributed non-randomly by cardinal direction within ponds because the data did not meet parametric assumptions even with transformations to test egg cluster distribution within ponds. Moreover, combining ponds for analysis was not considered appropriate because of the differences in the size and shape of quadrants and ponds in general. Deposition site data collected from the ENR pond were not included in the analysis because over 50% of the pond was covered with pollen in the spring, rendering subsurface visibility problematic for reliably identifying when and where egg clusters were laid.

To determine if the number of days until hatching differed throughout the season, I used linear regression with the number of days between Julian date of egg cluster deposition and Julian date of hatching ('egg cluster duration') as the dependent variable, and Julian day as the independent variable. The α value was set at 0.05. All statistical tests were performed using SPSS® version 13.0.

Results

In 2006, egg deposition by *R. pipiens* took place from April 24 to May 23, for a total of 30 days, except at the ENR pond where breeding was 3–4 weeks behind the others (evident from the timing of males calling). I am confident my methods did not affect breeding because adults were vocal during my surveys in the daytime, and were bold enough that I could watch pairs of breeding adults in amplexus (when a male clasps a female with his forelimbs; McLister 2003). There were no instances of pairs in amplexus breaking contact in my presence. If individuals were chorusing as I approached they sometimes stopped but would resume again in less than five minutes. Many adults were encountered calling in areas of high egg cluster density throughout the breeding season.

The water pH within 10 cm of the egg clusters ranged from 7.6 to 8.5 and all (n = 26) egg clusters were within 2–10 cm of the water's surface. Most egg clusters (80%) were attached to emergent vegetation and were within 2–3 m of the shore. All egg clusters that were present at the same time within the same quadrant in a pond were within 0.5 m of each other, but only two egg clusters were actually touching. *Rana pipiens* egg clusters were typically grouped in shallow areas of the ponds and were well exposed to sunlight (pers. obs). Egg clusters were not present in areas of the ponds without vegetation.

In all ponds (Figures 2.2, 2.3, 2.4, and 2.5), the south-east quadrant spanned from 76.7° to 160° relative to the pond centre and from North. The north-east quadrant encompassed 14.7° to 76.7°, the north-west quadrant 256.4° to 14.7°, and the south-west quadrant 160° to 256.4°. Quadrants are hereafter referred to by their primary cardinal directions; SE (south-east), NE (north-east), NW (north-west) and SW (south-west). The included length of the shoreline of each quadrant within each pond was the most similar at the Gap pond (Table 2.1), likely because this was the most circular breeding site.

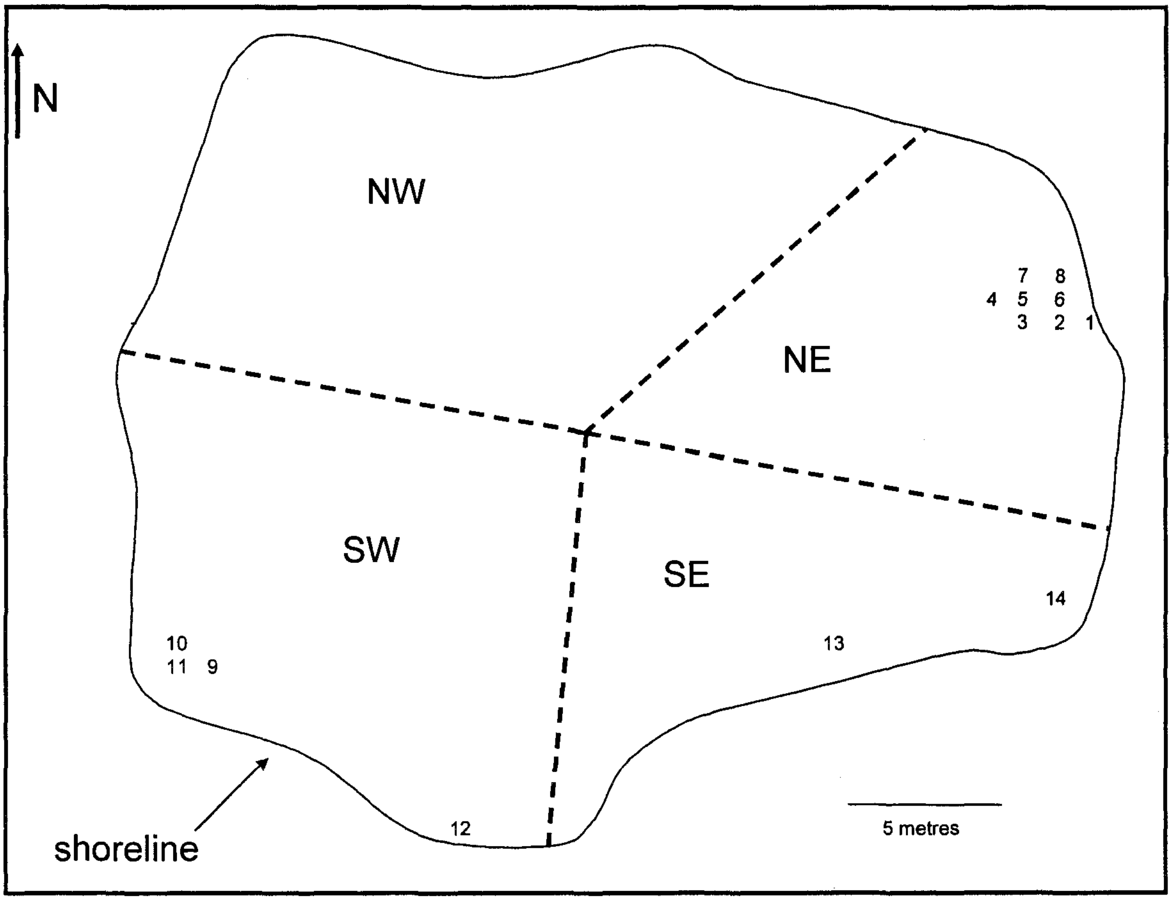


Figure 2.2. The RANA pond, with quadrants separated by dashed lines. Numbers within the pond indicate the number of the egg cluster (in the order in which they were located) at that location.

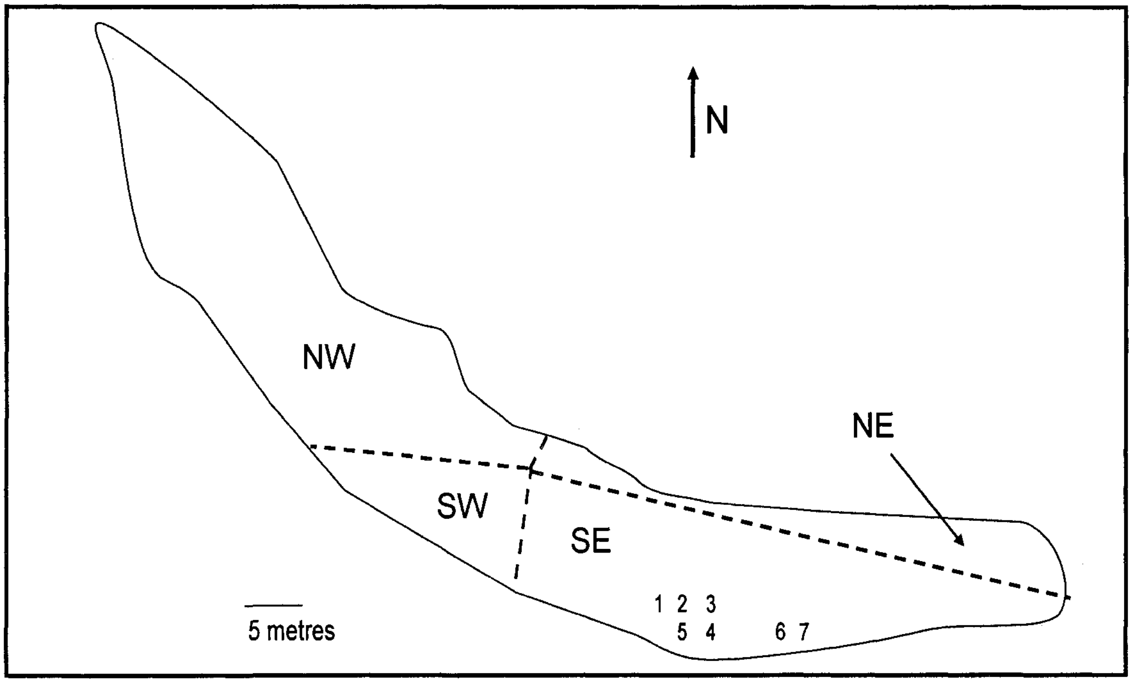


Figure 2.3. The BC pond, with quadrants separated by dashed lines. Numbers within the pond indicate the number of the egg cluster (in the order in which they were located) at that location.

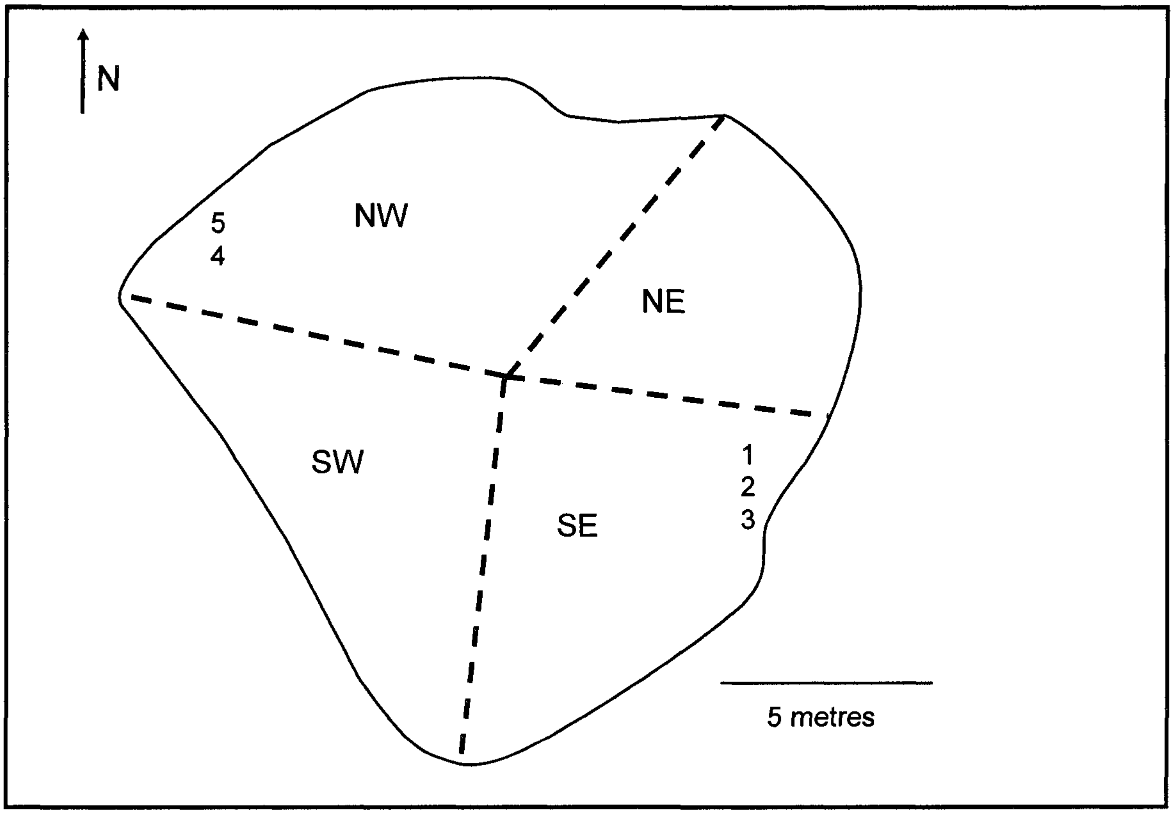


Figure 2.4. The Gap pond, with quadrants separated by dashed lines. Numbers within the pond indicate the number of the egg cluster (in the order in which they were located) at that location.

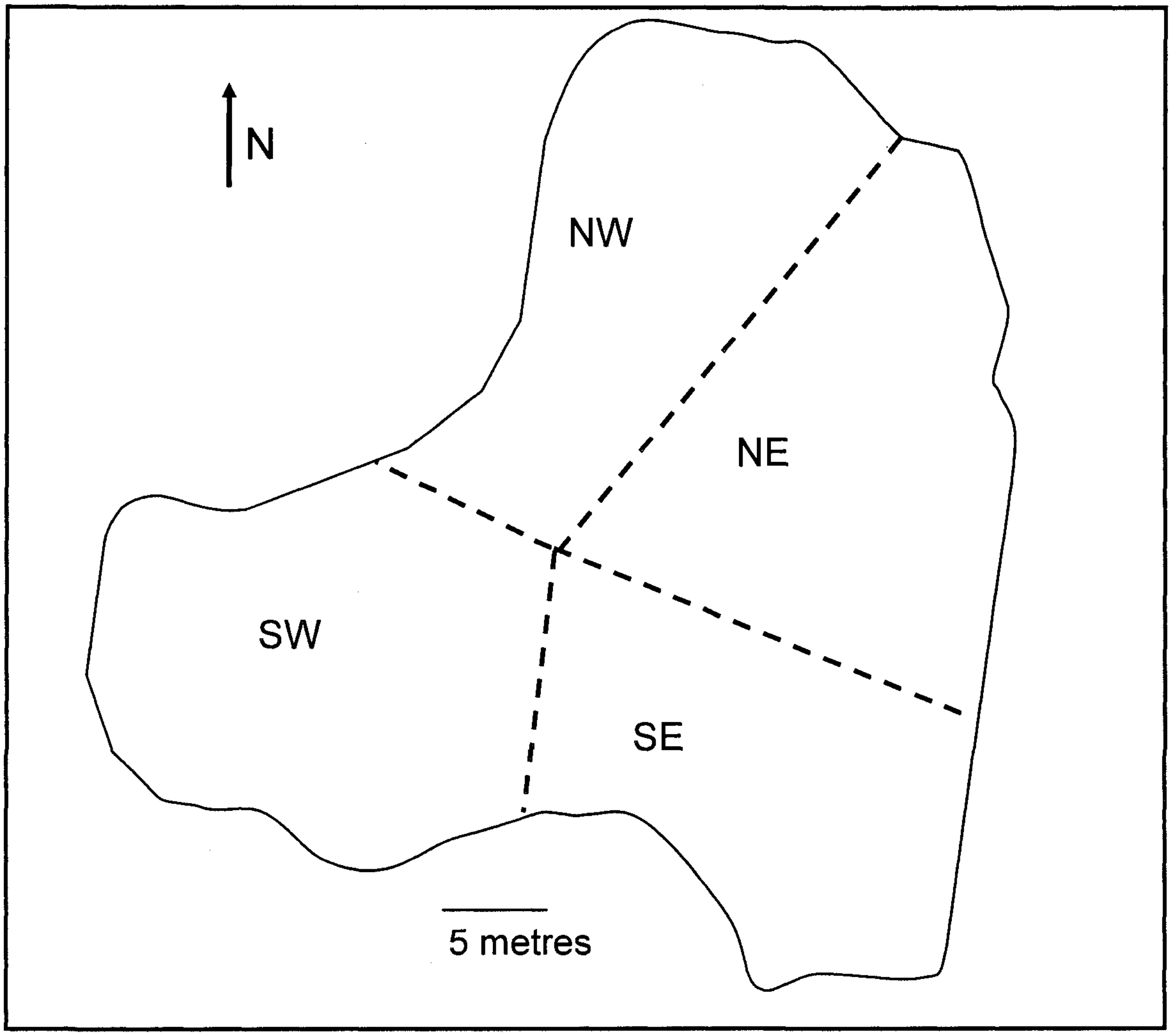


Figure 2.5 The ENR pond, with quadrants separated by dashed lines.

Table 2.1. Length of the shoreline and surface area (determined by forming the minimum convex polygon) of each quadrant in each pond at their maximum fill level.

	SE quadrant	**NE quadrant**	**NW quadrant**	**SW quadrant**	**Total**
RANA					
Length of shoreline (m)	19	20	34	27	100
Surface area (ha)	0.02	0.02	0.03	0.03	0.10
BC					
Length of shoreline (m)	48	48	86	10	192
Surface area (ha)	0.06	0.03	0.07	0.01	0.17
Gap					
Length of shoreline (m)	11	9	15	16	51
Surface area (ha)	0.01	0.01	0.01	0.01	0.04
ENR					
Length of shoreline (m)	42	32	42	72	188
Surface area (ha)	0.05	0.06	0.06	0.07	0.24

Of the three ponds, RANA was the most heavily utilized for egg deposition, with 14 egg clusters (Figure 2.2). The BC pond (Figure 2.3) received seven egg clusters, and the Gap pond (Figure 2.4) had five egg clusters. The number of egg clusters per quadrant was not equal. For example, in the RANA pond, the NW quadrant generally encompassed the longest shoreline compared to the other quadrants, yet no egg clusters were laid there (Figure 2.2). Instead, most egg clusters were laid in the NE quadrant, followed by the SW and SE quadrants respectively. Similarly, for the Gap pond, three of five egg clusters were laid in one of the quadrants with the shortest shorelines (the SE quadrant) compared to quadrants with a greater shoreline available. However, the surface areas of quadrants within the Gap pond were similar (Table 2.1). For the BC pond, the quadrant with nearly twice as much shoreline available and the largest surface area compared to other quadrants had no egg clusters. In fact, all seven egg clusters deposited in the BC pond were laid in the SE quadrant. In general, the shorelines of quadrants are more unequal in the larger ponds (i.e. BC and ENR) than in the smaller ponds, but other than the BC pond (because of its highly irregular shape), surface areas of quadrants were somewhat similar within ponds (Table 2.1).

In some ponds, the quadrants in which egg clusters were deposited appeared to shift as the breeding season progressed (Figure 2.6). In the RANA pond, adults initially laid eggs in the NE quadrant, within 0.5 m of each other. Later in the season deposition occurred in the SE and SW quadrants, and egg clusters were not in as close proximity to each other as they were earlier in the season. In the Gap pond, egg clusters were initially laid in the SE quadrant, whereas later in the season they were deposited in the NW

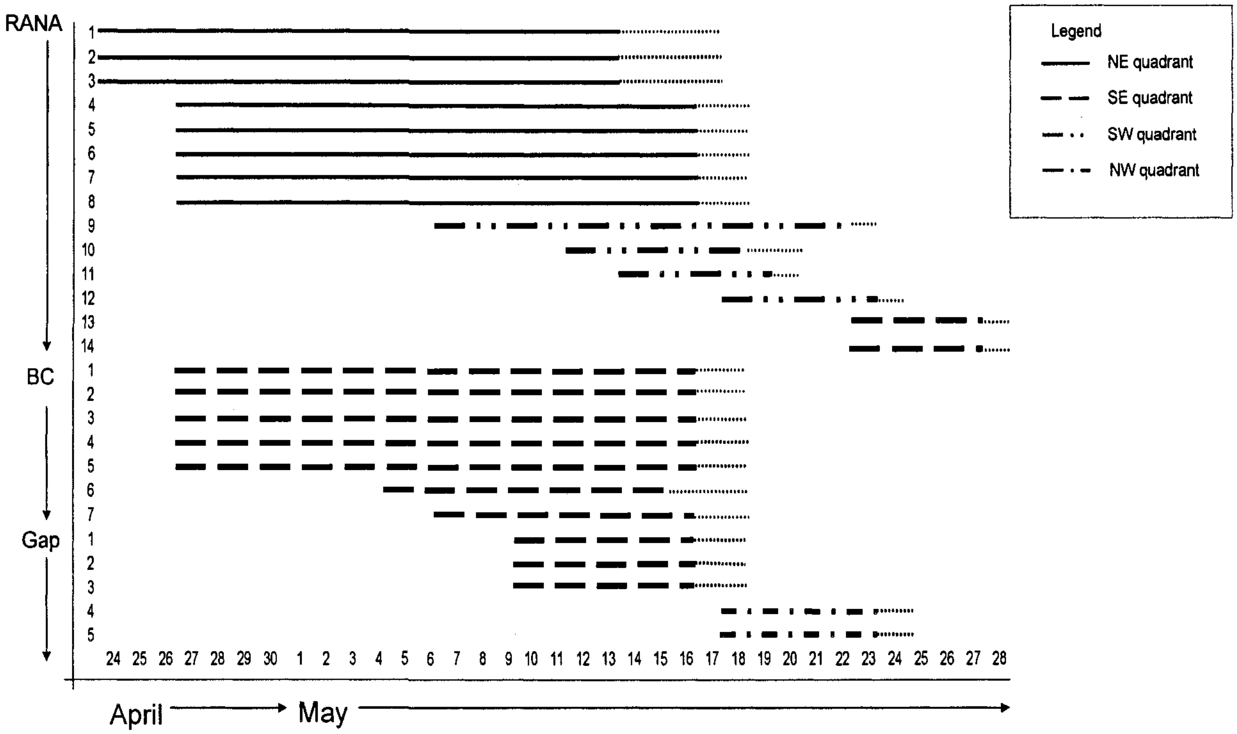


Figure 2.6. Frequency of oviposition and hatching in three ponds. Individually numbered egg clusters and their host pond are indicated on the Y axis, and date is indicated on the x axis. Lines within the figure indicate the length of time each egg cluster was present in the pond; thin dashed lines (············) indicate the hatching period associated with each egg cluster. The quadrant each egg cluster was found in is indicated by the dash styles (see Legend).

quadrant. Most egg clusters within the same quadrant at both the Gap and BC ponds were within 0.5 m of each other.

The range of mean daily air temperatures in which egg clusters were deposited was 5-17°C. Water temperatures within ponds varied between 5.7-25.8°C. Air and water temperatures fluctuated throughout the breeding season but overall they tended to increase over time. The regression showed that time from deposition to hatching ranged from 5–20 days, and was inversely correlated with the Julian day on which they were laid {$F_{1,24} = 236.6$, $r^2 = 0.908$ (unadjusted), $P < 0.01$; Figure 2.7}. The time until hatching was approximately four times longer for the earliest egg clusters laid (i.e. those laid between April 24 - May 3) than for the latest egg clusters laid (i.e those laid between May 4 - May 23); and this was consistent across ponds (Figure 2.7).

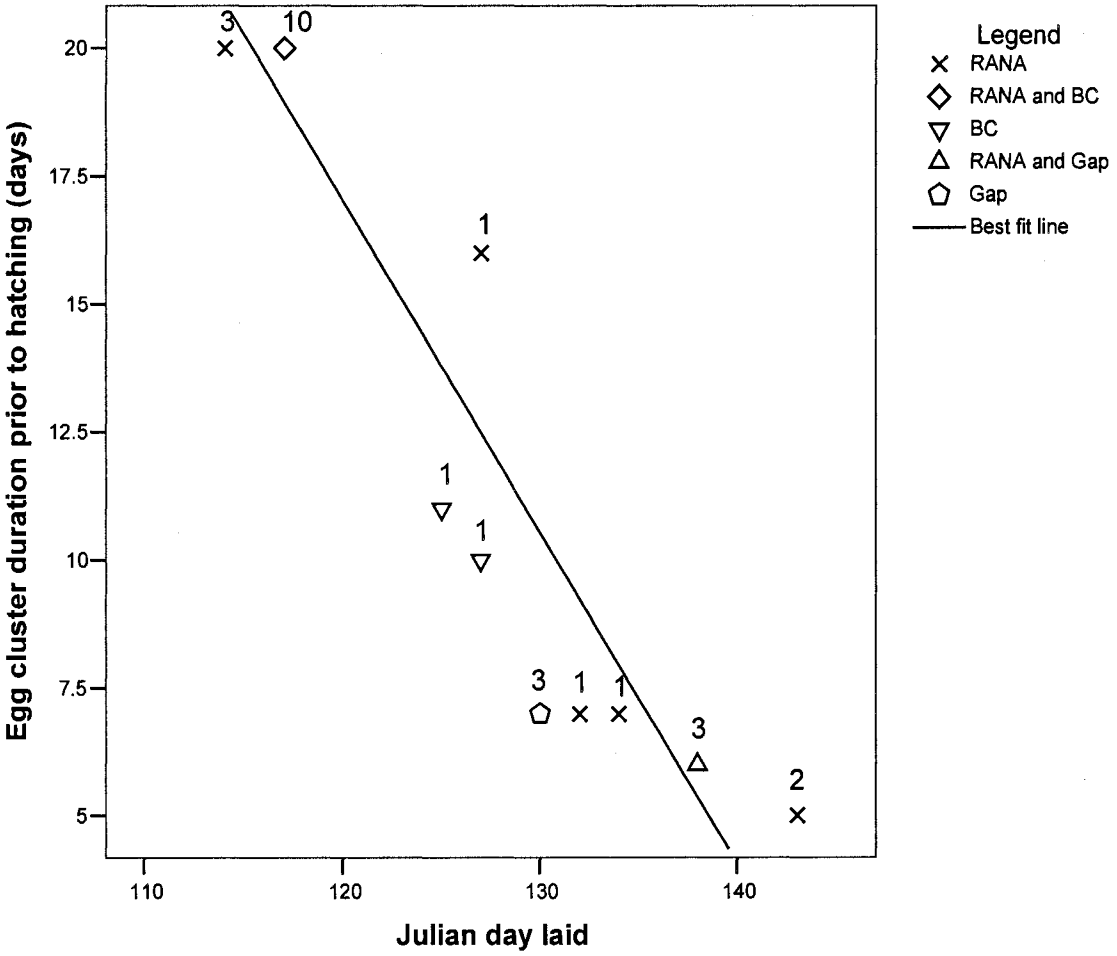


Figure 2.7. The number of days from deposition to hatching (egg cluster duration) for each egg cluster in the RANA, Gap and BC ponds over time (Julian day 110 = April 20). Symbols indicate the ponds in which the egg clusters were deposited (see Legend). Numbers above symbols indicate the number of egg clusters indicated by that symbol. The best fit line is indicated.

Discussion

In this chapter I examined oviposition site selection and the time until hatching of egg clusters for the duration of the breeding period in *R. pipiens*. The number of egg clusters was highest at the RANA pond when compared to the Gap and BC ponds. Within ponds, certain quadrants were more heavily used for egg deposition than others. The duration between deposition and hatching decreased as the season progressed. Below I address each of these findings in light of other studies and discuss timing as an important factor in egg cluster deposition. I conclude by commenting on topics requiring further research.

Due to small sample sizes I could not test statistically whether the distribution of egg clusters among quadrants within ponds differed; nevertheless, the trend was for certain quadrants to be used for egg cluster deposition more than others. Had adults deposited egg clusters randomly, the number of egg clusters across quadrants in each pond would have been more evenly distributed. In general, egg clusters were not deposited in areas within ponds that were heavily shaded or lacking vegetation. These observations agree with other studies of *R. pipiens*, including those of Merrell (1977), Hine et al. (1981), and Gilbert et al. (1994). Another potential explanation for the non-random distribution of egg clusters within ponds is that adults may simply deposit egg clusters in the section of the pond closest to where they entered from their overwintering site. Trapping adults as they enter the pond in the spring would reveal whether there is a relationship between adult immigration and egg cluster locations.

Non-random distributions of egg clusters within ponds have been documented in other amphibian species including the spotted salamander (*Ambystoma maculatum),* wood frog (*Rana sylvatica*), tree-hole breeding frog (*Phrynobatrachus guineensis*) and the common frog (*Rana temporaria*) (Howard 1980; Seale 1982; Waldman and Ryan 1983; Laurila and Aho 1997; Dougherty et al. 2005; Rudolf and Rödel 2005). Factors thought to influence these patterns include a preference for certain substrates (Seale 1982), sediment type (Rudolf and Rödel 2005), presence of conspecifics (Howard 1980), and predation (Laurila and Aho 1997). A common thread among these studies is that oviposition site selection in natural ponds is complex and cannot be explained by only a single factor. Clearly more studies, including both habitat parameters and intra- and interspecific interactions, are necessary.

Across the RANA, BC and Gap ponds, *R. pipiens* egg clusters took anywhere from 5–20 days to hatch. These results agree with other published reports on the topic (Wright 1914; Hine et al. 1981; Seburn and Seburn 1998). In addition, egg clusters laid later in the season did not take as long to hatch compared to those laid early in the season, supporting my predictions. This may be explained by the generally increasing air temperature that presumably led to increased water temperatures as the breeding season progressed. The rate of amphibian egg development accelerates and the time to hatching decreases in high versus low water temperatures (Hassinger 1970; Hine et al. 1981). Therefore, my results suggest that egg clusters deposited later in the season could have developed faster because of warmer surrounding temperatures within 10 cm (which is where water temperature was measured in this study) of the egg clusters, but more data are needed to test this hypothesis.

Temperature might be the most likely factor influencing the length of time between laying and hatching in many species, but other environmental factors could also affect the time until hatching of *R. pipiens* egg clusters. For example, Touchon et al. (2006) noted that anurans can alter their hatching time in response to the risk of infection by water mold. In other species, hatching has been induced by the presence of predators (Saenz et al 2003; Vonesh and Bolker 2005; Ireland et al. 2007). Whether extrinsic factors (besides the appropriate development of eggs) affect hatching time requires further investigation.

Since the time until hatching decreases as the breeding season progresses, one might expect other biotic conditions to vary within ponds over the season as well. For example, I did not expect to observe a difference in egg cluster placement as the season progressed (in the RANA and Gap ponds). The actual pattern may be because adults select deposition sites containing conspecifics only if the existing egg clusters are younger than a certain developmental stage (e.g. *Rana japonica*; Iwai et al. 2007). Hatchlings from eggs laid later may be competitively inferior and subsequently have lower chances of survival if placed in close proximity to eggs laid earlier (Petranka and Thomas 1995; Matsushima and Kawata 2005). Although cannibalism might be suspected as a reason to deposit eggs far from older clusters, I am confident that cannibalism was not occurring during this study because I never witnessed tadpoles feeding on newly deposited eggs laid late in the breeding season. Moreover, the feeding by *R. pipiens* tadpoles on conspecific tadpoles has never been documented. However, such factors are interesting because they suggest that adults are discriminating among sites based on the survival of later life stages, and not based solely on the physical characteristics of the pond. Experimental studies that test whether adjacent egg clusters of varying developmental stages result in different survival rates of later life stages will help clarify these relationships.

Other studies suggest that oviposition behaviour throughout the breeding season relates to environmental conditions within the ponds. Southern leopard frogs (*Rana sphenocephala*) lay eggs in communal clusters (egg clusters in physical contact, laid by many different females) when water temperatures are cold. During warm weather, however, egg clusters are deposited in isolation (spaced out from each other, not touching any other egg clusters; Caldwell 1986). The clumping of egg clusters confers advantages to embryos by increasing the ambient water temperature via an increase in the amount of metabolic heat produced by developing embryos (Hassinger 1970). This can then increase the rate of development (Howard 1980; Seale 1982). While I found egg clusters in the same quadrant were often within 0.5 m of each other, only two were actually touching, so I suggest that a thermal advantage due to clumping was not the driving factor behind a seasonal change in *R. pipiens* egg deposition. In fact, egg clusters deposited in isolation may benefit from higher rates of gas exchange in warmer water than those laid in clumps. This is because isolated egg clusters will have an increased available surface area: volume ratio for gas exchange compared to egg clusters that are touching each other (Caldwell 1986). As a result, isolated egg clusters are able to withstand lower oxygen tension than are communal clusters (Caldwell 1986). More research testing different environmental factors on the placement of eggs is required to help interpret *R. pipiens* egg deposition behaviour throughout the season.

Due to the small sample size, I cannot say with confidence whether egg cluster deposition sites differ among quadrants within ponds, and ultimately whether adult *R. pipiens* are actively selecting oviposition sites. There is the possibility that one or more egg clusters may have been overlooked, but I am confident that the risk of missing egg clusters was negligible because, except for the ENR pond, my study ponds were small and were completely visually accessible such that an observer could see all areas of each pond. Additionally, ponds were checked almost every day, at different times and under various weather conditions. Overall, it is difficult to draw conclusions from a single breeding season because of potential year to year fluctuations in characteristics of amphibian populations. In fact, Ficetola et al. (2006) found that microhabitat features that were important for agile frog (*Rana dalmatina*) egg cluster deposition in one year were not important the following year in the same pond. My research needs to be repeated as data from multiple seasons will increase the understanding of *R. pipiens* deposition behaviour.

In summary, a range of behavioural flexibility in oviposition patterns and variation in time to hatching appears to occur among the *R. pipiens* populations used in this study. However, it is not clear from my study how breeding *R. pipiens* detect suitable oviposition sites. As *R. pipiens* attach their egg clusters to vegetation, the structure of pond vegetation will constrain the location of deposition sites. A full description of the vegetation in breeding ponds will be of value to future research in *R. pipiens* deposition trends. Further, to understand the importance of oviposition site selection on reproductive success, it will be necessary to quantify egg, tadpole and frog survival (Ficetola et al. 2006). Temporally and spatially replicating this research will help resolve issues such as how the location and timing of deposition influences the persistence of *R. pipiens* populations.

This chapter described oviposition patterns of *R. pipiens* within ponds, but to completely understand the importance of natal sites, one must consider the next stages in their life cycle. In the next chapter I investigate the distribution of tadpoles within the same three ponds, and ultimately relate all of this information to observations made on patterns of metamorph emigration and dispersal.

CHAPTER 3: PHYSICAL FACTORS INFLUENCING *RANA PIPIENS* TADPOLE DISTRIBUTION WITHIN BREEDING PONDS

Introduction

Few studies have assessed the distribution of anuran larvae (tadpoles) within natural ponds, even though such distributions could have important consequences for tadpole growth and survival (Smith et al. 2003). Within wetlands, many different microhabitats can be recognized, which vary in important features such as water temperature, depth, vegetation and exposure to the sun. As these features could affect the survival and development of anuran egg clusters (Ficetola et al. 2006), one might also expect them to be important for tadpole survival and growth after hatching. Northern leopard frog (*Rana pipiens*) egg clusters are non-randomly distributed within ponds (Chapter 2), suggesting that the adults select particular egg-laying sites. The spatial distribution of resulting tadpoles in natural breeding ponds is not well known. Tadpoles are potentially able to self-select microhabitat. Whether they do so and whether tadpole distribution reflects or differs from egg cluster distribution is not known. Building upon results from Chapter 2, the current chapter explores the distribution of *R. pipiens* tadpoles within natural ponds.

Tadpoles have been used extensively for investigating microhabitat selection, both in laboratory and semi-natural conditions (i.e. cattle troughs, man-made ponds, or enclosures in natural ponds; Thurnheer and Reyer 2000; Haramura 2007). Physical factors such as photoperiod (Schley et al. 1998; Laurila et al. 2001), water temperature (Thurnheer and Reyer 2000), and water depth (Smith and Doupnik 2005) influence the spatial distribution of tadpoles. Under natural conditions though, tadpoles respond to a combination of environmental cues (Alford 1986). For instance, if the predation risk is high or if a pond is drying up quickly, tadpoles may select warm areas of waterbodies to speed up their growth in order to metamorphose quickly, allowing them a rapid escape from the aquatic habitat (Noland and Ultsch 1981). But if both food and predators are more abundant in warm areas, tadpoles must balance the benefits of foraging and increased growth rates with the risk of predation.

In this chapter I investigate the spatial distribution of *R. pipiens* tadpoles in naturally occurring ponds in relation to several predictor variables. Many factors (e.g. predation risk, food availability) likely influence tadpole distribution, but because this chapter is part of a broader project examining overlapping life stages at the same sites, it was necessary that I tested microhabitat variables that had minimal disturbance on the pond community. These included water temperature, water depth, and shade at evenly-distributed locations within breeding ponds. I predict that tadpoles will be associated with warm, un-shaded areas rather than cool, shaded areas of ponds because warmer temperatures accelerate growth in ectotherms (Smith-Gill and Berven 1979). Likewise, I predict that tadpole occurrence will be associated with shallower compared to deeper water because shallow water is generally warmer (Thurnheer and Reyer 2000). In order to compare tadpole distribution to that of other life stages, it was necessary to maintain a design similar to that used in other chapters. Therefore, I evaluate tadpole distribution relative to specific cardinal directions within a 360° arc in breeding ponds (i.e. quadrants; Chapter 2). There is no reason to expect that tadpoles should orient along particular cardinal directions within ponds, thus I predict that, when water temperature, depth and shade are accounted for tadpoles will be randomly distributed relative to quadrants. Finally, because most of the mortality in *R. pipiens* populations occurs in the tadpole stage (Merrell 1977), combined with metamorphosis later in the summer, I expected tadpole abundance to decrease as the summer progressed.

Methods

Survey technique

Chapter 2 outlines details on how study sites were established, their general characteristics, and how quadrants were designated within each pond. Preliminary observations suggested that tadpoles did not often swim more than 3–4 m in 15 min; and therefore tadpole observation points (stakes) were set-up at 5 m intervals around the shoreline of each pond. This helped ensure that tadpoles were not swimming from one observation point to the next, and therefore minimized counting tadpoles twice during a survey. Nonetheless, I noted any instances where I was uncertain if they swam between stakes and were potentially counted twice. The first stake was placed at a random location on the perimeter of each pond, and subsequent stakes were located every 5 m (except for the first and last stake which depended on the length of shoreline of each pond). The RANA, Gap, and BC ponds had 22, 10, and 26 stakes respectively. Since stakes were placed every 5 m, the number of stakes was proportional to the shoreline length in each quadrant. Being a field-based study, the ponds were irregularly shaped and as a result, the number of stakes in each quadrant was not equal.

For each survey, I counted and recorded the number of tadpoles within an approximately 2 m radius around each stake and within a 2 min observation period. *Rana pipiens* tadpoles are distinguished from other amphibian larvae by their cream-coloured belly, brown or gold spots, and a brown or grey body (Wright 1914). Tadpole surveys were conducted at each pond at least once a week. The order in which stakes were surveyed varied (i.e. clockwise or counter-clockwise around the pond) with each survey so that tadpole behaviour was not uniformly influenced by the direction that the stake was approached from. Effort was made to minimize the trampling of shoreline vegetation and the disturbance of tadpoles in the vicinity by not walking immediately next to the pond when travelling between stakes.

After each stake observation, the water depth (in cm) and water temperature (degrees Celsius) 10 cm below the water surface were measured at 1 m from the stake and perpendicular to the shore (Thurnheer and Reyer 2000). If the depth of the water was less than 10 cm at 1 m from the stake, the temperature was taken as close to the substrate as possible. At each stake observation, I also recorded whether the 2 m observation radius was shaded or not. Finally, instances in which visibility was poor due to heavy shading, pollen cover, or other reasons were recorded.

Half of the ENR (Eagles Nest Ranch) pond was covered in a mat of pollen all summer, rendering tadpole surveys impossible in these areas due to poor visibility. If there was no difference in water temperature between 'good' and 'poor' visibility stakes in this pond, the 'good' visibility stakes could have been used as a sub-sample of available stakes in the analysis. However, the mean water temperatures at stakes with pollen were significantly lower than at stakes without pollen (t-test, df = 110, t = -0.3402, $P < 0.05$). Consequently the ENR pond was not included in the analysis.

Statistical analyses

I used an information-theoretic approach to evaluate several *a priori* models of the factors influencing tadpole abundance at each stake. Under this approach, the models are ranked according to Akaike's Information Criterion (AIC); the model receiving the lowest AIC value is the model that best approximates the data (Quinn and Keough 2002). The AIC adjusts for the addition of parameters, and thus selects a model that fits the data well with a minimum number of parameters. I used generalized linear mixed models (GLMM) to determine which variables were the best predictors of tadpole abundance. This approach allowed me to account for repeated stake observations and for the nesting of stakes within pond. However, because of a large number of zeros in the data (i.e. there were many instances of "0" tadpoles at a stake) even the best model was found to have low predictive power (ie. linear and quadratic regressions of the predicted probabilities against the number of observed tadpoles had r^2 values < 0.3). As a result, I also used binary logistic regression as a second method of analysing the data. Because logistic regression uses presence/absence data and not abundance, it is not testing the same question as the GLMM, but logistic regression may be a more appropriate analytical approach when there is a high number of zeros in the data. However, logistic regression does not account for nested variables or the repeated observations, which is why both analyses complemented each other. To detect the potential bias that might be associated with the inability of the logistic regression models to incorporate 'stake' as a repeated measure or nested variable, GLMM models were run without 'stakes' having these designations. Test outcomes between these GLMM models were almost identical to those where 'stakes' had been identified as a repeated and nested variable. Since the repeated measures effect of 'stakes' and its nesting within ponds seems to be minor in terms of predicting the total number of tadpoles in the GLMM, the potential bias associated with a lack of repeated and nested designations in the logistic regression is likely also minor.

For the GLMM, I evaluated three alternative models for predictor variables that influenced tadpole abundance (Table 3.1). I initially ran the simplest model (single factors only) to reduce the risk of over fitting the data (i.e. where the addition of extra predictor variables, in this case in the form of interactions, could suggest a better model even when they actually add very little power) to the response variable (tadpole abundance; Quinn and Keough 2002). I ran a second model including all pair-wise interactions, since any of these variables could potentially be interacting, but I had no *a priori* reason to test certain interactions over others. A third model included Julian day, shade, water depth and temperature because these were the factors hypothesized *a priori* to influence tadpole abundance.

For the logistic regression, I combined all surveys in all ponds because although it suffers from a loss of independence between data points, it was the only measure by which I could ensure an adequate sample size given the number of predictor variables (Babyak 2004). Prior to conducting the regression I made scatter plots and calculated pairwise Pearson or Spearman (if variables were not normally distributed) correlations

Table 3.1. Abbreviations and descriptions of categorical and continuous variables included in the GLMM and logistic regression models that were used to test the distribution of *R. pipiens* tadpoles in three ponds.

Variable	**Description**
Categorical	
POND	whether the stake was in the RANA, Gap or BC pond
QUAD	whether the stake was in the NE, SE, SW or NW quadrant
SHADE	whether the 2 m observation area around the stake was 'shaded' or 'not-shaded' (instances where it was only partly shaded were considered as 'shaded')
Continuous	
JDAY	Julian day of observation
DEPTH	depth of water 1 m from stake
TEMP	temperature of water 1 m from stake and 10 cm below the surface

among all variables to test for multicollinearity (Haramura 2007). Multicollinearity occurs when predictor variables are correlated, which could reduce the predictive power of the model parameters and potentially mask the effect of significant factors (Quinn and Keough 2002). Because all correlations were below the critical value of 0.7 (Green 1979) and no linear relationships among independent variables were detected, multicollinearity was not considered a problem in the analysis.

The presence (1 or more tadpoles) or absence (no tadpoles) of tadpoles at each stake was used as the dependent variable. The same variables used in the GLMM were used in the logistic regression (Table 3.1). Reference categories for the categorical variables (that category to which all other categories of that variable are compared to) were the last category in an alphabetical list of each variable. Therefore, the RANA pond, the SW quadrant, and 'shaded' were the reference categories for each categorical variable. The multivariate model was run as a forward stepwise binary logistic regression with an alpha level of 0.05 for entry. I ran the same three models as for the GLMM (i.e. single factors only, single factors with all pair-wise interactions, and those single factors originally hypothesized to influence tadpole distribution). Only the results from the model with the highest percentage of correctly classified cases are presented.

For each variable retained in the final logistic regression model, the regression coefficient (B) with its corresponding Wald χ^2 statistic, and the odds ratio (Exp(B)) with 95% confidence intervals are reported (SPSS 1999). The percentage of cases that were correctly classified and the area under the ROC (Receiver Operating Characteristic) curve (AUC) are presented, the latter of which indicates the accuracy of the final model.

Results

Tadpoles were first seen hatching on May 14 at the RANA pond and on May 16 at the BC and Gap ponds. For the first 4–5 days after hatching tadpoles remained aggregated around the egg clusters from which they had emerged and fed on remaining jelly. They were not visible for the next 2–3 weeks, therefore, the first day of tadpole surveys in all three ponds was June 6. At this time all egg clusters in all ponds had hatched and tadpoles were large enough to identify as those of *R. pipiens*. Fifteen surveys were completed in total; five at the GAP pond, three at the BC pond and seven at the RANA pond. Most surveys were completed between 1000 and 1600 h. Only for five out of 263 stake observations (2%) were tadpoles potentially counted twice. I observed several known tadpole predators within ponds, including dragonfly (Anisoptera spp.) and damselfly (Zygoptera spp.) larvae, diving beetles (*Dytiscus* spp.) and both wandering (*Thamnophis elegans vagrans*) and red-sided (*Thamnophis radix*) garter snakes. Other amphibian species observed at the ponds included boreal chorus frogs (*Pseudacris maculata*) and tiger salamanders (*Ambystoma tigrinum*). Tadpoles were found at depths ranging from 4–53 cm, and at temperatures ranging from 6–28°C.

The date of the last tadpole survey differed between ponds because tadpoles were last observed on July 14 at the RANA pond, but the BC and Gap ponds were dry by July 12 and 28 respectively. Having dried early in the summer, no tadpoles survived in the BC pond. In the final days in which water remained in the Gap pond, the pond was reduced to a puddle approximately 1 m^2 in size. In that time frame I observed fewer than ten metamorphosed frogs and many dead tadpoles. Also, because all ponds shrunk due to drying throughout the summer, the number of stake observations per survey decreased across ponds as a result of an absence of water at certain stakes (Table 3.2).

Results from the GLMM

A total of 263 stake observations were used in the GLMM. Each observation at each stake was a datum point. The full model proved to be the best model for predicting tadpole abundance (Table 3.3). In this model, only some interactions were significant. These were QUAD*DEPTH (GLMM, $F_{3,122} = 2.7$, $P < 0.05$), QUAD*TEMP ($F_{3,207} = 2.8$, $P < 0.05$) and QUAD*JDAY ($F_{3,211} = 2.8$, $P < 0.05$; Table 3.4). Models lacking pair-wise interactions ranked the highest (i.e. they represented the worst fit of the data).

To help understand the significant interactions, I made graphs of each interaction. Tadpole abundance appears to be highest in shallow water although this effect depended on the quadrant (Figures 3.1, 3.2 and 3.3). Similarly, the temperature at which tadpole abundance is highest differs by quadrant (Figures 3.4, 3.5 and 3.6). Lastly, the change in the mean number of tadpoles over time depended on the quadrant (Table 3.5). For instance, towards the end of the tadpole season, the mean number of tadpoles seemed to drop in the SE and NE quadrants, but spiked in the SW and NW quadrants.

Results from the Logistic Regression

The same 263 observations used in the GLMM were used in the logistic regressions. Each observation at each stake was a datum point. Similar to the GLMM

Table 3.2. The number of stakes per quadrant in the Gap, BC and RANA ponds at the beginning of the season. Numbers in brackets indicate the number of stakes towards the end of the season (if different because of drying).

	Quadrant				
Pond	**SE**	**NE**	**NW**	**SW**	**Total**
RANA	4	4	8	6	22
BC	7 (6)	6 (3)	12 (8)	1	26 (18)
Gap	2	2 (0)	4 (3)	2 (1)	10 (6)
Total	13 (12)	12 (7)	24 (19)	9 (8)	58 (46)

Table 3.3. GLMM models of *R. pipiens* tadpole abundance in the Gap, BC and RANA ponds. Models are ranked according to Akaike's Information Criterion (AIC). Δ AIC = change in AIC from the best model.

Model	**No. parameters**	**AIC**	**Δ AIC**
POND QUAD SHADE JDAY DEPTH TEMP + all pair-wise interactions	44	1635.8	0.0
POND QUAD SHADE JDAY DEPTH TEMP	12	1667.6	31.8
SHADE JDAY DEPTH TEMP	7	1680.1	44.3

Table 3.4. GLMM results predicting tadpole abundance in the Gap, BC and RANA ponds.

Variable	Numerator df	Denominator df	F	*P.*
Pond	2	217.5	.4	.69
Quadrant	3	217.0	1.7	.17
Shade	1	221.0	1.0	.31
Depth	1	215.4	.1	.75
Jday	1	211.3	.1	.81
Temp	1	217.6	.3	.59
Pond * Quadrant	6	83.4	1.6	.16
Pond * Shade	2	198.5	.2	.83
Depth*Pond	2	175.5	.1	.90
Jday*Pond	2	213.7	.7	.51
Temp*Pond	2	215.7	1.9	.15
Quadrant*Shade	3	206.3	.5	.71
Depth*Quadrant	3	122.3	2.7	.05
Jday*Quadrant	3	211.2	2.8	.04
Temp*Quadrant	3	217.5	2.8	.04
Depth*Shade	1	212.0	1.6	.21
Jday*Shade	1	221.0	1.2	.27
Temp*Shade	1	219.1	.5	.47
Depth*Jday	1	214.6	.1	.76
Depth*Temp	1	211.1	.0	.98
Jday*Temp	1	217.5	.4	.53

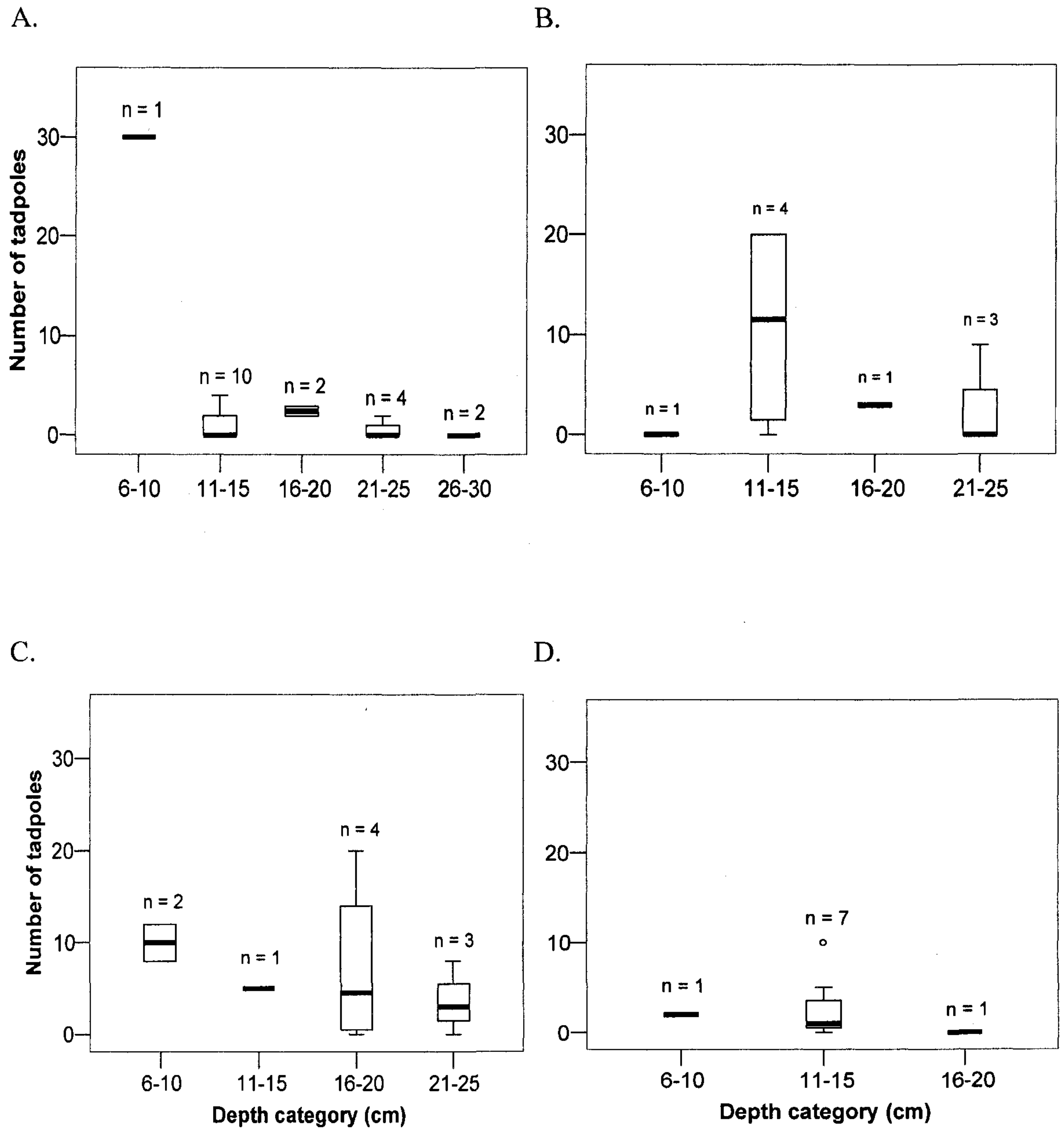


Figure 3.1. Boxplots showing the effect of water depth (cm) on the number of tadpoles in the NW (A), NE (B), SW (C) and SE (D) quadrants in the GAP pond. n = number of stake observations representing each category.

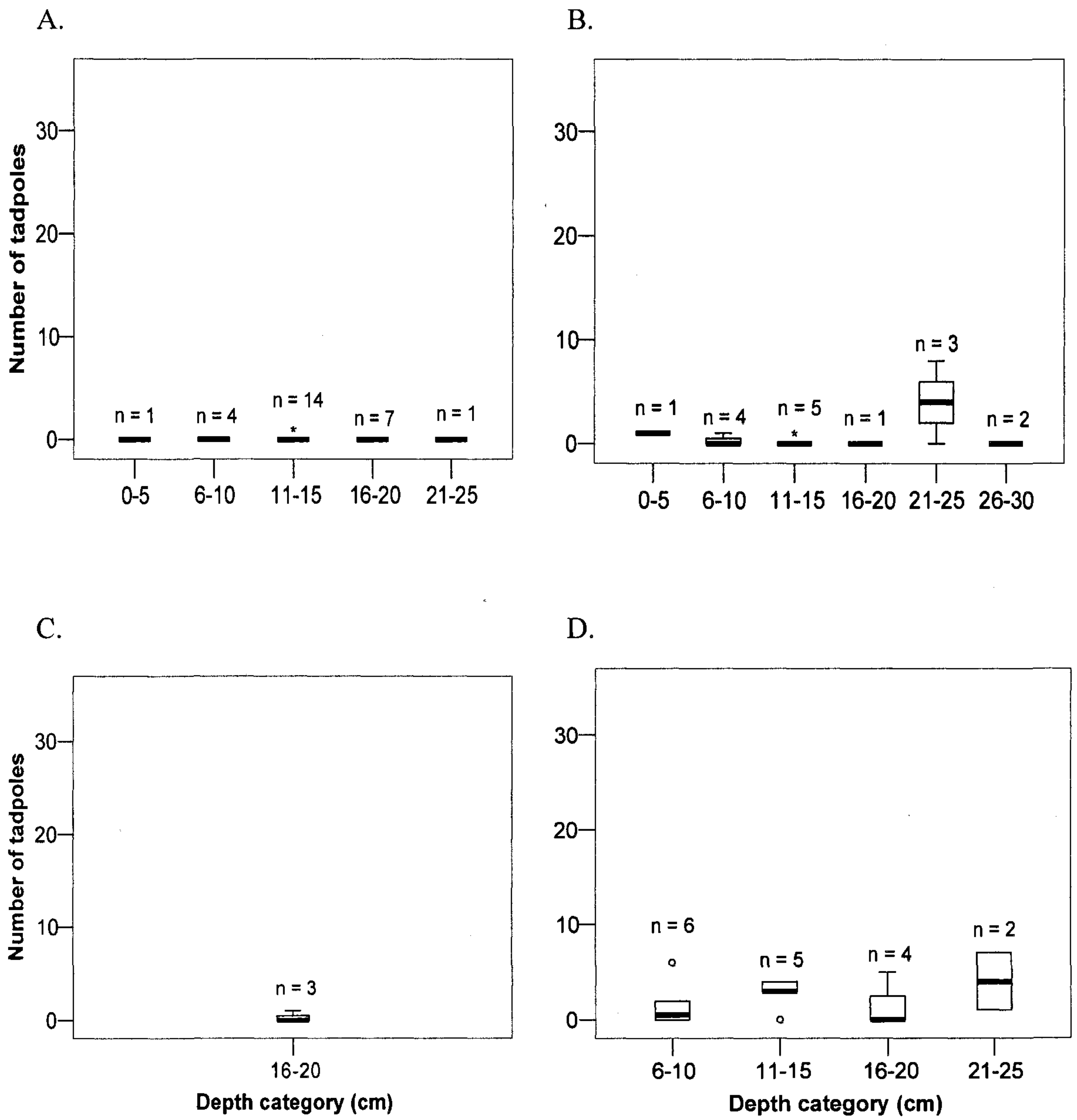


Figure 3.2. Boxplots showing the effect of water depth (cm) on the number of tadpoles in the NW (A), NE (B), SW (C) and SE (D) quadrants in the BC pond. n = number of stake observations representing each category.

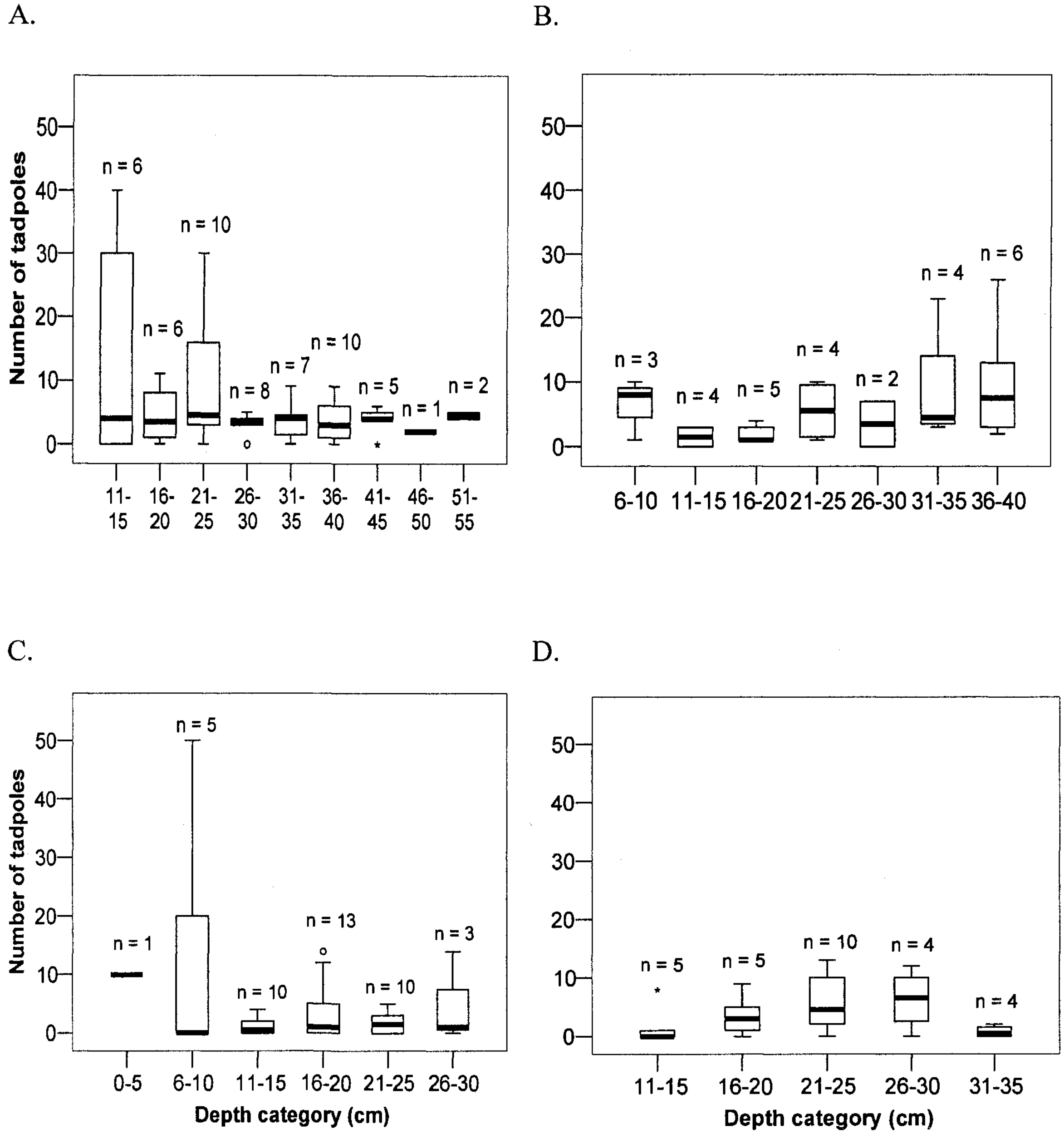


Figure 3.3. Boxplots showing the effect of water depth (cm) on the number of tadpoles in the NW (A), NE (B), SW (C) and SE (D) quadrants in the RANA pond. n = number of stake observations representing each category.

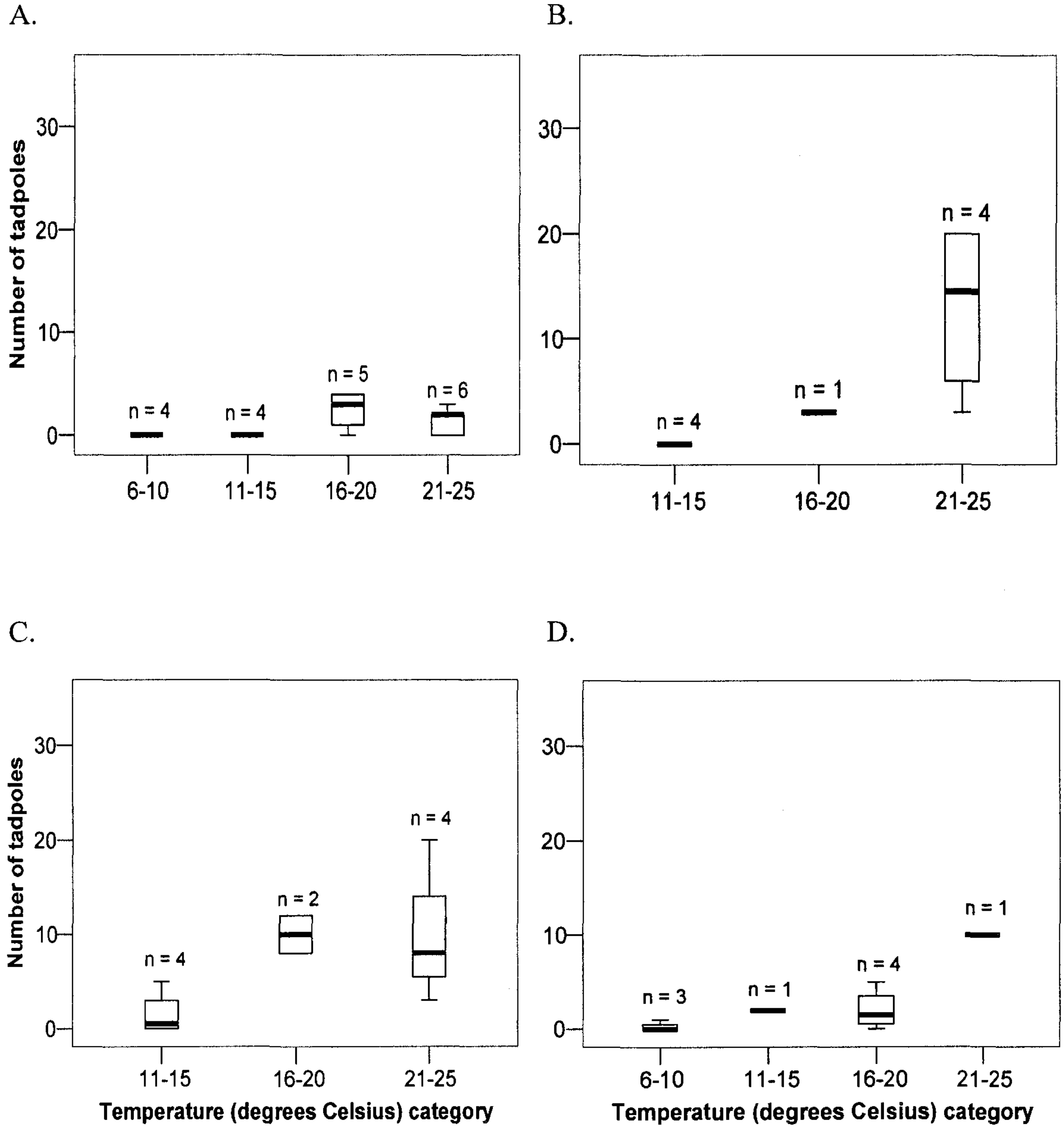


Figure 3.4. Boxplots showing the effect of water temperature (°C) on the number of tadpoles in the NW (A), NE (B), SW (C) and SE (D) quadrants in the GAP pond. n = number of stake observations representing each category.

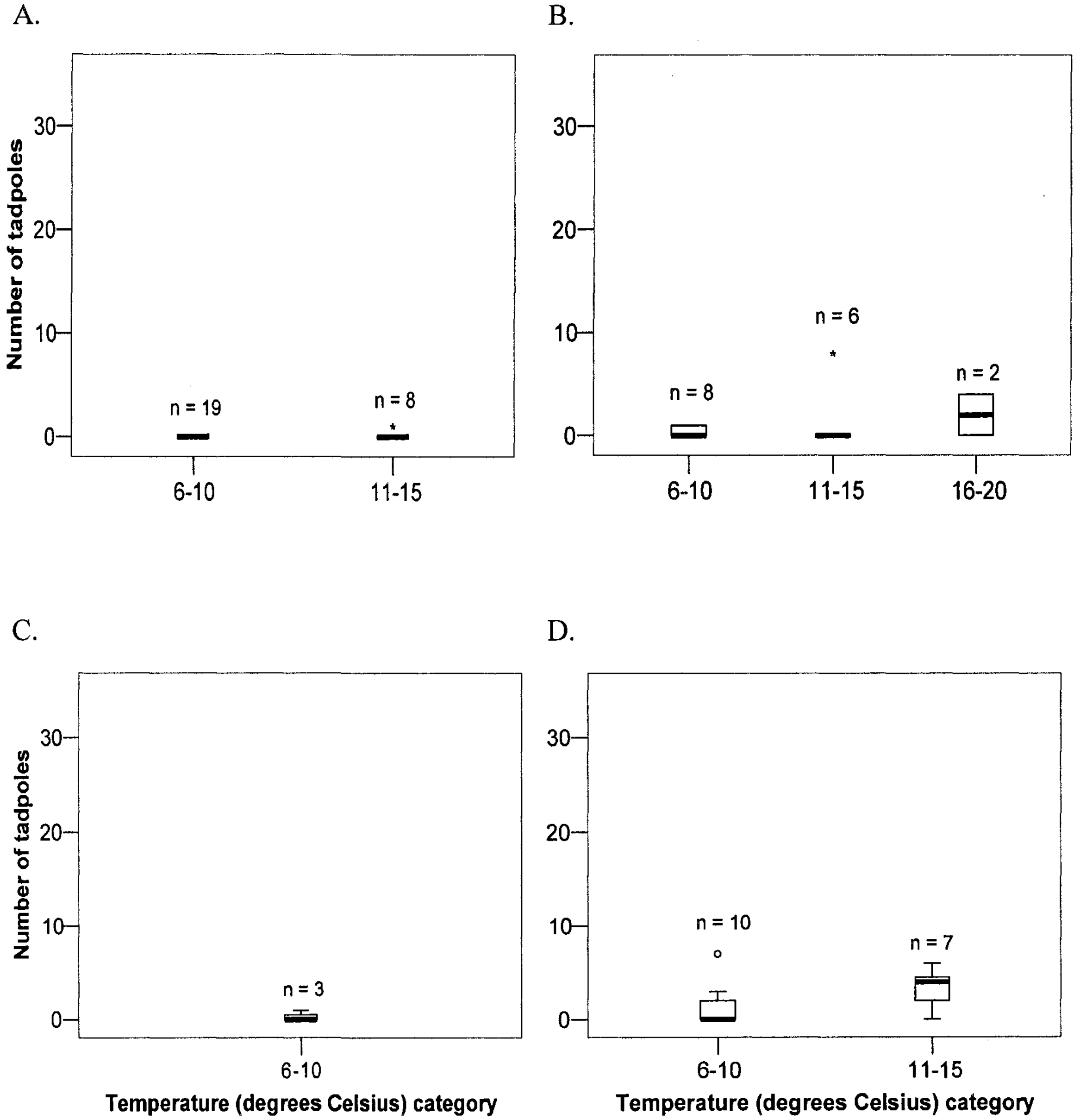


Figure 3.5. Boxplots showing the effect of water temperature (°C) on the number of tadpoles in the NW (A), NE (B), SW (C) and SE (D) quadrants in the BC pond. n = number of stake observations representing each category.

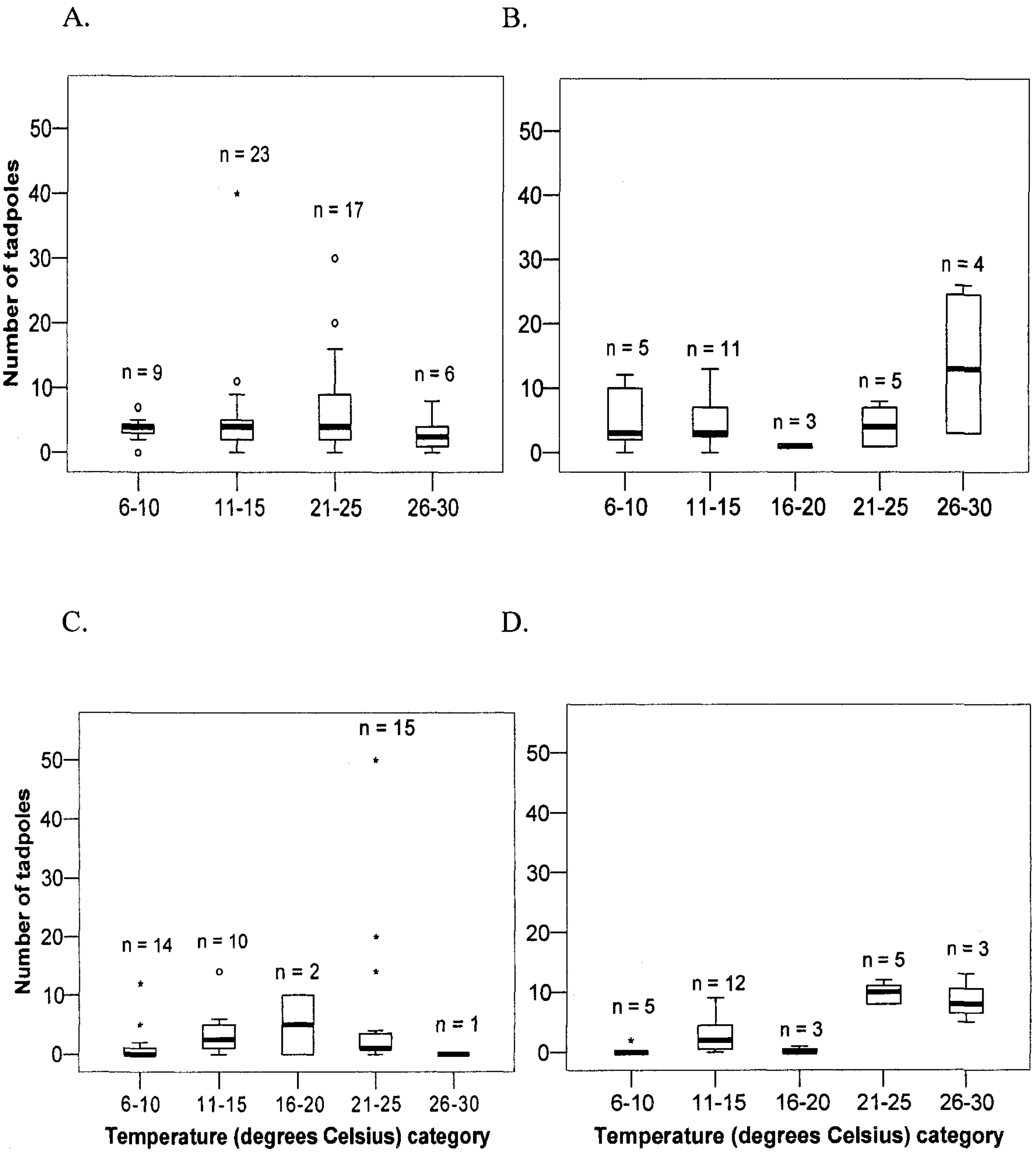


Figure 3.6. Boxplots showing the effect of water temperature (°C) on the number of tadpoles in the NW (A), NE (B), SW (C) and SE (D) quadrants in the RANA pond. n = number of stake observations representing each category.

Table 3.5. Summary table showing the distribution of egg clusters (Chapter 2) and tadpoles early and late in the season in the Gap, BC and RANA ponds. n = number of stake observations.

Pond	Quadrant	Number of egg clusters	Time in season			
			Early (June 6 – 29)		Late (June 30 – July 21)	
			n	Mean (± 1 SE) number of tadpoles	n	Mean (± 1 SE) number of tadpoles
GAP	NW	2	16	0.6 (± 0.3)	3	12.3 (± 8.8)
	NE	0	8	6.5 (± 3.1)	1	3 (± 0.0)
	SW	0	8	4.6 (± 1.6)	2	14.0 (± 6.0)
	SE	3	8	2.4 (± 1.2)	1	2 (± 0.0)
BC	NW	0	27	0.04 (± 0.04)	N/A	
	NE	0	16	0.9 (± 0.5)	N/A	
	SW	0	3	0.3 (± 0.3)	N/A	
	SE	7	17	2.1 (± 0.6)	N/A	
RANA	NW	0	47	4.5 (± 0.9)	8	12.6 (± 4.3)
	NE	8	24	6.4 (± 1.4)	4	1 (± 0.0)
	SW	4	36	2.4 (± 0.6)	6	13.7 (± 7.9)
	SE	2	24	4.5 (± 0.9)	4	0.3 (± 0.3)

results, the model that included all pair-wise interactions had the highest percentage of cases correctly classified (80.6% correct) compared to the single factor model (74.1% correct) or the hypothesized model (74.1% correct). Tadpole presence was influenced only by several interactions and no single factors. The interactions of QUAD*POND, QUAD*JDAY, QUAD*SHADE and JDAY*TEMP were significant predictors of tadpole presence (Table 3.6). Compared to the SW quadrant in the RANA pond, the odds of a tadpole being present were between 0.01 and 0.1 times lower in the NE and NW quadrants in the Gap and BC ponds. As the tadpole season progressed, the odds of finding a tadpole in the NE quadrant increased by a factor of slightly over 1.0 compared to the SW quadrant each day. Tadpole presence was influenced by the presence of shade, but the effect depended on the quadrant. Compared to the shaded stakes in the SW quadrant, the odds of finding a tadpole were 5.7 and 12.8 times higher at non-shaded stakes in the SE and NW quadrants respectively. Lastly, for every 1°C increase in water temperature and each additional day of the tadpole season, the odds of a tadpole being present at a stake increased by a factor of very slightly over 1.0 (Figures 3.7, 3.8, 3.9 and 3.10).

The only interaction that was significant in both analyses was QUAD*JDAY. Because the two analyses tested for factors influencing tadpole distribution in different ways (i.e. abundance versus presence/absence), caution is taken in interpreting results relative to each analysis.

Table 3.6. Estimated logistic regression coefficients for significant variables predicting the presence or absence of *R. pipiens* tadpoles at each stake in the RANA, Gap and BC ponds. Sample size is 263 stake observations.

Variable	**Final Model Step 6**		
	B	**Wald**	**Exp(B), (95% C.I.)**
Constant	- 1.9	12.1 **	0.2
QUAD [b] * POND [a]		30.8**	
QUAD(SE) by POND(GAP)	- 0.2	0.03	0.9, (0.1–5.3)
QUAD(SE) by POND(BC)	- 0.02	0.0	1.0, (0.2–4.4)
QUAD(NE) by POND(GAP)	- 2.6	6.5**	0.1, (0.0-0.5)
QUAD(NE) by POND(BC)	- 3.1	9.0**	0.1, (0.0–0.3)
QUAD(NW) by POND(GAP)	- 2.3	10.1**	0.1, (0.0–0.4)
QUAD(NW) by POND(BC)	- 4.3	14.0**	0.01, (0.0–0.1)
QUAD [b]*JDAY		9.3*	
QUAD(SE) by JDAY	- 0.0	0.7	0.997, (0.9–1.00)
QUAD(NE) by JDAY	0.02	7.4**	1.02, (1.01–1.04)
QUAD(NW) by JDAY	- 0.0	0.6	0.997, (0.9 – 1.0)
QUAD [b]*SHADE [c]		20.6**	
QUAD(SE) by SHADE	1.7	6.1**	5.7, (1.4–22.4)
QUAD(NE) by SHADE	- 1.9	2.6	0.2, (0.0–1.5)
QUAD(NW) by SHADE	2.6	12.0**	12.8, (3.0–54.1)
JDAY*TEMP	0.0	23.0**	1.001, (1.001–1.0001)
N	263		
Model χ^2 [df]	128.9[13]		
% Correctly Classified	80.6		
Nagelkerke R^2	0.5		
Area under the curve (AUC)	0.9		

* $P < 0.05$

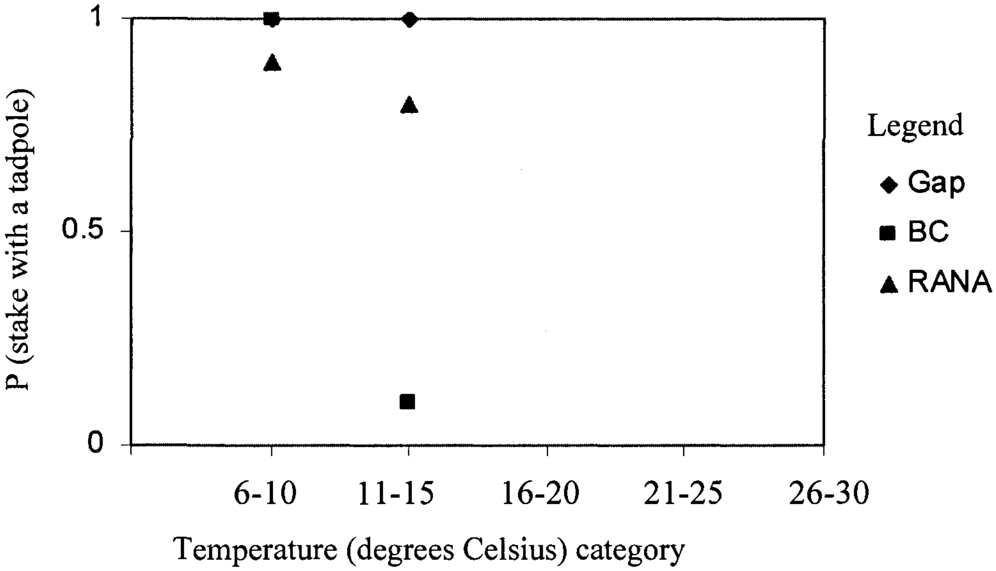
** $P < 0.01$

[a] Pond reference is 'RANA'

[b] Quadrant reference is 'SW quadrant'

[c] Shade reference is 'shaded'

A.



B.

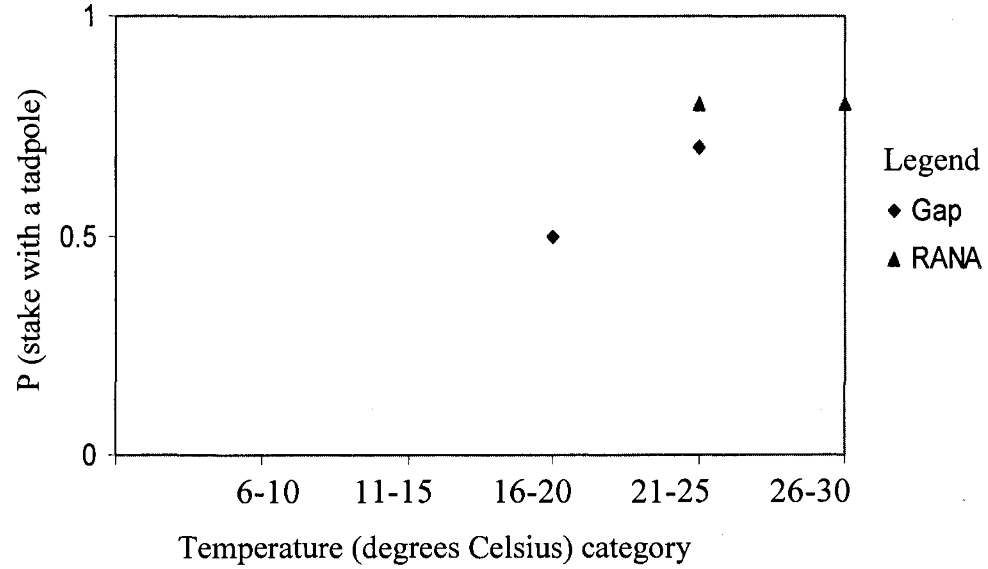


Figure 3.7. Probability (P) of a tadpole being present at a stake in the NW quadrant in each of the Gap, BC and RANA ponds at different water temperatures (°C). Figure is split into stake observations early (June 6 – 29; A) and late in the season (June 30 – July 21; B).

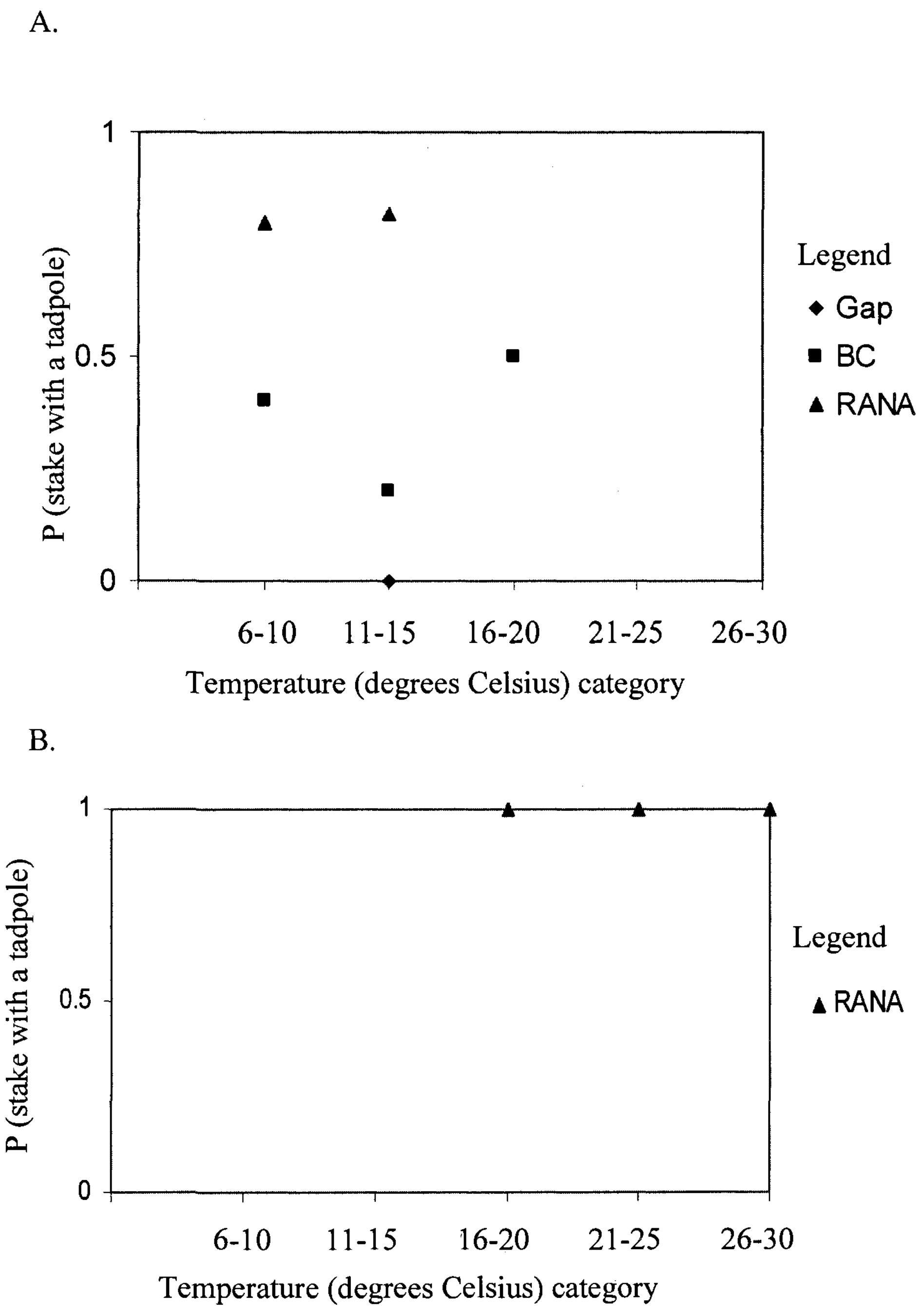


Figure 3.8. Probability (P) of a tadpole being present at a stake in the NE quadrant in each of the Gap, BC and RANA ponds at different water temperatures (°C). Figure is split into stake observations early (June 6 – 29; A) and late in the season (June 30 – July 21; B).

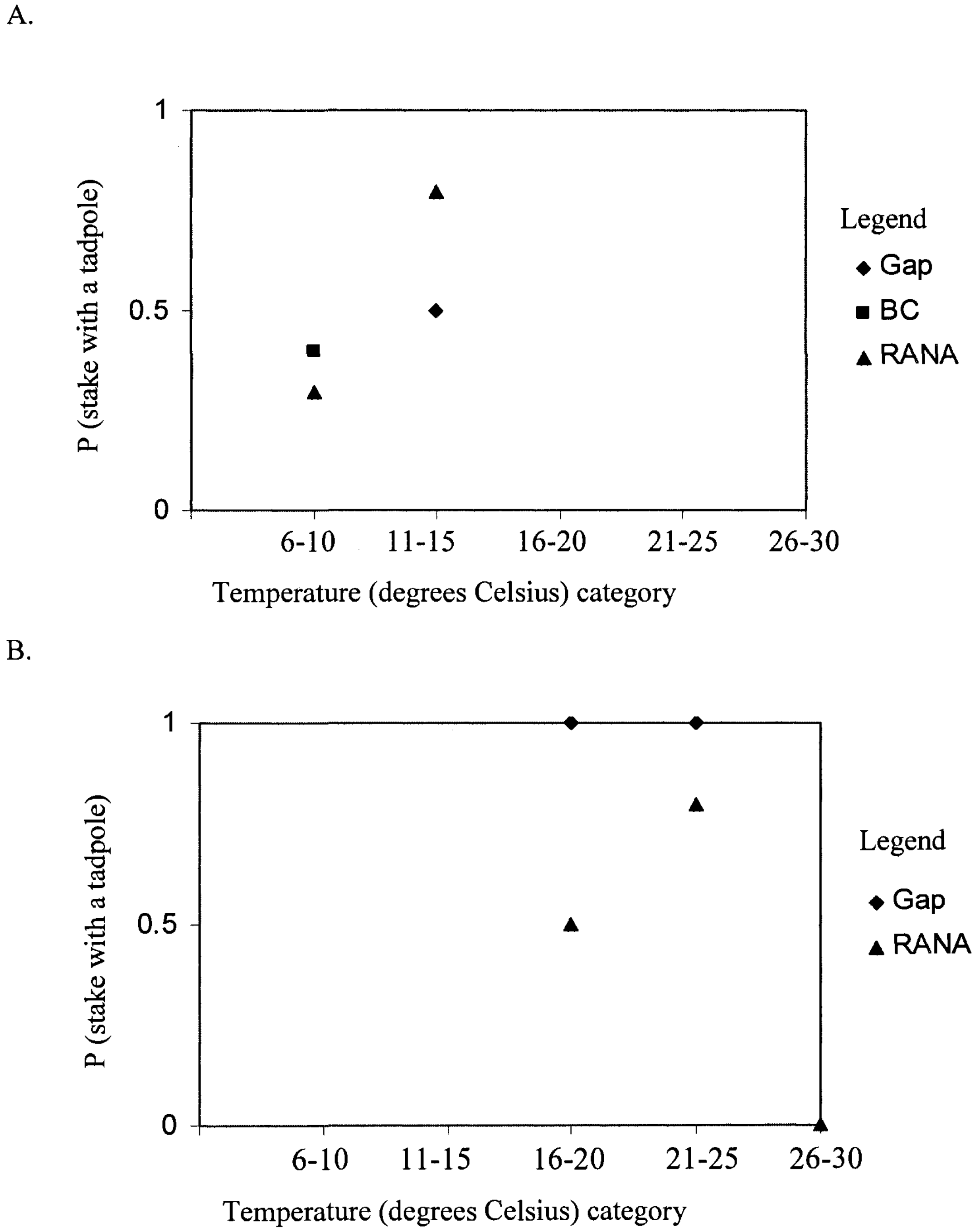


Figure 3.9. Probability (P) of a tadpole being present at a stake in the SW quadrant in each of the Gap, BC and RANA ponds at different water temperatures (°C). Figure is split into stake observations early (June 6 – 29; A) and late in the season (June 30 – July 21; B).

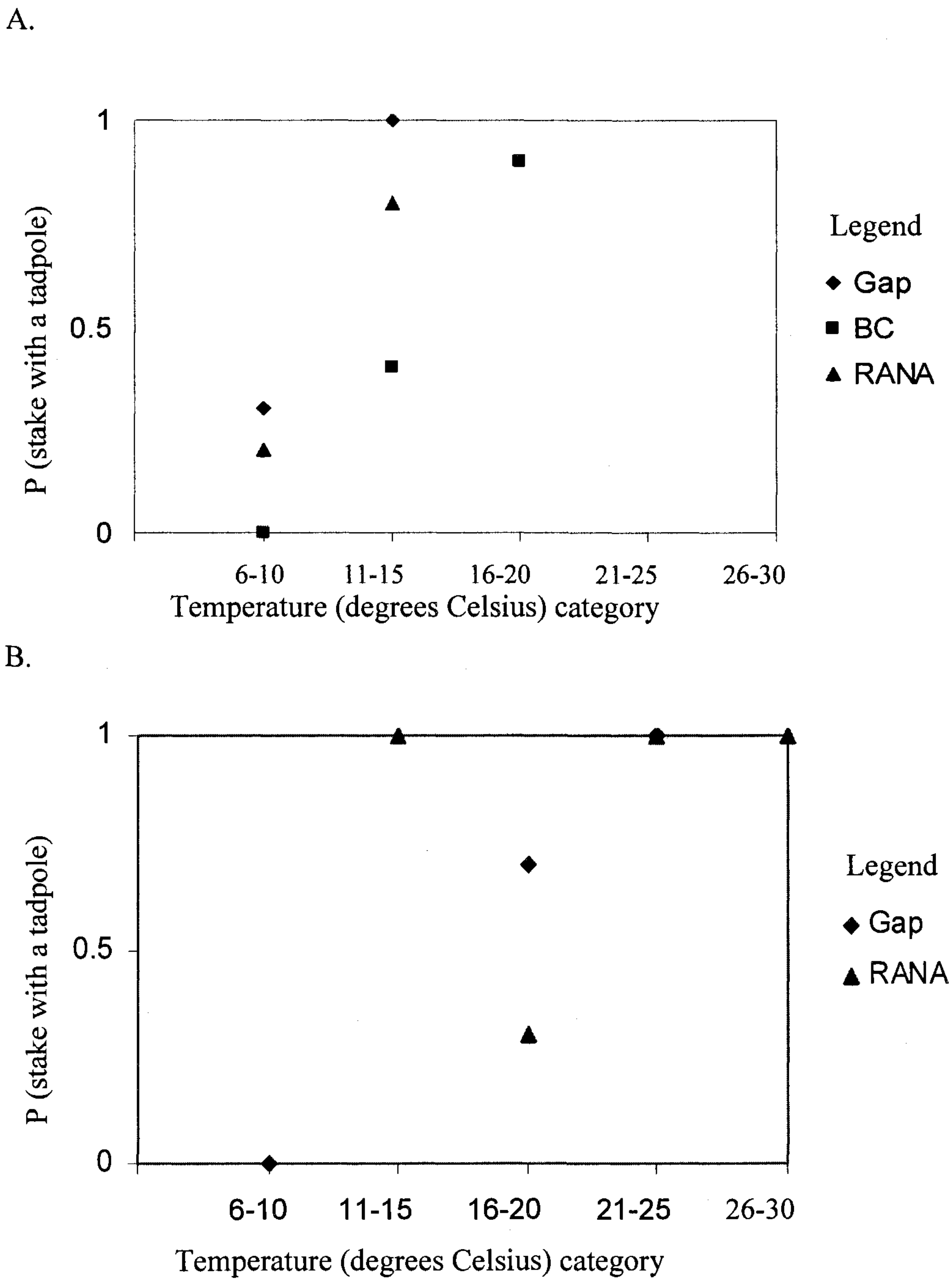


Figure 3.10. Probability (P) of a tadpole being present at a stake in the SE quadrant in each of the Gap, BC and RANA ponds at different water temperatures (°C). Figure is split into stake observations early (June 6 – 29; A) and late in the season (June 30 – July 21; B).

Discussion

The tadpole stage is particularly prone to high rates of mortality (Lane and Mahony, 2002). Consequently, there should be strong selective pressure for tadpoles to discriminate among available microhabitats. Similar to the results for *R. pipiens* egg clusters, tadpoles were non-randomly distributed within ponds, but this was dependent on a variety of factors. In general, tadpoles were more likely to be found in shallow, warm, non-shaded areas within ponds compared to deep, cool, shaded areas. However, the influence of these factors depended on the quadrant, the pond, and the day tadpoles were surveyed. These findings are discussed in detail below, along with proposed mechanisms for each. I conclude with limitations of this study and areas where further research is required.

My findings that the presence but not abundance of *R. pipiens* tadpoles depends on the pond could be due to several factors. There were more egg clusters/m^2 laid in the RANA pond than the Gap and BC ponds (Chapter 2), which could explain why tadpoles were more likely to be seen in the RANA pond. However, if they were more spread out in the RANA pond compared to larger tadpole concentrations in certain areas of the Gap and BC ponds, this might explain why the abundance did not differ significantly between ponds. Also, tadpoles could have been less likely to be seen in the Gap and BC ponds compared to the RANA pond because the Gap and BC ponds dried before most tadpoles had undergone metamorphosis. The drying of ponds has resulted in complete mortality prior to metamorphosis for other *R. pipiens* populations (Merrell 1977, Hine et al. 1981) and for other anurans (Seale 1982, Crump 1991, Murphy 2003, Egan and Paton 2004), but how drying affects the long-term sustainability of *R. pipiens* populations in the

Cypress Hills is unknown. Long-term monitoring of these sites might provide some answers. Lastly, the effect of pond on the presence of tadpoles was quadrant-dependent, which suggests that these ponds were potentially spatially heterogeneous. This finding might provide some insight as to whether tadpoles associate with their natal sites.

The occupancy of quadrants by *R. pipiens* tadpoles was not similar to the egg cluster pattern presented in Chapter 2. Most egg clusters were laid in the NE, SE and SE quadrants of the RANA, Gap and BC ponds respectively. However, there was no strong association with tadpoles for these quadrants in any pond, which suggests that other than the period immediately following hatching, *R. pipiens* tadpoles do not associate with their natal sites in these ponds. These results disagree with those of Pfennig (1990) who found that, in a similar sized pond as that used in my study, spadefoot toad (*Spea multiplicatus*) tadpoles tend to remain near their natal site. Perhaps differences between studies in relation to ecological factors such as water chemistry, temperature and other species present in the ponds will lead to different conclusions, and it might be advantageous for some species to associate with oviposition sites but not others. My results suggest that adult *R. pipiens* may select egg cluster deposition sites in order to maximize the survival of eggs, but that sites, although speculative, may not have been chosen with the intent of tadpoles remaining at natal sites. Since eggs are a fixed life-stage and tadpoles are mobile, *R. pipiens* parental investment in deposition sites might be more important than investment in suitable tadpole locations, because presumably tadpoles are able to protect themselves through mobility.

Both presence and abundance of *R. pipiens* tadpoles varied according to Julian day, but the direction of the effect depended on the quadrant. Mortality is generally very high for anuran tadpoles due to predation (Thurnheer and Reyer 2000). Perhaps tadpole predators were non-randomly distributed within ponds, which would explain why tadpole abundance may have increased or decreased throughout the season depending on the quadrant. Also, increased activity or a stronger affinity for the waters' edge by older, larger tadpoles later in the season (Golden et al. 2001) could make them more visible. This would increase the probability of seeing them, but these effects may not be uniform across quadrants within ponds. Quadrants also dried unevenly throughout the season; therefore, tadpole abundance necessarily increased in certain areas of ponds compared to others as the season progressed. Although tadpole abundance was higher in the SW and NW quadrants later in the season compared to the other quadrants, tadpole presence increased in the NE compared to the SW quadrant towards the end of the season. This suggests that tadpoles may have been more spread out in the NE quadrant towards the end of the season, but that they may have been at fewer sites but in larger aggregations in the SW and NW quadrants. Lastly, the quadrants are not independent of each other, therefore, if tadpole abundance increases in some quadrants it must decrease in others. Although a more in-depth approach is needed to determine how tadpole distribution is related to predator distribution, pond-drying and their effect on activity levels, my results suggest that the non-random distribution of *R. pipiens* tadpoles within these ponds changes throughout the season.

The observation that, in general, *R. pipiens* tadpoles selected warm areas compared to cool areas of these particular ponds corroborates the findings of other research. A preference for higher temperatures may be the most important factor influencing tadpole distribution (Beiswenger 1977; Schley et al. 1998; Thurnheer and

Reyer 2000; Haramura 2007). The reason why tadpoles select warm temperatures is not well understood, but evidence suggests that warm temperatures result in a higher metabolic rate and faster development (Noland and Ultsch, 1981; Alford 1999). Rapid growth and development are especially important for species living in temporary habitats where pools dry up quickly (Semlitsch and Wilbur 1988), or potentially in aquatic habitats with a large number of larval predators. The extent to which tadpole density increased with increasing temperature depended on the quadrant within ponds, which suggests that other factors (e.g. oxygen levels; Moore and Townsend 1998) that are not uniformly distributed within ponds may work in concert with temperature to influence tadpole abundance. Further, tadpoles were more likely to be present as water temperature increased, but only as the season progressed. Combined with the result that tadpole presence also depended on Julian day, but that the effect differed by quadrant (as discussed above), suggests that these three factors (quadrant, Julian Day and water temperature) likely work together to influence tadpole presence. However, I did not have enough data to test three-way interactions, but increased sampling intensity (i.e. more data) may clarify this relationship.

The presence of *R. pipiens* tadpoles did not depend on water depth, but where they did occur, their abundance generally decreased in most quadrants with increasing water depth. Although an affinity for shallow water is often explained by deeper zones being cooler than shallower zones (Schley et al. 1998), my results did not find a significant interaction between water depth and temperature. However, I only recorded water temperature at a depth of 10 cm, which might not represent the actual temperature experienced by tadpoles if they are not located at a depth of 10 cm below the surface.

Also, different approaches to measuring 'shallow' versus 'deep' make direct comparisons among other studies and mine difficult. Further, my results suggest that the affinity for certain depths is quadrant-dependent and, therefore, depth by itself is not an accurate predictor of high tadpole densities and other factors should be considered.

In some quadrants, *R. pipiens* tadpoles were more likely to be present in non-shaded areas compared to shaded areas. This is usually explained by the increase in water temperature that is generated from increased light intensity (Beiswenger 1977) but water temperature and shade did not interact in this study. Any instances where the observation area was partly shaded was included in the 'shaded' category for analysis; therefore such areas may have been warmer than expected given that they were not entirely shaded. Light exerts a strong, positive effect on periphyton, a major food source for anuran tadpoles (Kim and Richardson 1999) which could help explain the association of *R. pipiens* tadpoles with non-shaded areas, but this remains speculative as food availability was not measured in these ponds. Also, the effect of shade on tadpole presence depended on the quadrant, which may suggest that quadrants did not receive equal amounts of sunlight, or sunlight alone is not a major driving force behind tadpole spatial distribution. Finally, although shade was important for predicting tadpole presence, it did not influence tadpole abundance. Additional research is needed to determine the importance of shade on tadpole distribution and how other factors not tested in this study (e.g. food availability) interact with light to influence tadpoles.

This study demonstrated that temperature, light, depth, pond, day and quadrant within these ponds interact in some way to influence the spatial distribution of *R. pipiens* tadpoles, but other aspects of tadpole ecology were not examined and warrant further investigation for *R. pipiens*. Most surveys in this study were conducted at midday, when the sun was generally overhead, but previous research has shown that anuran tadpoles undergo daily cycles dependent on the weather (Beiswenger 1977; Warkentin 1992) that may change throughout the season (Floyd 1984). Research examining the joint effects of time of day, day in the season, and weather conditions on tadpole distributions will help shed light on these relationships. Although I could not determine the age of tadpoles, there is evidence that age-specific tadpole activity is strongly regulated by competition and predation pressures (Morin 1983; Alford 1986; Lawler 1989; Barreto and Moreira 1996; Azevedo-Ramos et al. 1999; Babbitt 2001; Spieler 2003; Watkins and McPeek 2006). For instance, physiological changes associated with the onset of metamorphosis, including a major shift in diet, will alter their behaviour and susceptibility to predation (Bovbjerg and Bovbjerg 1964; Golden et al. 2001). Therefore, it may be more appropriate to examine spatial distribution among animals of the same age and/or developmental stage only. Lastly, how other amphibian species (e.g. *Pseudacris maculata, Ambystoma tigrinum*) using these ponds influence the activity of *R. pipiens* tadpoles and their resulting spatial distribution has not been investigated but could play a major role, especially if they compete for similar resources or prey upon each other.

This is one of the few studies that address the spatial distribution of tadpoles within natural breeding ponds. Some of my results challenge those of laboratory experiments on *R. pipiens* tadpoles. For instance, Noland and Ultsch (1981) found that *R. pipiens* tadpoles decrease in abundance with increasing water temperature in a laboratory setting and my study found the opposite relationship. Whereas naturally occurring ponds are spatially heterogeneous environments (Wilbur 1997), laboratory studies are able to control nuisance variables (e.g. food availability) that are not being tested for, which makes the interpretation of results potentially simpler. However, studies in the wild may offer a more realistic overview of natural tadpole distribution than laboratory-based investigations (Bridges 2002; Halverson et al. 2006). More studies that compare results from both artificial and natural settings will help clarify the most appropriate experimental designs, in order to maximize their practicality and value.

Conditions during early development can have considerable effects on the later performance of individuals and cohorts (Beckerman et al. 2002). Therefore it could be argued that one cannot fully understand the implications of tadpole behaviour and microhabitat selection without studying the next life-stage. The next chapter explores the spatial distribution and emigration patterns of metamorphosed frogs at the RANA pond and whether it reflects or differs from egg cluster (Chapter 2) or tadpole distribution.

CHAPTER 4: EMIGRATION BEHAVIOUR OF METAMORPH *RANA PIPIENS* AND CONSERVATION IMPLICATIONS

Introduction

Dispersal regulates animal populations through immigration and emigration, and has important consequences for gene flow, life history traits and both population and community dynamics (Clobert et al. 2001; Mazerolle and Vos 2006). For pond-breeding amphibians, dispersal of newly-metamorphosed individuals (metamorphs) plays an important role in linking habitats and isolated amphibian populations (deMaynadier and Hunter 1999; Marsh and Trenham 2001). Little is known about metamorph dispersal and how they choose their emigration direction (Rothermel and Semlitsch 2002). I previously demonstrated that northern leopard frog (*Rana pipiens*) egg deposition sites and tadpole distributions are non-random in their natal ponds, and also that these distributions are not related to each other (Chapters 2 and 3). In this chapter, I build on previous chapters by examining whether pre-metamorph life stages influence the subsequent orientation of metamorphosed individuals. More specifically, this chapter explores the terrestrial distribution of *R. pipiens* metamorphs before and during emigration, relates the distribution of early life history stages to each other, and highlights how studies of emigration in free-living animals may contribute to conservation efforts.

Amphibian orientation

The locomotory behaviour of anuran amphibians includes striking mass movements of reproductive individuals to their breeding pond (Sinsch 1990), as well as dispersal distances of over 800 m by metamorphs (Dole 1971). To accomplish these movements, orientation mechanisms are used by amphibians and these have been well documented and include: celestial (Dole 1972), acoustic (Dole 1972; Sinsch 1991), chemical (Oldham 1967; Dole 1972; McGregor 1989; Berven and Grudzien 1990; Joly and Miaud 1993; Sjogren-Gulve 1998; Hayward et al. 2000; Waldman and Bishop 2004), visual (Berven and Grudzien 1990; Malmgren 2002; Rothermel 2004), and magnetic compass cues (Deutschlander et al. 2000; Freake et al. 2002). Adult anurans have the ability to use many orientation cues to which they have been exposed earlier in life (Hayward et al. 2000), and indeed they exhibit remarkable homing and navigation abilities (Phillips et al. 1995; Pough et al. 2001). However, newly-metamorphosed individuals emigrating from their natal pond for the first time have no previous knowledge of the surrounding terrestrial habitat, other than cues they are able to detect during their larval period.

The orientation ability of metamorphs is a key issue, especially considering the costs associated with terrestrial activity (Mazerolle and Vos 2006). Ectothermy adds to the cost of dispersal because it forces anurans to invest significant time in behavioural temperature regulation to avoid both low and high ambient temperatures while on land (Sinsch 1991). Furthermore, the limited terrestrial activity of anurans relative to other vertebrates may be due to their highly permeable skin, which makes them extremely sensitive to changes in microclimate (deMaynadier and Hunter 1999). Metamorphs are particularly vulnerable to desiccation while on land because of their greater surface area to volume ratio compared to adults (Ray 1958; Matsuda and Richardson 2005). Long excursions resulting from incorrect orientation could lead metamorphs into hostile environments and lessen their chances of survival (Mazerolle and Vos 2006). The terrestrial emigration abilities of metamorph *R. pipiens* have never been formally tested, but are important because populations are dwindling in Alberta (Roberts 1992) and the recolonization and long-term persistence of their populations may rely on successful metamorph dispersal (Seburn et al. 1997; Johnson 2003; Rittenhouse and Semlitsch 2006). Another unexplored aspect of movement behaviour relates to whether these patterns are fixed or are subject to variation in metamorphs. If local environmental conditions are unpredictable, it may be advantageous to develop some sensitivity to cues related to changing environmental factors (Massot et al. 2002). A potential first step in testing this is by observing whether movements are repetitive at the same site, where local conditions are assumed to be relatively stable.

Distribution of early life stages

The emerging importance of metamorph orientation behaviour to sustaining amphibian populations has encouraged experimental approaches to understanding emigration behaviour. Most studies, however, have considered the metamorph life-stage only, and do not take into account the prior experience of these individuals in their natal habitats. For instance, Rothermel (2004) and Patrick et al. (2007) translocated tadpoles, just prior to metamorphosis, from their natal site to artificial sites (i.e. wading pools) to test the ability of resulting metamorphs to orient towards suitable habitat. The discovery that metamorphs did not orient towards safe habitat led to the conclusion that they have a limited perceptual range (Rothermel 2004) or use indirect cues (e.g. the sun's position) to orient (Patrick et al. 2007). In fact, Patrick et al. (2007) found that translocated metamorphs emigrated in a similar direction as did non-translocated metamorphs from the same natal site in previous years. These authors did not consider that translocated individuals might be orienting according to cues detected during the tadpole period at their natal site.

Given that some tadpole species show strong associations with familiar environmental cues to which they are exposed after hatching (Pfennig 1990; Hayward et al. 2000), early experiences during the aquatic life stages may influence metamorph orientation as well. However, if the availability or distribution of suitable terrestrial sites around the natal pond changes throughout egg and tadpole development, metamorphs may benefit by only responding to cues just prior to emigration. Whichever the case, the interpretations of experimental studies could be missing a key aspect of the development of orientation behaviour. Comparing the natural spatial distributions of egg clusters, tadpoles and metamorphs within the same pond can provide some insight into when orientation towards the terrestrial environment begins, and therefore help improve hypothesis development for emigration studies.

Body size effects

In addition to orientation towards suitable habitats, one of the most important factors affecting anuran success in terrestrial habitats is body size (Smith 1987). Larger metamorphs enjoy increased survival, earlier age at first reproduction, and larger size as adults relative to smaller metamorphs (Berven 1990). Extreme phenotypic plasticity in size at (and timing of) metamorphosis has been demonstrated many times in anurans (e.g. Loman 2002; Boone 2005; Schiesari et al. 2006) and has been found to be affected by temperature (Loman 2002), intra-specific competition (Healy 1975; Reques and Tejedo 1995; Loman 2002), food (Newman 1989; Murray 1990; Doughty and Roberts 2003; Rudolf and Rödel 2007), the length of time a pond holds water (Semlitsch and Wilbur 1988; Newman 1989; Altwegg 2002), predators (Altwegg 2002; Smith et al. 2005) and egg size (Loman 2002). However, whether larger metamorphs orient differently to smaller metamorphs has received less attention.

Conservation relevance

In the wake of worldwide amphibian declines, information on amphibian spatial distributions and emigration behaviour is critical for conservation programs such as reintroductions (Semlitsch 2002; Johnson 2003). Reintroducing animals to their previously occupied range has become a popular tool in amphibian management (Dodd and Seigel 1991), but the success of such programs varies. For instance, 5,000–6,000 eggs of the natterjack toad (*Bufo calamita*) were released in two consecutive years in Great Britain, which resulted in 14 successfully restored populations out of 20 attempts

(Denton et al. 1997). The release of some 4,000 metamorphs led to the successful reintroduction of European tree frogs (*Hyla arborea*) in Latvia (Zvirgzds 1998). In contrast, the release of half a million tadpoles, metamorphs, juveniles and adults of Houston's toad (*Bufo houstonensis*) in Texas has apparently not resulted in any new populations (government reports cited by Dodd and Seigel 1991). Clearly, reintroduction success does not depend solely on the life-stage or number of animals released. Rather, one factor that is associated with unsuccessful reintroductions is the lack of necessary information concerning the habitat requirements and dispersal abilities of the species (Macdonald and Johnson 2001; Pilliod et al. 2002; Semlitsch 2002). Metamorph emigration studies can help to determine whether metamorphs are able to detect suitable habitat or whether they need to be 'guided' in the right direction in order to maximize survival following reintroduction.

Rana pipiens has vanished from much of its historic range in Alberta (Roberts 1992). Between 1999 and 2004 more than 13,000 metamorphs were reintroduced across three sites within the historical range of the species in Alberta (Kendell 2004). Three years later, there is no evidence to suggest that any of the reintroductions were successful (i.e. second generation adults breeding; Semlitsch 2002). Metamorphs are the main dispersal agent in anuran populations (Berven and Grudzien 1990), suggesting that their orientation towards suitable habitat is important for population persistence. Therefore, a potential contributing factor to the low reintroduction success rate is that the natural orientation mechanisms of reintroduced metamorphs may be disrupted during capture, handling, and manipulations associated with the reintroduction, leading them into unsuitable habitat. If this is the case, it is possible that reintroduction sites provide excellent conditions for survival, but animals are 'doomed' prior to reintroduction because of the procedures used to move them. One way to gain insight into the movement behaviour of reintroduced animals is to consider the distribution and emigration patterns of free-living animals under similar circumstances (Stamps and Swaisgood 2007). My study applied similar procedures to those used in previous *R. pipiens* reintroductions to investigate naturally occurring *R. pipiens* populations. It not only tests whether emigration direction is consistent, but also helps determine whether handling disrupts metamorph orientation.

Objectives and Hypotheses

This chapter addresses some of the aforementioned unexplored areas of amphibian ecology as they relate to naturally occurring *R. pipiens* populations. For the first objective I investigate the spatial distribution of metamorphs prior to emigration. Similar to the hypotheses for tadpoles, I predict that metamorphs will be associated more often with warm, un-shaded areas of breeding ponds compared to cool, shaded areas, because warmer temperatures foster accelerated growth rates in ectotherms (Smith-Gill and Berven 1979). I also predict that metamorphs will be associated with shallower versus deeper water because it is assumed that catching terrestrial prey is easier if in shallow rather than deeper water. This is because presumably the transportation costs from water to land will be less than if metamorphs are in deeper water. I predict that metamorphs will be randomly distributed according to quadrant within ponds (Chapter 2), when water temperature and depth are accounted for. Finally, since breeding ponds are unsuitable as overwintering sites, necessitating metamorphs to emigrate from the pond to an overwintering site (Merrell 1977), I predict that metamorph abundance at breeding ponds will decrease as the summer progresses.

Secondly, I determine whether the natural emigration patterns of metamorphs differ between two years. Assuming metamorphs perceive and respond to cues in either the aquatic environment as tadpoles and/or the terrestrial environment as frogs, I predict that most metamorphs in a given year will emigrate in the same direction. Furthermore, unless there are obvious physical changes to the pond or surrounding landscape between years, I predict that emigration direction will not differ between years.

Thirdly, using results from previous chapters I examine whether the spatial distribution of the egg cluster or tadpole life stage predicts the distribution or emigration direction of metamorphs. The influence of environmental cues detected throughout development could improve the quality, quantity and nature of available information for terrestrial emigration (Massot et al. 2002). However, because terrestrial environments likely present new challenges for frogs (e.g. different prey requirements) which are not necessarily related to those of their natal or larval sites, I predict that distributions of the aquatic life stages will not be related to the terrestrial distribution of metamorphs.

For the fourth objective I investigate whether metamorph body size influences emigration direction. Because there is no reason to assume that different sized animals should orient using different mechanisms, I predict that emigration direction will not be influenced by body size.

For the fifth objective I determine whether metamorph body size differs depending on the timing of emigration. I predict that emigrants leaving the breeding pond later in the season will be larger than earlier emigrants because they will likely have had more time to feed and therefore increase in body size.

For the sixth objective I investigate whether metamorph body size differs from year to year. I predict that body size will differ between years only if the number of metamorphs emigrating from the pond also differs, and that a greater metamorph density would result in smaller-sized metamorphs.

I determine whether metamorph emigration direction is persistent for the seventh objective. This also relates to whether handling affects their natural movement patterns. Assuming that the "urge to disperse" is retained throughout the dispersal season (Dole 1971) and after metamorphs are returned to their pond following their first emigration attempt, I predict that metamorphs will emigrate in a similar direction to that taken initially. This is because I assume that any environmental cues used in orientation persist at the pond between captures. Further, I assume that metamorphs will not be discouraged from emigrating towards a certain direction if their first attempt was unsuccessful or was a negative experience because even if capture and handling cause acute stress (Teixeira et al. 2007), it has been shown to not suppress natural behaviour in other amphibians and reptiles in the time period used in this study (e.g. Moore et al. 2000; Massot et al. 2002). That is, if emigration is random amongst all first-time captures, then recaptured metamorphs will also emigrate randomly. But if metamorphs exhibit directional movements from the pond, recaptured metamorphs will emigrate in a similar direction relative to their first capture.

Lastly, I explore the relationship between weather variables and metamorph emigration for the eighth objective. Both warm conditions and rainfall are considered to be the most important environmental cues stimulating amphibian migrations (Semlitsch and Ryan 1999). Therefore, I predict that metamorph emigration will be associated with warm, humid conditions.

Methods

Survey technique

The study sites used in this research are described in Chapter 2. The distribution of metamorphs prior to emigration was evaluated at the Researching Amphibian Numbers in Alberta (RANA) and ENR (Eagles Nest Ranch) ponds in 2006. Complete drying of the BC (Battle Creek) and Gap ponds by July precluded their use in this study. Metamorph survey methods were identical to those used for tadpoles (Chapter 3). In short, I counted the number of metamorphs at 5 m intervals around the shoreline of each breeding pond. Since stakes were placed every 5 m around the shoreline (or as close as possible for the first and last stake installed), the number of stakes in each quadrant approximated the length of available shoreline in each quadrant. Water temperature, depth, and whether the area around the stake was shaded or not were also recorded at each stake observation. If stakes were approached slowly, metamorphs exhibited minimal signs of disturbance (i.e. rarely jumping > 1 m), meaning that the risk of counting metamorphs twice during one survey was low. However, these instances were noted. Because I conducted surveys and trapped immigrants and emigrants (see below) concurrently at the RANA pond, any immigrant metamorphs or sub-adults may have been included in the surveys because they were not distinguishable from resident frogs.

Ponds within 1 km of the ENR pond showed no signs of successful breeding; therefore, I assumed that the ENR pond was the natal site of all observed metamorphs (refer to Chapter 3 for further details on survey methods).

Metamorph emigration

I investigated metamorph emigration at the RANA pond using drift fence and pitfall traps in both 2005 and 2006. The BC pond was also set-up for this part of the study but dried in both years before tadpoles metamorphosed. Time constraints and a beaver dam prevented installation of pitfall traps and drift fences at the Gap and ENR ponds respectively. Therefore, further descriptions and analyses regarding trapping refer to the RANA pond only. Pitfall traps and drift fences are widely used for monitoring terrestrial amphibians because of very low mortality and escape rates (Brown 1997; Adams and Freedman 1999; Perkins and Hunter 2002). The pond was completely encircled with black silt fencing that stood approximately 90 cm above ground, attached to wooden stakes. The bottom of the fencing was buried 10–20 cm below ground to prevent frogs from bypassing the fence below. The fence was installed as close to the water's edge as possible, contingent on pond topography and confidence that traps would not be flooded.

Pitfall traps consisted of PVC (poly-vinyl chloride) pipe 14.5 cm wide and 36.4 ± 0.7 cm deep (the depth varied depending on obstacles in the ground such as large roots and rocks). This pitfall trap design is extremely effective in catching anurans (Moulton et al. 1996; Crawford and Kurta 2000; Wahbe et al. 2004; Rice et al. 2006) and has increased the capture efficiency of *R. pipiens* at the RANA pond in recent years

(Wilkinson and Kempin 2004). Trap dimensions were also tested at the Calgary Zoo prior to installation to ensure that metamorph *R. pipiens* could not escape. Pairs of pitfall traps were installed at approximately 10 m intervals along the fence, on the inside and outside of the fence in order to catch both immigrating and emigrating animals. The exact distance between pairs of traps was dependent on vegetation and subsurface obstacles such as roots and rocks. Traps were dug into the ground so that the rims were flush with the soil surface (Corn 1994). Sixteen pairs of traps were in place in 2005, with an additional pair of traps installed in 2006 to fill a large gap in trap distribution around the fence (large rocks under the soil prevented installation in certain areas in 2005). Each trap contained a moist sponge, a rock for perching, and a stick to allow small mammals to escape (Greenberg et al. 1994; Perkins and Hunter 2002; Rothermel and Semlitsch 2006).

A common assumption of drift fence studies is that frogs wander in a fairly straight line from the pond and fall into one of the two traps closest to their point of exodus (e.g. Malmgren 2002). However, this has never been formally tested for *R. pipiens* and metamorph movement around the edge of the pond on land prior to reaching the fence could confound measures of emigration direction from the pond. To minimise such movement, four fences were installed perpendicular from the waters' edge to the outer fence, dividing the traps around the pond into four quadrants (see Chapter 2 for details concerning the designation of quadrant locations; Figure 4.1).

Traps were opened before metamorphosis in both years (i.e. end of June). Preliminary observations in 2005 revealed that metamorph emigration occurs mainly at night. Accordingly, traps were checked between 0700 and 0900 hrs every day. Whether metamorphs left the pond just after dusk or just before dawn or any time in-between

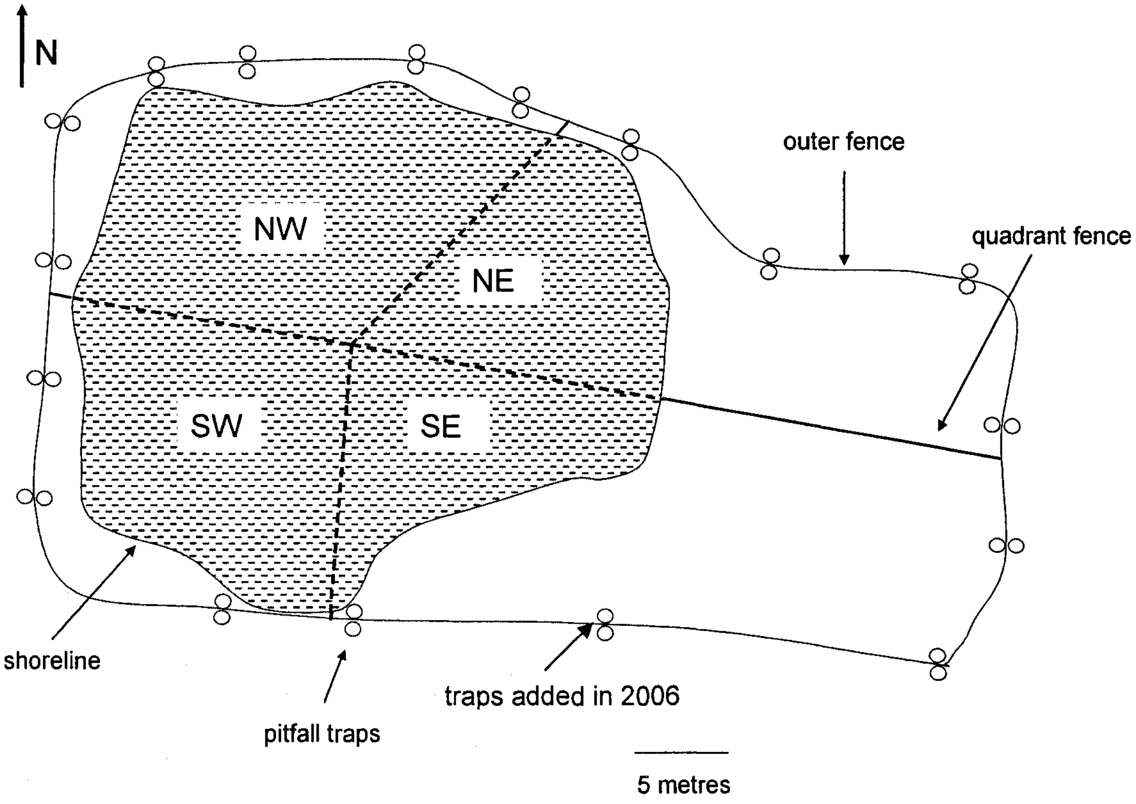


Figure 4.1. Diagram of the set-up of fencing and pitfall traps at the RANA pond in 2005 and 2006. The shoreline location was determined at the beginning of the 2006 breeding season and the location of fences and traps (except for the added traps in 2006) were identical in both 2005 and 2006. The hatched area indicates the pond, and dashed lines within the confines of the pond demarcate quadrant designations.

could not be determined.

Measurements and marking procedures

For each *R. pipiens* caught, I recorded the trap number, quadrant and age based on size (metamorphs are < 50 mm; Merrell 1970; Hine et al. 1981). Metamorphs were weighed (to the nearest 0.1 g) using a Pesola spring scale, and their snout-vent length (SVL; from the tip of their rostrum to the cloaca) was measured to the nearest 0.01 mm using dial calipers (OEM Pro Am Tools, Inc). Every metamorph was marked with Visible Implant Elastomer (VIE; Northwest Marine Technology, Inc). VIE is a two-part silicone based material that consists of elastomer and curing agent at a ratio of 10:1 mixed immediately before use (Northwest Marine Technology 2003). The mixture is then loaded into a syringe and injected into the webbing of the hind feet as a liquid that soon cures into a pliable, biocompatible solid (Nauwelaerts et al. 2000). To prevent transfer of potential amphibian diseases, the syringe was sterilized with alcohol prior to and after injection into each metamorph. VIE tags are rapidly applied and externally visible, and amphibian studies have shown that they exhibit high retention rates (up until at least four months; Nauwelaerts et al. 2000; Marold 2001; Northwest Marine Technology 2003). A colour unique to the quadrant in which the animal was caught was implanted in the webbing of the right hind foot.

To evaluate handling effects on natural movement patterns, all metamorphs were released back into the pond after processing. The quadrant into which they were released changed each day, moving in a counter-clockwise direction. All recaptured metamorphs were marked on the left hind foot with the colour demarcating the quadrant in which it was recaptured. I assumed that metamorphs did not behaviourally avoid the direction in which they first emerged (see Introduction), and that any cues used for orienting were still present in the environment after their replacement back into the pond. Upon recapture, the previous mark and new trap number were recorded. Their weight and SVL was measured and recorded. Any recaptured metamorphs (including 2nd, 3rd, or 4th time caught) were moistened with pond water and released at least 5 m from the fence on the opposite side from where they were caught, in line with their direction of travel prior to recapture (i.e. frogs caught in outside traps were released on the inside and vice-versa). Any unmarked frogs entering the RANA pond throughout the summer (i.e. caught in pitfall traps outside the fence) were assumed to have originated from other waterbodies and were given a unique mark to differentiate them from the RANA pond metamorphs.

Traps were closed on September 10 in 2005 because resident muskrats (*Ondatra zibethicus*) in the pond began filling traps with vegetation, creating animal welfare concerns. At this time, the majority of metamorphs were assumed to have left the pond because < 15% of the season's maximum number of metamorphs was still able to be counted at the pond. Traps were closed on September 7 in 2006 because no metamorphs had been captured in the preceding 7 days, and surveys revealed < 3% of the season's maximum number of metamorphs remained. These metamorphs may not have emerged for several weeks and it was calculated that their capture would not have altered the results. After traps were closed in 2006, four person days were spent using dip nets to capture the remaining metamorphs in the pond, which were measured, weighed, marked uniquely from frogs caught in traps (in order to discriminate among trapped and non-trapped metamorphs in a later part of this study), and released back into the pond. During the off-season, all traps were filled with soil and closed with coverboards to prevent animals from entering, and the fence was opened at approximately 15 m intervals to allow animal movements to and from the pond. To confirm that metamorphs did not overwinter in the pond, surveys for metamorphs were conducted at the pond twice in October 2006.

Habitat and environmental variables

To evaluate whether metamorphs orient towards certain microhabitat features upon emigration, a suite of habitat variables was measured at each trap. Habitat characteristics were evaluated within a 5 m radius centered on each trap using point-intercept sampling (Block and Morrison 1998). Specifically, four transects were extended at 90° intervals, starting at 45° from the fenceline. At 1 m intervals on each transect, starting 1 m from each trap, I recorded habitat type (i.e. grass, pond, soil, road, branch, dead wood, dead grass) and maximum vegetation height above ground (cm), resulting in 20 observation points for each trap. Any points with vegetation taller than 1 m were lumped as '> 1m'. Shoreline slope was also measured perpendicular to each trap using a clinometer (Suunto PM-5). To determine if distance to the pond influenced the number of metamorphs captured, I measured the shortest distance between the pond and each trap (cm). Lastly, I measured the length of fence in between traps (m). Halving these distances yielded two fence lengths for each trap, which were added together to give the total length of fence associated with each trap.

Before each trap check, weather conditions for the preceding 24 hrs (or since the last trap check) were collected. The maximum and minimum air and water temperature (°C) and humidity (%) were recorded (Oregon Scientific Thermohygrometer model THGR268). Precipitation (mm) using a rain gauge (Tru-Check ®) was recorded and emptied at each trap check. Because of instrument malfunctions, data were supplemented from the nearest weather station (see Chapter 2 for details).

Statistical analyses

Metamorph surveys

As for analyses of tadpole distribution (Chapter 3), I used an information-theoretic approach to evaluate several *a priori* models of the factors influencing metamorph abundance at each stake. The models are ranked according to Akaike's Information Criterion (AIC); the model receiving the lowest AIC value is the model that best approximates the data (Quinn and Keough 2002). Again, I used generalized linear mixed models (GLMM) which allowed me to account for repeated stake observations and for the nesting of stakes within pond. Like the tadpole data, there was a large number of zeros in the dataset, and even the best model was found to have low predictive power (ie. linear and quadratic regressions of the predicted probabilities against the number of observed metamorphs had r^2 values < 0.4). Therefore, binary logistic regression was also used. Similar to the tadpole analyses, the repeated measures and nested effect of 'stakes' seems to be minor in terms of predicting the total number of metamorphs in the GLMM. Therefore, the potential bias associated with a lack of repeated and nested variable designation in the logistic regression is likely also minor. Refer to Chapter 3 for additional details on these analyses.

I evaluated three alternative models using a GLMM for predictor variables that influenced metamorph abundance (Table 4.1). I initially ran the simplest model (single factors only) to reduce the risk of over fitting the data. I ran a second model including all pair-wise interactions, since any of these variables could potentially be interacting, but I had no *a priori* reason to test certain interactions over others. A third model included Julian day, shade, water depth and temperature because these were the factors hypothesized *a priori* to influence metamorph abundance.

Similar to the tadpole surveys, I combined all surveys in all ponds for the logistic regression models for metamorph distribution. Scatter plots were made and pairwise Pearson or Spearman (if variables were not normally distributed) correlations among all variables were calculated in order to test for multicollinearity (Haramura 2007). All correlations were below the critical value of 0.7 (Green 1979) and no linear relationships among independent variables were detected; therefore, multicollinearity was not considered a problem in the analysis.

The presence (1 or more metamorphs) or absence (no metamorphs) of metamorphs at each stake was used as the dependent variable. The same variables used in the GLMM were used in the logistic regression. Reference categories for the categorical variables (that category to which all other categories of that variable are compared) were the last category in an alphabetical list of each variable. Therefore, the RANA pond, the SW quadrant, and 'shaded' were the reference categories for each categorical variable. The multivariate model was run as a forward stepwise binary logistic regression with an

Table 4.1. Abbreviations and descriptions of categorical and continuous variables included in the GLMM and logistic regression models that were used to test the distribution of *R. pipiens* metamorphs in two ponds.

Variable	**Description**
Categorical	
POND	whether the stake was in the RANA or ENR pond
QUAD	whether the stake was in the NE, SE, SW or NW quadrant
SHADE	whether the 2 m observation area around the stake was 'shaded' or 'not-shaded' (instances where it was only partly shaded were considered as 'shaded')
Continuous	
JDAY	Julian day of observation
DEPTH	depth of water 1 m from stake
TEMP	temperature of water 1 m from stake and 10 cm below the surface

alpha level of 0.05 for entry. I ran the same three models as for the GLMM (i.e. single factors only, single factors with all pair-wise interactions, and those single factors originally hypothesized to influence metamorph distribution). Only the results from the model with the highest percentage of correctly classified cases are presented.

For each variable retained in the final logistic regression model, the regression coefficient (B) with its corresponding Wald χ^2 statistic, and the odds ratio (Exp(B)) with 95% confidence intervals are reported (SPSS 1999). The percentage of cases that were correctly classified and the area under the ROC (Receiver Operating Characteristic) curve (AUC) are presented, the latter of which indicates the accuracy of the final model.

Metamorph emigration

Using the total number of metamorphs per trap as the dependent variable, I evaluated metamorph emigration in relation to several predictor variables (Table 4.2). I could not include all categorical and continuous predictor variables in one model because small sample size data ($n = 16$ traps in 2005 and $n = 17$ traps in 2006) were not normally distributed. Non-parametric analyses were used to evaluate categorical variables because I could not achieve normality of the data in both years, even after applying log and square-root transformations. Multiple tests were run on the same data sets because multi-factorial testing of non-parametric data was not possible. Each year was analysed separately because the number of traps differed between years. The significance level was set *a priori* at $\alpha = 0.05$ for all statistical tests.

To determine whether traps next to a quadrant fence ('corner' traps) had the same

Table 4.2. Descriptions of all variables used in trap analyses.

Variable name	**Categorical / Continuous**	**Definition**	***Number of trap categories in 2005***	***Number of trap categories in 2006***
Total metamorphs per trap	Continuous	Total number of emigrating metamorphs caught in each trap	16	17
Quadrant	Categorical	¼ Section of pond with varying number of traps, divided with fence spanning from outer fence to shore of pond	4	4
Habitat	Categorical	Traps are divided into 2 habitat categories – 'treed' indicates trap primarily surrounded with branches > 1m tall, 'open' indicates traps surrounded primarily with either low-lying or no vegetation	2	2
Slope	Continuous	Slope of pond shoreline at 90° angle to each trap	16	17
Distance to pond (m)	Continuous	Shortest distance between pond and trap	16	17
Length of fence (m)	Continuous	The length of fenceline associated with each trap	16	17

probability of catching metamorphs as traps in the middle of quadrants, I tested if there was a difference in the number of metamorphs caught in corner traps versus middle traps using a Mann-Whitney U test for each year. To evaluate the direction of emigration from the pond each year, I used Kruskal-Wallis tests that considered the total metamorphs per trap as the dependent variable and quadrant as the independent variable. To determine if the number of trapped metamorphs differed between years, I used a paired Wilcoxon signed ranks test, the non-parametric equivalent to a paired t-test. For this, I used the total number of metamorphs per trap as the dependent variable, and the trap as the paired independent variable for the two years. The added trap in 2006 was excluded from this analysis.

To determine if emigration direction depended on habitat characteristics surrounding the pond, I collapsed the point-sampling data into two categories, because otherwise there were too many variables for the number of cases (Babyak 2004). I divided the point-sampling data into either treed (having branches) or non-treed (grass, pond, dead wood, road, dead grass, soil) groups, which became the two new habitat categories (referred to as 'treed' and 'open'). Using the 20 points for each trap, any traps with 10 or more points recorded as having branches (which coincidentally were always > 1 m in height) were considered 'treed' and the rest were 'open' traps. I used Mann-Whitney U tests to compare the number of metamorphs caught in treed versus open traps each year. Significant results were explored further. If any of the categorical variable analyses differed (in terms of significance) by year, I re-ran the analysis excluding captures from the added trap in 2006 to determine if the added trap was the reason for the discrepancy.

I used linear regression to explore whether emigration direction was dependent on several continuous variables (shoreline slope, distance to the pond, and length of fence). Again, the total metamorphs per trap was the dependent variable and each year was analysed separately. I could not use a multiple regression including all continuous variables because I did not have the minimum of 10 to 15 observations per predictor variable that would allow proper estimates of the model (Babyak 2004). Accordingly, I included one variable for each regression analysis.

Using data from Chapters 2 and 3, I conducted a qualitative analysis of whether spatial distributions of early life stages (egg clusters, tadpoles, metamorphs) were similar in 2006. Different methods of data collection for different life stages prevented statistical analysis (e.g. tadpoles were analysed according to stakes whereas metamorph emigration was analysed according to trap).

Circular statistics

Most studies examining amphibian dispersal use circular statistics to test the null hypothesis that individual movements from the pond are uniformly distributed, treating single observations of migrating individuals from the pond as movement vectors for certain directions (e.g. Dodd and Cade 1998; Malmgren 2002; Rothermel 2004; Vasconcelos and Calhoun 2004; Marty et al. 2005; Patrick et al. 2007). Data are considered to be grouped when the circle (in this case, the pond) is subdivided into arcs of equal length (Batschelet 1981). The number of groups around the pond is equal to the number of pitfall traps. To meet the assumptions of circular statistics, all groups must be of equal size. Since the RANA pond is not circular and because the fence is not uniformly distant from the pond, I determined that circular statistical analyses were inappropriate for my data (hence the above mentioned analyses). However, in order to compare my data to those from similar studies (in which ponds are also not always circular; e.g. Vasconcelos and Calhoun 2004) I used circular statistics secondarily to test for directionality by approximating the designation of trap-groups as accurately as possible (Table 4.3). Note that the angles of each trap-group were different each year because of different numbers of traps.

For the circular statistical analyses, the mean vector length (*r*) is reported, and indicates the degree to which observations are clustered around the mean. The value of *r* ranges from 0 to 1; the larger the *r* value the greater the degree of clustering around the mean (Batschelet 1981). I multiplied *r* by a correction factor of 1.0073 in 2005 and 1.0051 in 2006 to account for the grouping of data (Batschelet 1981; correction factors differed each year because of different numbers of traps). I used the Rayleigh test to determine whether the direction of movement out of the pond was random or directional. If the mean angles of emigration from the RANA pond are significant in both years, 95% confidence limits will be constructed to evaluate whether the angles differed between years. The significance level was set at $\alpha = 0.05$ and I used Oriana software (Version 2.02, Kovach Computing Services) to graph and run circular statistics.

Table 4.3. Actual angles of each trap relative to the centre of the RANA pond, and the angles used for the purpose of circular statistics for both 2005 and 2006 data. Corrected angles indicate the approximation of trap-groups as close as possible to their actual angle, in order to create equal-sized groups that are necessary for circular statistical analyses.

Trap	Actual angle relative to the centre of the pond and North	Corrected angles for analysis (2005)	Corrected angles for analysis (2006)
1	214.6	215	214.2
2	236.4	237.5	235.2
3	262.9	260	256.2
4	287.2	282.5	277.2
5	302.96	305	298.2
6	317.5	327.5	319.2
7	346.4	350	340.2
8	6.7	12.5	1.2
9	25.2	35	22.2
10	52.6	57.5	43.2
11	58.2	80	64.2
12	73.4	102.5	85.2
13	85.7	125	106.2
14	98.7	147.5	127.2
15	156.8	170	169.2
16	178.3	192.5	190.2
17	118.7	N/A	148.2

Body size analysis

To evaluate whether emigration direction, timing of emigration, or year influenced metamorph size, I used a univariate general linear model (GLM ANOVA) that included the three independent variables and all possible interactions. Trap captures were combined for each quadrant each year, and quadrant was used to indicate emigration direction. To determine if metamorph size depended on the timing of emigration, the number of days between when the first metamorph was caught and when traps were closed was halved. I used the 2006 data to determine the number of days in each half of the season because I was more certain that the data accurately represented the entire emergence season compared to the data for 2005. This was because in 2006 traps were closed when only 3% of the metamorph population remained in the RANA pond, whereas traps were closed in 2005 due to animal welfare concerns and time constraints prevented determining the exact number of remaining metamorphs. As a result, metamorphs caught in the first 25 days of trapping for both years were considered 'early' emigrants, and those caught in the last 25 days of trapping were considered 'late' emigrants. Finally, I included year in the model to determine if metamorph body size from the RANA pond fluctuated between the two years. Two extreme outliers found in the 2006 data were removed from the analysis. I achieved homoscedasity for the overall model, but I could not achieve normality for the 'late' emigrants in 2006 using any data transformation. However, since this was the only group out of four that was not normally distributed, and because non-parametric tests do not allow inclusion of all interactions, using a general linear model was considered the most appropriate statistical analysis despite this non-normality. I used SVL solely as an indication of body size, because the weight data could have been confounded by the timing of a frogs' last meal and because SVL is used more often in the literature to test for body size effects in frogs and salamanders (e.g. Semlitsch et al. 1988; Sjogren-Gulve 1998; deMaynadier and Hunter 1999; Mazerolle 2001; Loman 2002; Rothermel 2004; Vasconcelos and Calhoun 2004; Chelgren et al. 2006), thus simplifying direct comparisons between other studies and mine.

Handling effects

A Chi-square test was used to determine whether observed emigrations between captures differed from what was expected in 2006. I did not have enough metamorph recaptures in 2005 (4 in total) to warrant such statistical tests. For the 2006 data, I used a chi-square analysis to test whether metamorphs emigrated from the pond consistently into the same or a different quadrant from the initial capture. Observed values were the number of metamorphs recaptured in either the same or different quadrants, and expected values were calculated based on the total number of recaptures. The null hypothesis (i.e. expected values) for this test was that 25% of recaptures will be caught in the same quadrant and 75% will be caught in a different quadrant.

Weather

To test for associations between weather cues and amphibian movements in 2005 and 2006, I calculated Spearman rank correlation coefficients between daily metamorph counts (first captures only) and weather data for the preceding 24 hr period (using weather data collected at the pond and from the nearest Environment Canada weather station; Chapter 2).

Results

Metamorph surveys

In 2006, metamorphs were first seen on July 11 at the RANA pond and on July 27 at the ENR pond. Since most metamorphs had left both ponds by the end of August, the last surveys took place on August 24 and 28 for the ENR and RANA ponds respectively. Twenty surveys were completed in total; eight at the ENR pond and twelve at the RANA pond. Most metamorph surveys were completed between 1000 and 1600 hr. Only 14 out of 508 (3%) observations recorded the potential for counting a metamorph twice. When both ponds are combined, the NE and SW quadrants had the fewest and the most stakes, respectively (Table 4.4). However, since both ponds shrunk throughout the summer because of falling water levels, the number of stake observations per survey decreased as a result of an absence of water at certain stakes.

A total of 508 stake observations were used in the GLMM. Each observation at each stake was a datum point. The full model proved to be the best model predicting metamorph abundance (Table 4.5). In this model, the single factors that were significant

Table 4.4. The number of stakes per quadrant in the RANA and ENR ponds at the beginning of the metamorph season. Because the ponds are not perfectly circular, the number of stakes per quadrant is not equal within ponds. Numbers in brackets indicate the number of stakes towards the end of the season (if it is different) due to falling water levels.

	Quadrant				
Pond	**SE**	**NE**	**NW**	**SW**	**Total**
RANA	4 (2)	4 (2)	8	6 (5)	22 (17)
ENR	9 (8)	5	10	14 (11)	38 (34)
Total	13 (10)	9 (7)	18	20 (16)	60 (51)

Table 4.5. Models of *Rana pipiens* metamorph abundance in the RANA and ENR ponds. Models are ranked according to Akaike's Information Criterion (AIC). Δ AIC = change in AIC from the best model.

Model	**No. parameters**	**AIC**	**Δ AIC**
POND QUAD SHADE JDAY DEPTH TEMP + all pair-wise interactions	36	2946.3	0.0
POND QUAD SHADE JDAY DEPTH TEMP	11	2958.4	12.1
SHADE JDAY DEPTH TEMP	7	2970.3	24

included QUAD (GLMM, $F_{3,454} = 3.9$, $P < 0.01$), SHADE (GLMM, $F_{1,446} = 5.1$, $P < 0.05$), DEPTH (GLMM, $F_{1,461} = 11.6$, $P < 0.01$) and TEMP (GLMM, $F_{1,437} = 16.0$, $P < 0.01$). Significant interactions included POND*QUAD (GLMM, $F_{3,58} = 4.2$, $P < 0.01$), JDAY*QUAD (GLMM, $F_{3,451} = 3.9$, $P < 0.01$), TEMP*SHADE (GLMM, $F_{1,457} = 5.9$, $P < 0.05$), JDAY*DEPTH (GLMM, $F_{1,461} = 12.0$, $P < 0.01$) and JDAY*TEMP (GLMM, $F_{1,437} = 14.6$, $P < 0.01$; Table 4.6). Models lacking pair-wise interactions ranked the highest (i.e. they had the worst fit of the data).

To help understand the significant variables, I made graphs of all significant interactions. Metamorph abundance was higher in the RANA pond compared to the ENR pond in all quadrants except the NW quadrant (Figure 4.2). Initially, metamorph abundance increases in all quadrants throughout the summer then gradually decreases, but this pattern depends on the quadrant (Table 4.7). Although metamorphs were generally more abundant in warm, non-shaded areas compared to cooler, shaded areas, at the lowest and highest temperatures their abundance depended on whether they were in the shade (Figures 4.3 and 4.4). Although metamorph numbers were generally higher earlier in the season compared to later, this effect depended on the water depth (Figures 4.5 and 4.6). Similarly, it appears as though metamorphs were generally in higher densities in shallower water, although this depended on the day. Despite the general decrease in the number of metamorphs as the summer progressed, their numbers were high later in the season when water temperatures were warm (Figures 4.7 and 4.8).

The same 508 stake observations used in the GLMM were used in the logistic regression models, each stake observation representing a datum point. The single factor regression model proved to be the best model with 77.0% of cases correctly classified

Table 4.6. GLMM results predicting metamorph abundance in the ENR and RANA ponds.

Variable	Numerator df	Denominator df	F	*P.*
Pond	1	1	.0	1.00
Quadrant	3	453.8	3.9	.01
Shade	1	445.6	5.1	.02
Jday	1	447.0	.2	.68
Depth	1	461.0	11.6	.00
Temp	1	437.4	16.0	.00
Pond * Quadrant	3	58.3	4.2	1.00
Pond * Shade	1	448.7	.8	.36
Jday*Pond	1	443.8	.1	.79
Depth*Pond	1	474.0	.5	.50
Temp*Pond	1	450.2	1.8	.18
Quadrant * Shade	3	442.6	.7	.55
Jday*Quadrant	3	451.5	.0	.01
Depth*Quadrant	1	471.8	1.7	.17
Temp*Quadrant	3	449.4	.0	.25
Jday*Shade	1	445.4	3.3	.07
Depth*Shade	1	450.3	.1	.79
Temp*Shade	1	456.7	5.9	.02
Jday*Depth	1	461.5	12.0	.00
Jday*Temp	1	436.9	14.6	.00
Depth*Temp	1	448.5	.2	.68

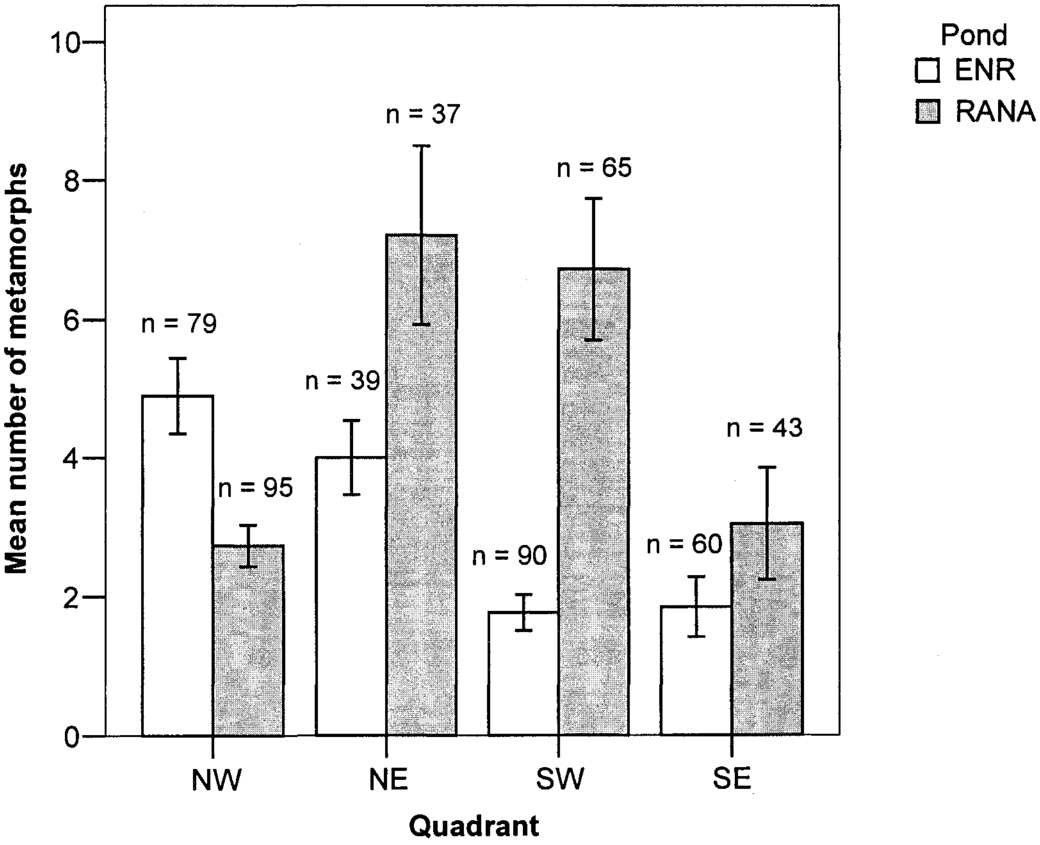


Figure 4.2. The mean (± 1 SE) number of metamorphs per quadrant in each pond. n = the number of stake observations representing each category.

Table 4.7. Summary table showing the distribution of egg clusters (Chapter 2), tadpoles (Chapter 3) and metamorphs early and late in the Gap, BC, RANA and ENR ponds in 2006, as well as number of trap captures in the RANA pond in 2006. n = number of stake observations.

Pond	Quadrant	Number of egg clusters	Tadpoles				Metamorphs					
			Time in season									
			Early (June 6 – 29)		Late (June 30 – July 21)		Early (July 11 – Aug 4)		Late (Aug 5 – 28)		Traps	
			n	Mean (± 1 SE) number of tadpoles	n	Mean (± 1 SE) number of tadpoles	n	Mean (± 1 SE) number of metamorphs	n	Mean (± 1 SE) number of metamorphs	Number of traps	Mean (± 1 SE) number of metamorphs
GAP	NW	2	16	0.6 (± 0.3)	3	12.3 (± 8.8)	N/A		N/A			
	NE	0	8	6.5 (± 3.1)	1	3 (± 0.0)	N/A		N/A			
	SW	0	8	4.6 (± 1.6)	2	14.0 (± 6.0)	N/A		N/A			
	SE	3	8	2.4 (± 1.2)	1	2 (± 0.0)	N/A		N/A			
BC	NW	0	27	0.04 (± 0.04)	N/A		N/A		N/A			
	NE	0	16	0.9 (± 0.5)	N/A		N/A		N/A			
	SW	0	3	0.3 (± 0.3)	N/A		N/A		N/A			
	SE	7	17	2.1 (± 0.6)	N/A		N/A		N/A			
RANA	NW	0	47	4.5 (± 0.9)	8	12.6 (± 4.3)	40	3.3 (± 0.6)	55	2.4 (± 0.3)	6	8.5 (± 8.1)
	NE	8	24	6.4 (± 1.4)	4	1 (± 0.0)	20	8.9 (± 1.8)	17	5.2 (± 1.8)	4	6.5 (± 2.1)
	SW	4	36	2.4 (± 0.6)	6	13.7 (± 7.9)	30	10.7 (± 1.8)	35	3.3 (± 0.7)	3	159.7 (± 57.4)
	SE	2	24	4.5 (± 0.9)	4	0.3 (± 0.3)	20	5.0 (± 1.5)	23	1.4 (± 0.5)	4	25.8 (± 9.0)
ENR	NW	N/A					20	11.1 (±)1.1	59	2.8 (± 0.3)		
	NE	N/A					9	4.1 (± 0.6)	30	4.0 (± 0.7)		
	SW	N/A					23	3.0 (± 0.8)	67	1.3 (± 0.2)		
	SE	N/A					12	4.5 (± 1.8)	48	1.2 (± 0.2)		

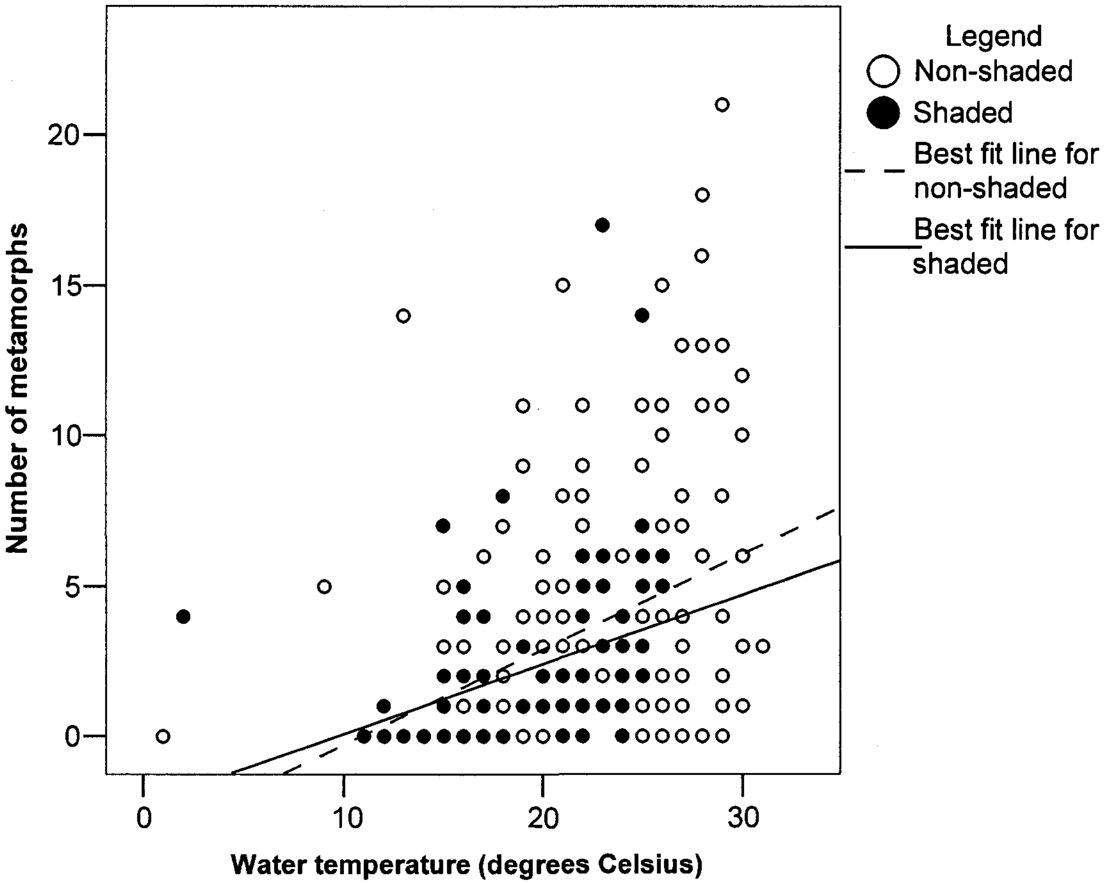


Figure 4.3. Scatterplot showing the effect of water temperature on the number of metamorphs in the ENR pond. Data points and best fit lines identified by the shade category (see Legend).

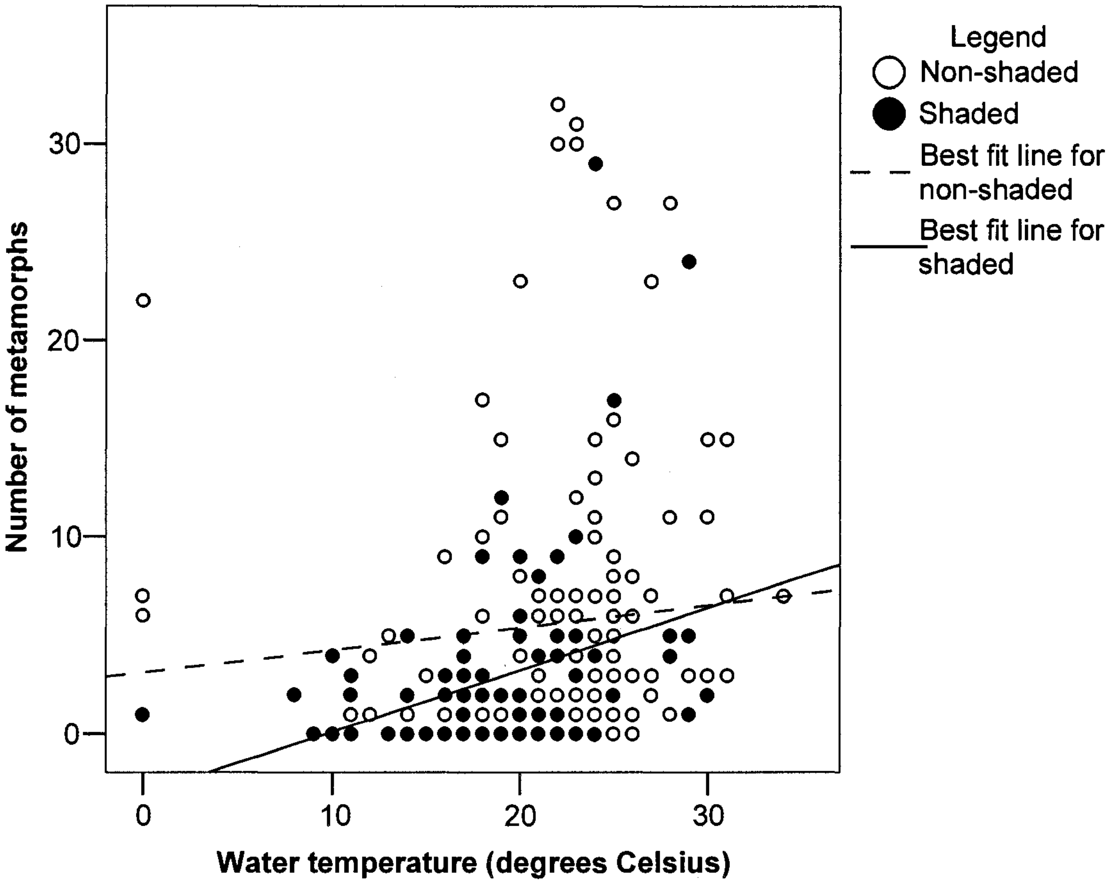


Figure 4.4. Scatterplot showing the effect of water temperature on the number of metamorphs in the RANA pond. Data points and best fit lines identified by the shade category (see Legend).

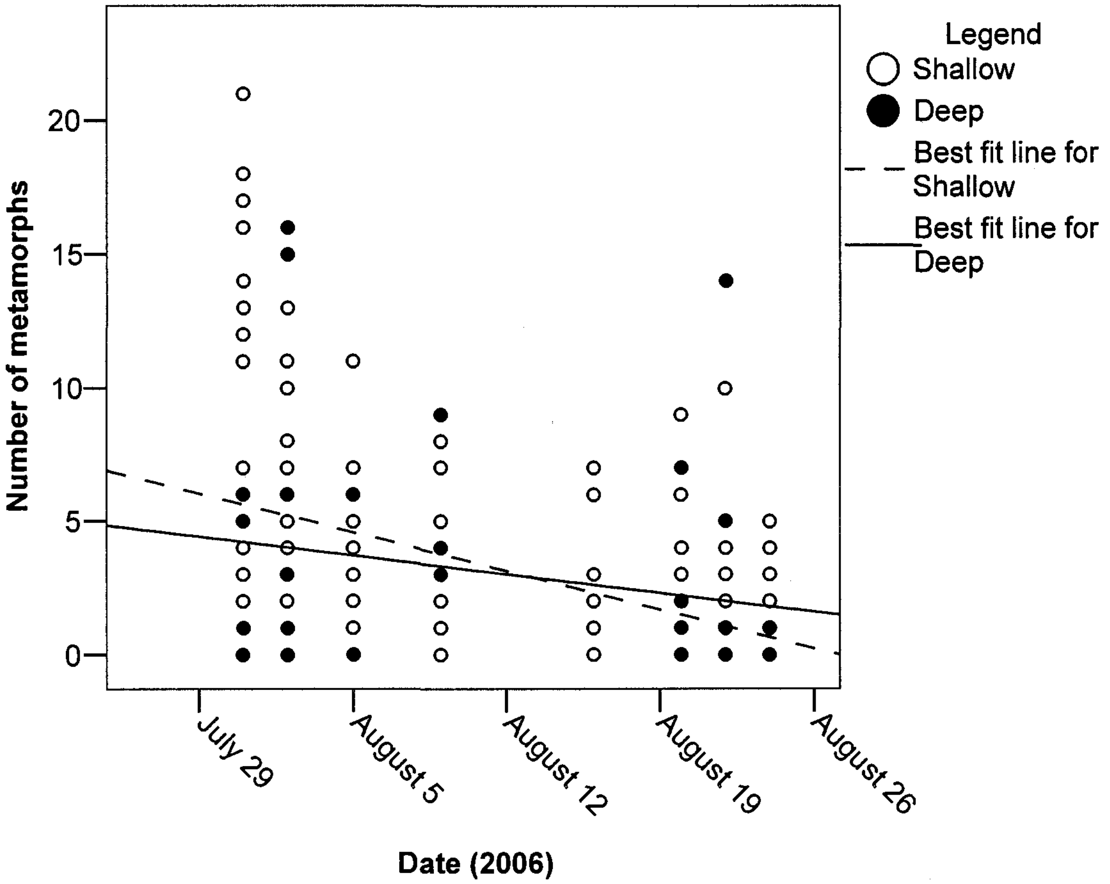


Figure 4.5. Scatterplot showing the effect of date (2006) and water depth (cm) on the number of metamorphs in the ENR pond. Water depth is divided into shallow (0-17 cm) and deep (18-34 cm) categories and best fit lines for each category are shown (see Legend).

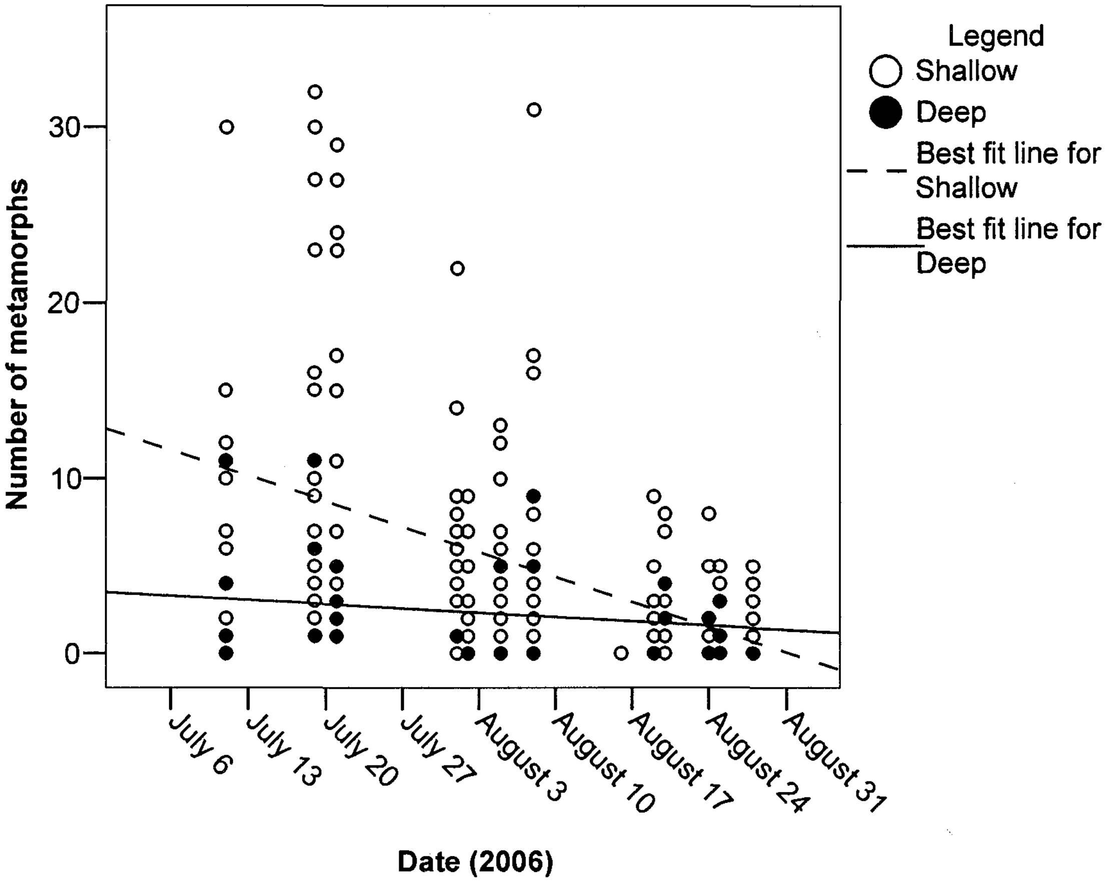


Figure 4.6. Scatterplot showing the effect of Date (2006) and water depth (cm) on the number of metamorphs in the RANA pond. Water depth is divided into shallow (0-17 cm) and deep (18-34 cm) categories and best fit lines for each category are shown (see Legend).

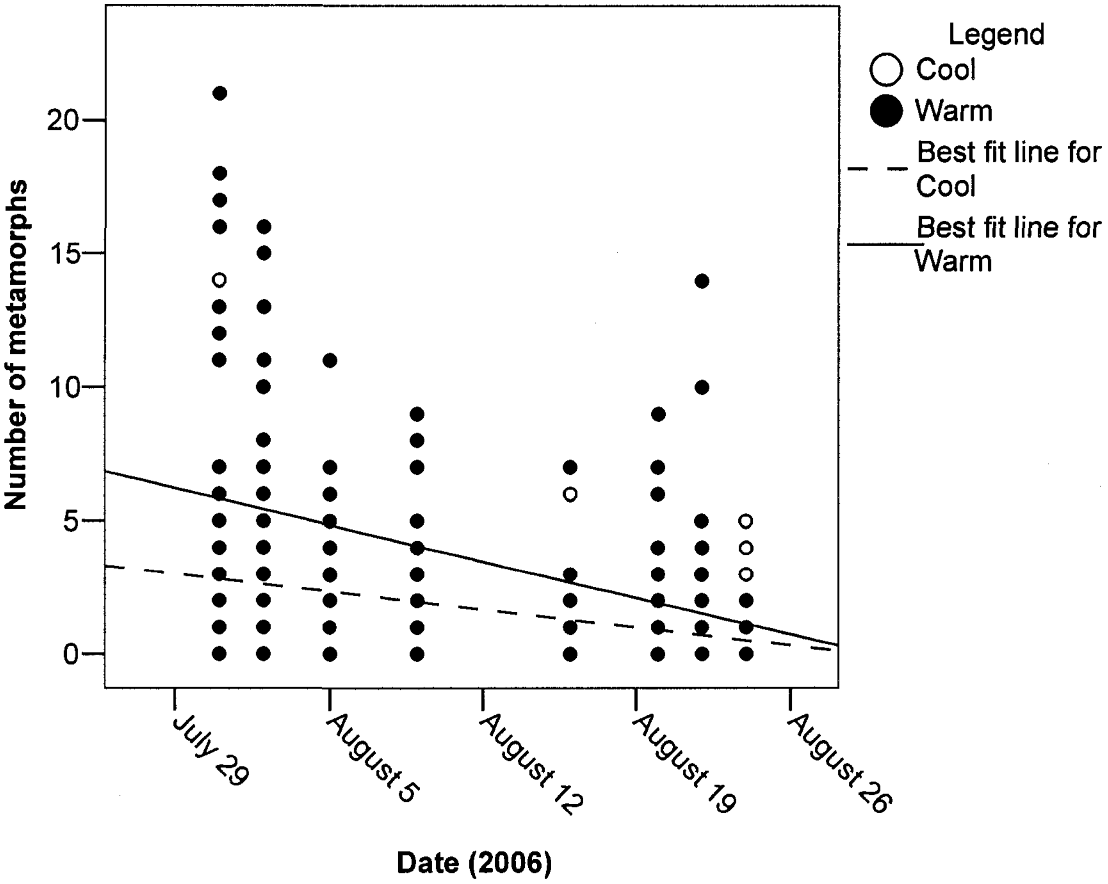


Figure 4.7. Scatterplot showing the effect of Date (2006) and water temperature (°C) on the number of metamorphs in the ENR pond. Water temperature is divided into cool (0-17 °C) and warm (18-34°C) categories and best fit lines for each category are shown (see Legend).

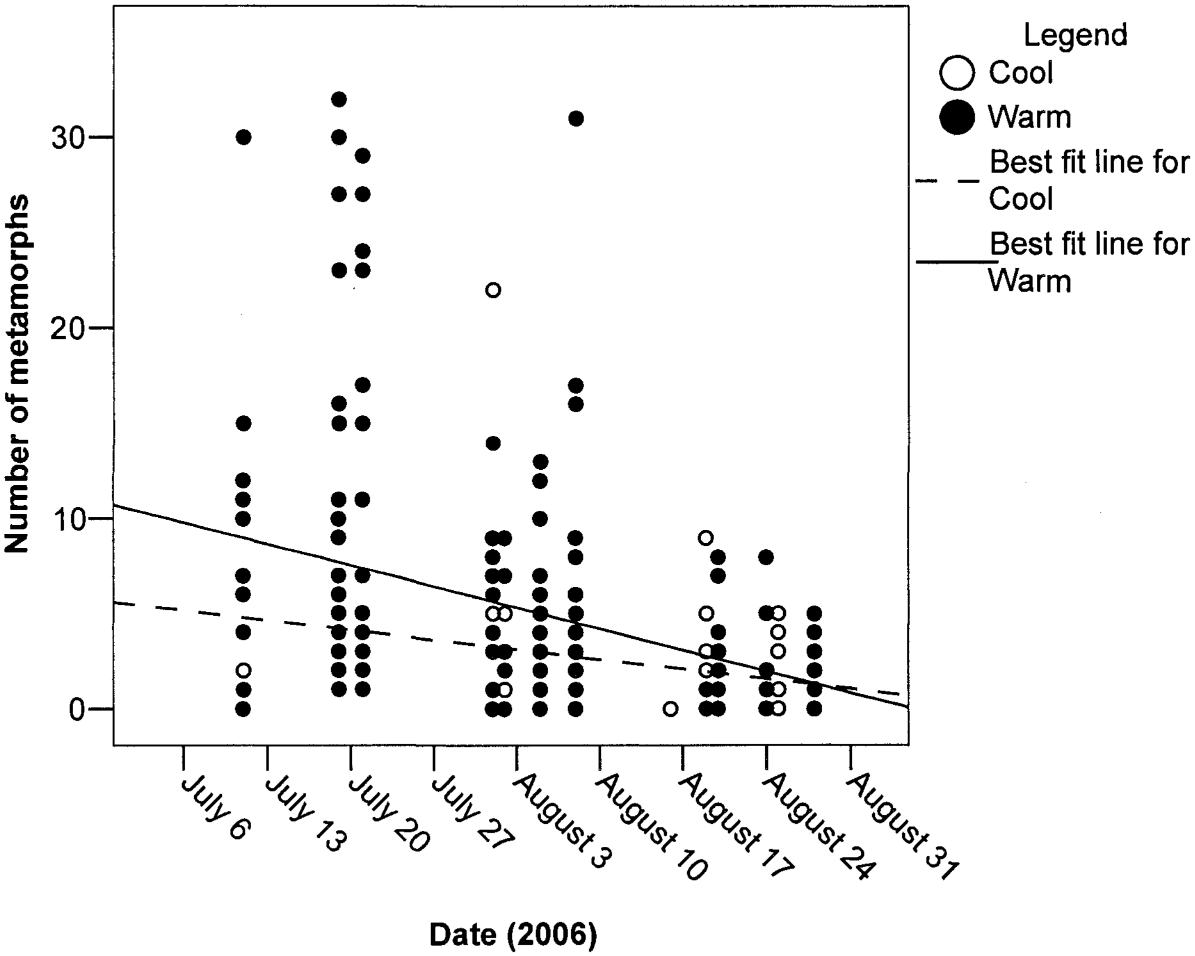


Figure 4.8. Scatterplot showing the effect of Date (2006) and water temperature (°C) on the number of metamorphs in the RANA pond. Water temperature is divided into cool (0-17 °C) and warm (18-34°C) categories and best fit lines for each category are shown (see Legend).

compared to the number of cases correctly classified with the full model (including all single factors and pair-wise interactions; 74.8%) and the hypothesized model (75%). The best model predicting the presence of metamorphs on the shoreline of ponds included QUAD, JDAY, DEPTH and TEMP as significant predictor variables (Table 4.8). The odds of finding a metamorph in the NW quadrant was 1.8 times greater compared to the SW quadrant, but the highest odds were for the NE quadrant where there is 2.9 times the chance of finding a metamorph (compared to the SW quadrant). The odds of metamorph presence decreased by a factor of slightly below 1.0 each day compared to the previous day. With each centimetre increase in water depth, the odds of finding a metamorph decreased by a factor of 0.9 (metamorphs were located at the maximum and minimum range of depths tested, ranging from 2-34 cm). With a one degree increase in water temperature, the odds of finding a metamorph increased by a factor of 1.1 within the minimum (0°C) and maximum (34°C) range of temperatures tested.

Metamorph emigration

The onset of emigration at the RANA pond differed between years, beginning on August 4 in 2005 and July 13 in 2006. A total of 53 and 659 *R. pipiens* metamorphs were caught in pitfall traps in 2005 and 2006 respectively. Few metamorphs returned to the pond (after being released on the opposite side of the fence) in both years (3 in 2005 and 20 in 2006), suggesting that trap captures represented primarily emigrating metamorphs. Thirty-one metamorphs or sub-adults immigrated to the RANA pond in 2006 and only one was already marked, indicating that it was a metamorph from 2005. Of the 87 metamorphs remaining in the RANA pond when traps were closed in 2006,

Table 4.8. Estimated logistic regression coefficients for significant variables predicting the presence or absence of *R. pipiens* metamorphs at each stake in the RANA and ENR ponds in 2006.

Variable	**Final Model Step 4**		
	B	**Wald**	**Exp(B), (95% C.I.)**
Constant	6.4	7.7 **	577.3
Quadrant [b]		22.4**	
SE quadrant	-0.6	3.7	0.6, (0.3–1.0)
NE quadrant	1.1	7.2**	2.9, (1.3–6.1)
NW quadrant	0.6	4.7*	1.8, (1.1–3.2)
Julian Day	-0.0	11.1**	0.97, (0.95–1.0)
Water Depth	-0.0	9.7**	0.9, (0.9–1.0)
Water temperature	0.1	22.9 **	1.1, (1.1–1.2)
N	508		
Model χ^2 [df]	85.7[6]		
% Correctly Classified	77.0		
Nagelkerke R^2	0.2		
Area under the curve (ROC)	0.8		

* $P < 0.05$

** $P < 0.01$

[a] Pond reference is "RANA"

[b] Quadrant reference is "SW quadrant"

[c] Shade reference is "shaded"

67 (77%) had at least one mark indicating they had attempted emigration at least once. Forty-three (49%) had two marks indicating they had emigrated twice but had since returned to the pond.

There was no difference in capture rate between traps adjacent to a barrier fence ('corner' traps that sectioned the pond into quadrants) and those in the middle of a quadrant in either year (in 2005: Mann Whitney U test, U = 16, $P > 0.05$, Figure 4.9; in 2006: U = 11, $P > 0.05$, Figure 4.10). As a result, all traps were assumed to have an equal chance of capturing metamorphs.

Emigration patterns were significantly non-random according to quadrant in both years (Table 4.9). In 2005, the mean (± SE) number of metamorphs per trap was highest in the SW quadrant (7.0 ± 3.6 metamorphs/trap, n = 3), followed by the SE quadrant (6.3 ± 0.3 metamorphs/trap, n = 3) and the NE quadrant (2.8 ± 1.8 metamorphs/trap, n = 4). The NW quadrant caught the fewest metamorphs (0.3 ± 0.2 metamorphs/trap, n = 6).

The results were similar in 2006, with the exception of the NE and NW quadrants. The highest mean number of metamorphs caught in 2006 was in the SW quadrant (159.7 ± 57.4 metamorphs /trap, n = 3), followed by the SE quadrant (25.8 ± 8.96 metamorphs/trap, n = 4) and the NW quadrant (8.5 ± 8.1 metamorphs/trap, n = 6). The NE quadrant had the lowest mean number of metamorphs (6.5 ± 2.1 metamorphs/trap, n = 4; Figure 4.11). The number of metamorphs caught in 2005 (n = 53) was significantly less than the number caught in 2006 (n = 659; Wilcoxon matched signed ranks test, $P < 0.05$).

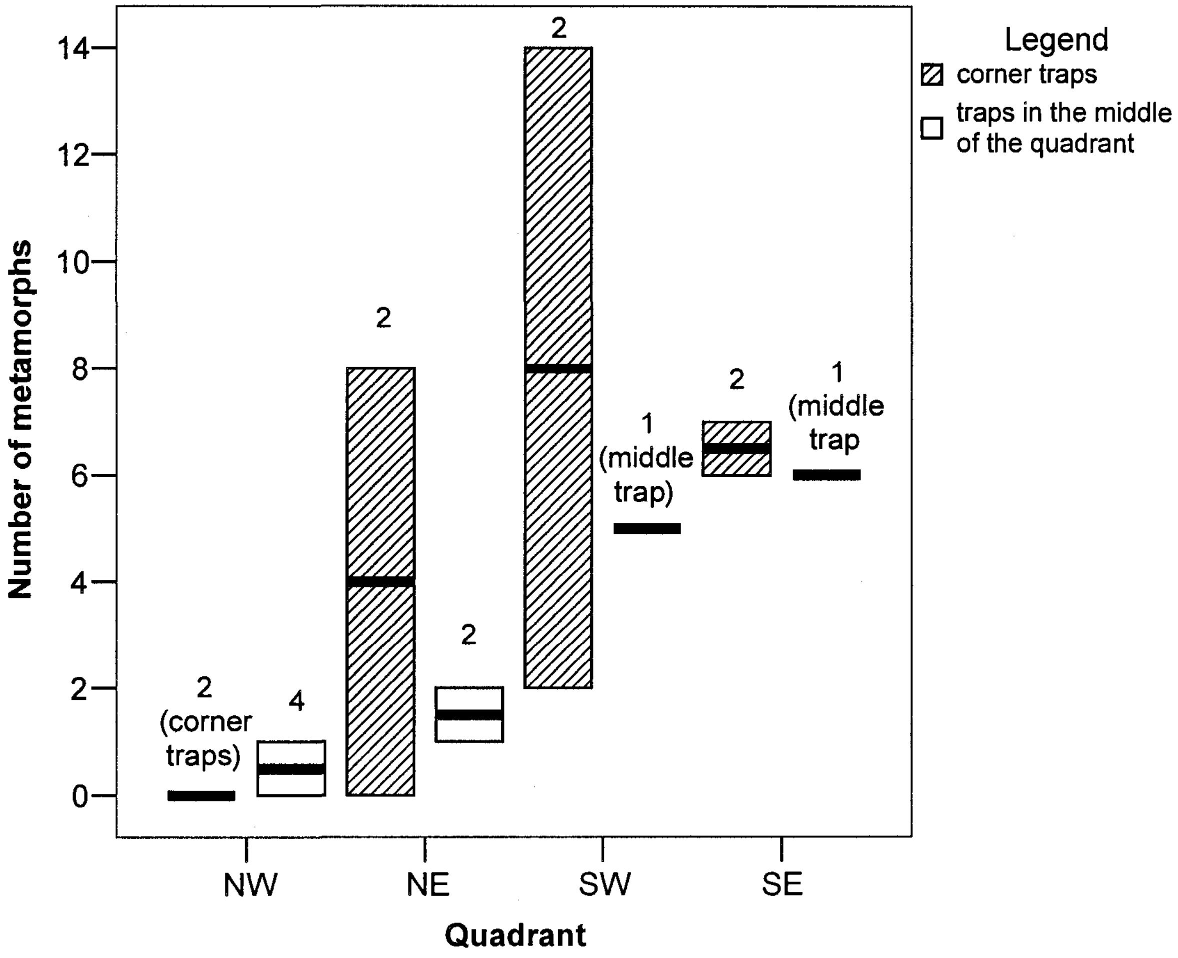


Figure 4.9. Boxplot showing the number of metamorphs caught per quadrant, segregated by traps adjacent to a barrier fence ('corner' traps) and traps in the middle of quadrants in 2005. Numbers indicate the number of traps in that category.

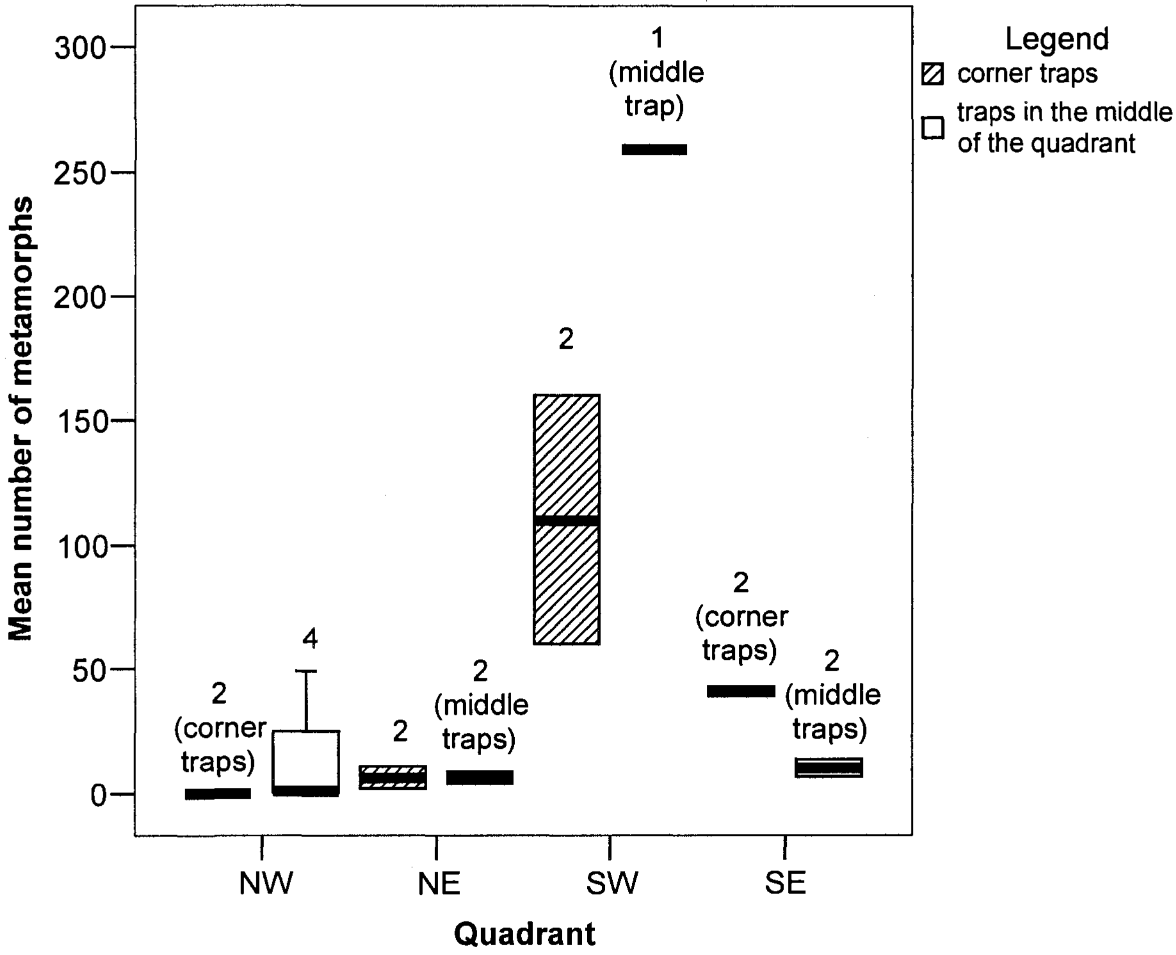


Figure 4.10. Boxplot showing the number of metamorphs caught per quadrant, segregated by traps adjacent to a barrier fence ('corner' traps) and traps in the middle of quadrants in 2006. Numbers indicate the number of traps in that category.

Table 4.9. Categorical variables used to predict the number of metamorphs caught in traps. Variables were tested with a Kruskal-Wallis (K-W) or a Mann-Whitney U (M-W) test. n indicates the number of traps in each quadrant for the K-W tests, or the number of traps in the treed (n_1) or open (n_2) categories for the M-W tests, and df indicates degrees of freedom.

Year	**Variable**	**Test**	**n_1, n_2, n_3, n_4**	**df**	**Test statistic** χ^2 **for K-W test or Z for M-W test**	***P***
2005	Quadrant	K-W	3, 4, 6, 3	3	9.4	< 0.05
2005	Habitat	M-W	6, 10	1	- 1.5	> 0.05
2006	Quadrant	K-W	4, 4, 6, 3	3	10.7	< 0.05
2006	Habitat	M-W	7, 10	1	- 2.3	< 0.05

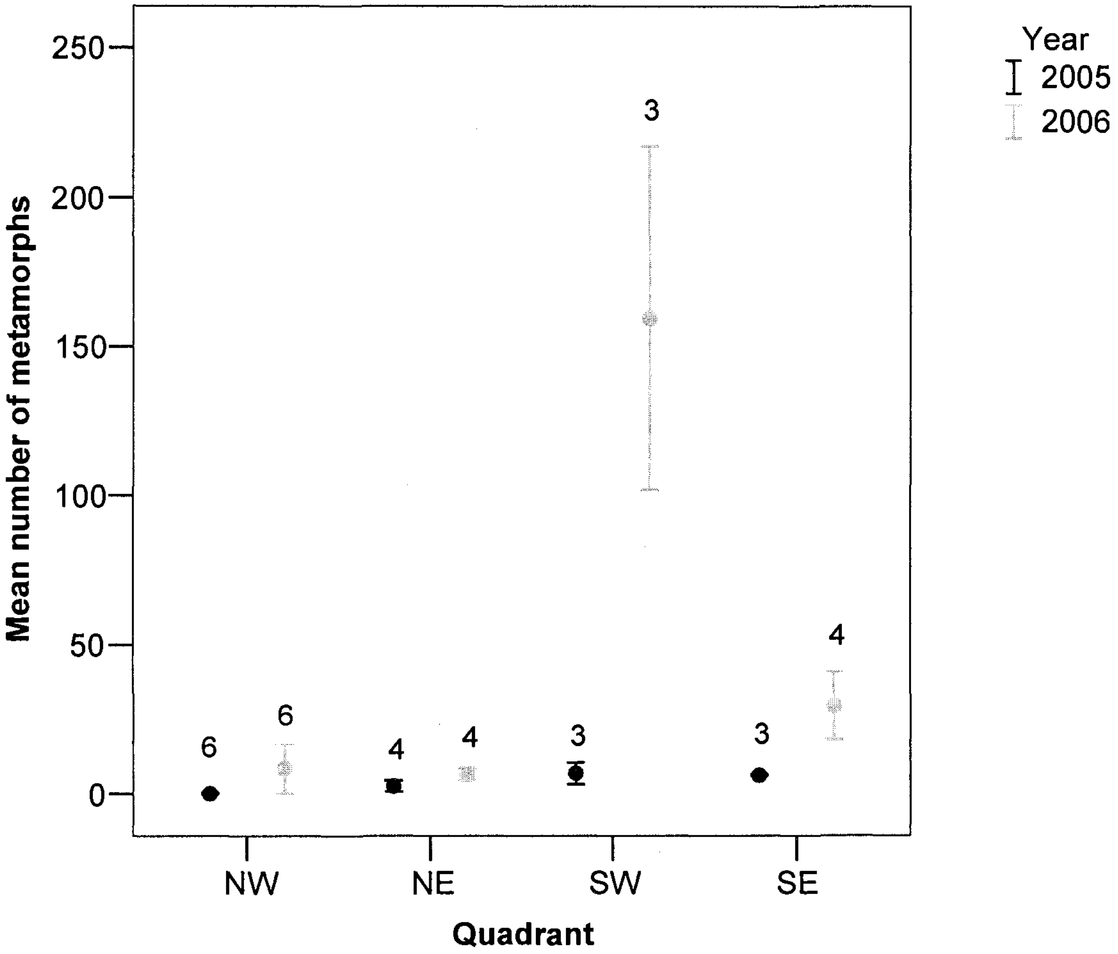


Figure 4.11. The mean (± 1 SE) number of *R. pipiens* metamorphs caught per quadrant in 2005 and 2006. Numbers indicate the number of traps in that quadrant each year.

Metamorph orientation did not differ between treed and open traps in 2005 (n = 53 metamorphs) but did differ in 2006 (n = 659 metamorphs), with a greater mean number of metamorphs caught in treed traps (mean ± SE: 62.7 ± 33.6 metamorphs/trap, n = 7) compared to open traps (22.0 ± 15.9 metamorphs/trap, n = 10; Table 4.9). This result did not change when the trap added in 2006 was excluded from the analysis. Although there were more treed (n = 5) than open (n = 2) traps in the south quadrants, but more open (n = 8) than treed (n = 2) traps in the north quadrants, I cannot determine if the quadrant effect (as described above) is driven by the surrounding habitat because the favorable habitat type is concentrated primarily in the favored emergence quadrants. Trap captures were not influenced by shoreline slope, distance to the pond, or length of the fence in either year (Table 4.10); therefore I did not explore these variables further.

In 2006, metamorph distribution on the shoreline was similar to the location of oviposition sites, but emigration direction was not associated with the distribution of any earlier life stage at the RANA pond. Most egg clusters in the RANA pond were laid in the NE quadrant (Chapter 2), whereas tadpoles distributed themselves according to different physical factors within ponds that varied by quadrant, but not according to their natal sites (Chapter 3). Prior to emigration, metamorphs were associated more often with the NE quadrant than any other quadrant but most metamorphs emigrated towards the two southern quadrants (Figure 4.11).

Table 4.10. Results from linear regression analyses of continuous variables used to predict number of metamorphs caught in traps in both years (n = sample size).

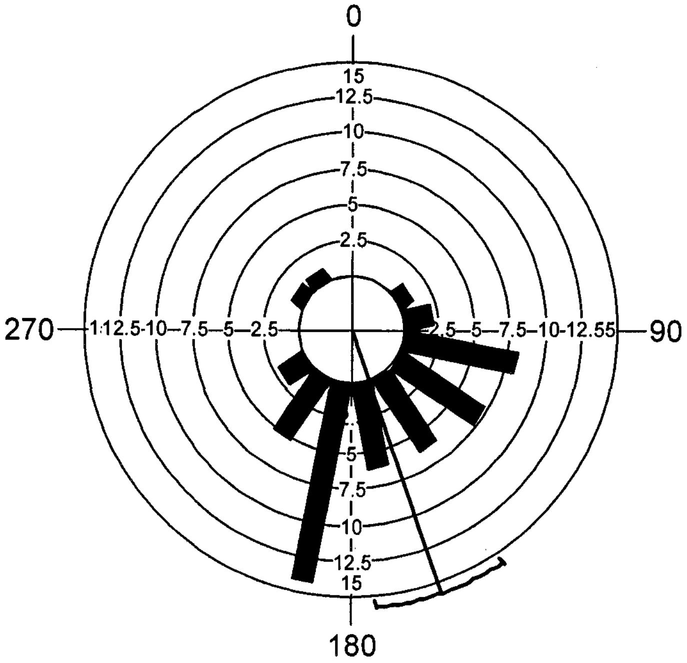
Year	**Variable**	**R-Squared**	**n**	***P***
2005	Slope	0.2	16	> 0.05
2005	Distance to pond (m)	0.1	16	> 0.05
2005	Length of fence (m)	0.2	16	> 0.05
2006	Slope	0.1	17	> 0.05
2006	Distance to pond (m)	0.0	17	> 0.05
2006	Length of fence (m)	0.0	17	> 0.05

Circular statistics

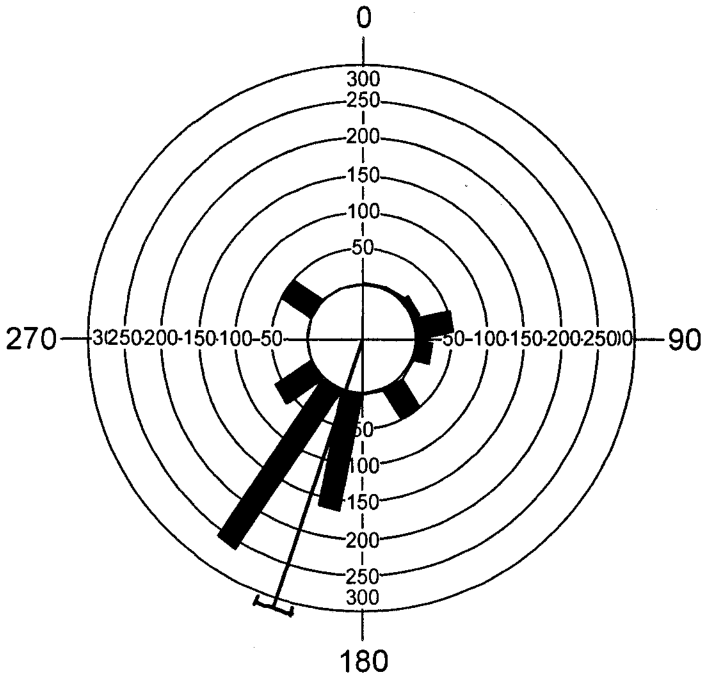
In both years, emigration from the RANA pond was significantly different from a random circular distribution, according to Rayleigh tests corrected for grouping: 2005 ($r = 0.7$, $P < 0.01$, n = 53), 2006 ($r = 0.8$, $P < 0.01$, n = 659). Orientation patterns had approximately unimodal distributions with preferred directions towards the south (160.6° in 2005 and 203° in 2006). The 95% confidence limits of each year did not overlap, indicating that the angles of emigration were significantly different between years, although only by 1 or 2 traps (Figure 4.12).

Body size

'Early' emigrants were trapped in 2005 between August 4 and August 28, and in 2006 between July 13 and August 4. 'Late' emigrants were trapped between August 29 and September 10 in 2005 and between August 5 and September 7 in 2006. All 53 metamorphs caught in 2005 were used in the GLM, but in 2006 22 metamorphs (of 659) were excluded due to missing data (n = 20) or because they were extreme outliers (n = 2). The SVL of metamorphs caught in each quadrant did not differ between quadrants (GLM, $F_{0.05,1,675} = 0.4$, $P > 0.05$) and the interaction between quadrant and year was not significant (GLM, $F_{0.05,1,675} = 0.4$, $P > 0.05$). However, emigrants in 2005 were significantly larger (mean ± SE; SVL = 36.8 ± 3.6 mm) compared to emigrants in 2006 (SVL = 33.9 ± 2.8 mm; GLM, $F_{0.05,1,675} = 15.9$, $P < 0.01$).



A.



B.

Figure 4.12. Circular diagram indicating the orientation of all first captures of metamorph *R. pipiens* in 2005 (A) and 2006 (B). The length of the bars indicates the total number of captures in each trap, with the abundance scale indicated on the concentric circles. The

solid line represents the mean vector with the 95% confidence limit indicated by the arc outside the circle.

Size differed significantly between early and late emigrants (GLM, $F_{0.05,1,675} = 7.1$, $P < 0.01$), but this depended on the year (GLM, $F_{0.05,1,675} = 16.1$, $P < 0.01$; Figure 4.13). Early emigrants were larger in 2005 (mean SVL ± SE; 38.8 ± 0.4 mm), compared to later emigrants (SVL = 34.2 ± 3.28 mm). Conversely, early emigrants were smaller than later emigrants in 2006 (SVL = 33.8 ± 0.11 mm for early emigrants and 34.7 ± 2.4 mm for late emigrants).

Handling effects

There were 4 recaptures in 2005 and 133 recaptures in 2006, which represents a 7.5% and 20.2% recapture rate respectively. I could not statistically analyze the results from 2005 because the sample size was too small. However, in 2005 three of the four recaptures were initially caught in the SE quadrant and recaptured in the SW quadrant, and the fourth metamorph was initially caught in the SW quadrant and recaptured in the SE quadrant. In 2006, the distribution of recaptures was significantly different from random ($\chi^2 = 33.9$, df = 1, $P < 0.01$). Metamorphs were caught more often in the same quadrant as their first capture compared to a different quadrant (Table 4.11). Only 6.8% of second captures were in a quadrant opposite to that of their first capture. Because I did not know individuals, I could not determine which quadrant metamorphs were released in when returned to the pond, and whether that would influence their second emergence direction. To account for this, the release quadrant changed in a counter-clockwise direction every capture day.

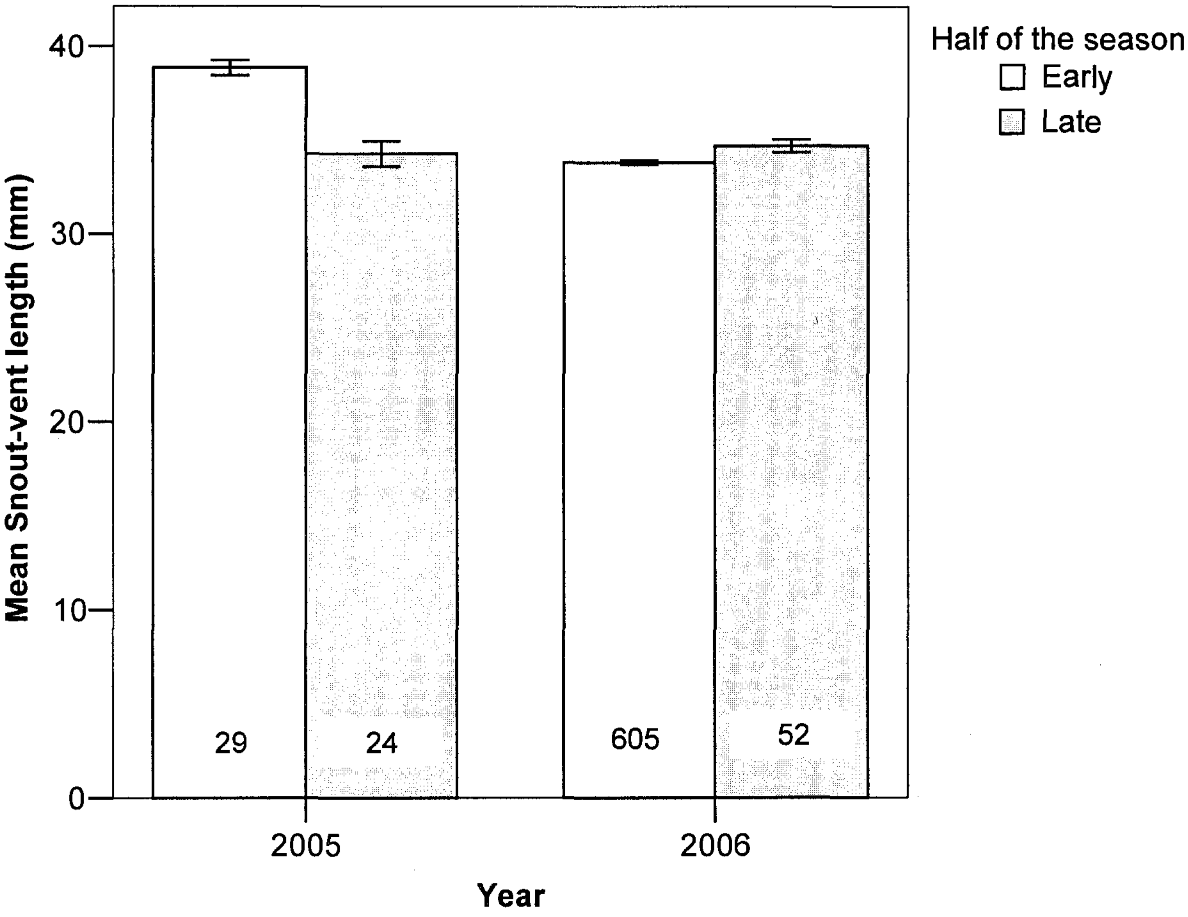


Figure 4.13. Mean (± 1 SE) snout-vent length (SVL; mm) of metamorph *R. pipiens* in 2005 and 2006, segregated into early and late in the season. Numbers indicate the number of metamorphs in that category.

Table 4.11. The distribution of second captures in 2006. Numbers indicate the first and second quadrant of all recaptures. Numbers in parentheses indicate the percentage of second captures that were recaptured in the same quadrant as their first capture (e.g. 37.5% of recaptures that were initially caught in the SE quadrant were recaptured in the SE quadrant). Shaded cells indicate the second captures that were caught in the same quadrant as the first capture (i.e. if all second captures emigrated in a similar direction as their initial capture, these cells would be 100%).

	Second quadrant			
First quadrant	**SE (%)**	**NE (%)**	**NW (%)**	**SW (%)**
SE	6 (37.5)	1 (6.3)	3 (18.8)	6 (37.5)
NE	1 (16.7)	0 (0)	1 (16.7)	4 (66.7)
NW	0 (0)	0 (0)	1 (25)	3 (75)
SW	26 (24.3)	3 (2.8)	23 (21.5)	55 (51.4)

Weather

There were no correlations between environmental variables and frog captures in either year (Spearmans correlation; $-0.5 \leq \rho \leq 0.5$). With the exception of one week in August, mean weekly air temperatures in July and August were 2-7°C higher in 2006 than in 2005. In the primary dispersal months of July and August, there was a total of 72 mm of rain in 2005 whereas there was no precipitation recorded in 2006 during the same period.

Discussion

Overview of results

In this chapter I have documented the spatial distribution of metamorph *R. pipiens* around breeding ponds both prior to and during emigration. Using observational surveys around the RANA and ENR ponds in 2006, metamorphs were generally found more often on the north shorelines relative to south shorelines and in non-shaded, shallow, warm water prior to exodus from the pond. Their numbers generally decreased as the summer progressed. Emigration direction was consistently non-random and oriented towards the south, but the mean direction differed slightly between years. Results from 2006 indicate that, out of all of the stages studied up until now (egg cluster, tadpole and metamorph distribution both prior to and during emigration), only the egg cluster sites (Chapter 2) and metamorphs on the shoreline were distributed similarly (both were located primarily on the north sides of the RANA pond). Also, metamorph production and their size at emigration depended on the timing of emigration and differed between years. Finally, results suggest that metamorph emigration direction is consistent and that the capture and handling of metamorphs does not alter their natural orientation behaviour. These findings contribute to an unexplored area of amphibian ecology and highlight the need for increased testing of common assumptions associated with amphibian conservation strategies.

Metamorph surveys

Preliminary observations found that *R. pipiens* metamorphs do not leave the breeding pond immediately following metamorphosis, but instead remain at the pond for up to two months. Similar findings have been observed for newly metamorphosed *Bufo calamita* (Boomsma and Arntzen 1985; Tejedo and Reques 1994). Their affinity for breeding ponds can be explained by a high desiccation risk for metamorphs immediately following metamorphosis (Ray 1958).

At the RANA and ENR ponds, metamorph *R. pipiens* were more likely to be found in the NE quadrant relative to the SW quadrant. This cannot be attributed to more shoreline available in the NE quadrant because the NE quadrant had the fewest number of stakes compared to all other quadrants. Also, metamorph abundance was higher in the RANA compared to the ENR pond in all quadrants except the NW quadrant. These results suggest that factors that were not tested for, such as prey density or abundance of hiding spots for metamorphs, might have influenced orientation towards the north shorelines, and especially the northwest shoreline in the ENR pond. A more controlled experiment is needed to address whether metamorphs orient according to certain cardinal directions and whether this depends on the pond.

Metamorph presence and abundance was generally higher in warm, shallow areas of the ponds. As with tadpoles (Chapter 3), selection for warm areas of ponds can be explained by the enhancement of locomotor, metabolic and sensory abilities (and hence increased ability to capture food and evade predation) in ectotherms when they are exposed to warm temperatures (Downes and Shine 1998). Even though shallow water is often associated with warmer temperatures (Thurnheer and Reyer 2000), these two variables were not highly correlated and did not interact in my study. However, depth was also an important predictor of metamorph presence and abundance, thus, both factors are important in metamorph microhabitat selection. Although the pattern was not very clear for metamorph abundance, they were more likely to be present in shallow compared to deeper water. Shallow water may provide more energetically appropriate habitat for frogs to emerge to catch terrestrial prey compared to deeper water.

Although the presence of metamorphs was not influenced by shade, where they did occur, their abundance was higher in non-shaded areas compared to shaded areas. Basking behaviour has been shown to increase digestion rates in anurans (Lillywhite et al. 1973). This could lead to faster energy release and sequestering, and emptying of the stomach to allow metamorphs to continue foraging in order to increase in size (Lamoureux et al. 2002) but this has not been tested in *R. pipiens*. The presence of shade interacted with temperature to influence metamorph abundance, which suggests that even if nearby pond water is relatively cold, metamorphs may seek out sunny shoreline spots to thermoregulate. My methods did not discriminate between metamorphs in or out of the water; therefore, if they were on the shoreline the temperature of the water would presumably not influence them if their intent was to control their body temperature via sunlight. Metamorphs may have also occurred in high densities in shaded water if the location only recently became shaded because of changes in the sun's position, and the water temperature had not yet cooled.

Lastly, metamorph presence and abundance generally decreased over time, which is easily explained by emigration from the ponds as the summer progressed. This effect, however, depended on several factors. Even slight variation in the time of day that surveys were completed may have influenced the degree of shading or other factors important to metamorphs in the quadrants relative to each other, which could explain why the decrease in abundance over time varied by quadrant. As previously mentioned, both water depth and temperature also influenced metamorph abundance. Their interaction with Julian day suggests that even though metamorph abundance generally decreased over time, in areas where conditions were ideal (i.e. locations with shallow, warm water) metamorph numbers were higher than would have been expected given the time in the season.

Whereas metamorphs are associated with the north sides of ponds before emigration, their actual emigration direction tended to be towards the south (at the RANA pond). This suggests that metamorph spatial distribution prior to leaving the breeding pond is not a reliable indicator of the direction metamorphs will travel upon emigration.

Metamorph emigration

Numerous studies have shown that metamorphs orient non-randomly from their natal pond (Dodd and Cade 1998; Sjogren-Gulve 1998; Malmgren 2002; Greenberg and Tanner 2004; Vasconcelos and Calhoun 2004), but the underlying basis for natural dispersal patterns remains largely unknown. The non-random emigration patterns of metamorph *Rana pipiens* in both years agree with my predictions and suggest that cues are used for orientation. Metamorphs may emigrate southward because Battle Creek, a potential overwintering site and the closest known waterbody, is located 160 m south of the RANA pond (and evidence in Chapter 5 suggests at least some metamorphs go there). Given that *R. pipiens* metamorphs generally move during the warmest and driest time of the year, directional movement towards the nearest water source could decrease the likelihood of desiccation (Merrell 1977). However, metamorph spotted salamanders (*Ambystoma maculatum*) and wood frogs (*Rana sylvatica*) have failed to exhibit oriented movement towards wetlands only 50 m away (Rothermel 2004). Moreover, in experimental arenas, dehydrated *Bufo calamita* were not able to detect water 1 m away without prior experience of its location (Dall'Antonia and Sinsch 2001). Therefore, it may be unreasonable to assume that metamorph *R. pipiens* are capable of detecting and orienting towards water at a distance of 160 m in this study. Emigration directed towards the creek may simply be an artefact of orientation related to the physical features immediately surrounding the pond.

Strong directional selection for the south side of the RANA pond could be explained by metamorph preference for treed areas compared to open areas adjacent to the pond when emigrating in 2006. The lack of statistical significance of emigration towards treed areas in 2005 could be due to the lower metamorph numbers (i.e. a lower sample size and therefore lower power of the test) that year, because there were no obvious physical changes in the vegetation surrounding the pond from one year to the next, and the quadrant with the highest number of captures (SW) did not change between years. Orientation towards closed canopy or forested areas adjacent to ponds has been demonstrated numerous times for amphibians (e.g. deMaynadier and Hunter 1999; Malmgren 2002; Vasconcelos and Calhoun 2004; Marty et al. 2005; Jenkins et al. 2006; Rittenhouse and Semlitsch 2006) and can be explained by greater dehydration rates experienced by metamorphs in open, grassier areas compared to treed areas (Rothermel and Semlitsch 2002; Rittenhouse et al. 2004). Interestingly, the occurrence of *R. pipiens* is usually associated with open areas (Werner and Glennemeier 1999; Guerry and Hunter 2002). Compared to the north-facing slopes in the Cypress Hills which consist primarily of lodgepole pine *(Pinus contorta)* and white spruce *(Picea glauca)* forest, vegetation on south-facing slopes, which is where the RANA pond is located, is more consistent with grasslands surrounding the park. Therefore, even though the south side of the RANA pond might be more heavily vegetated relative to the north side, although speculative, the entire landscape surrounding the RANA pond may be considered an 'open' habitat compared to habitat at other amphibian sites. Orientation is likely habitat-dependent, but more research is needed at other breeding sites to help clarify these results.

A third possibility that could explain the non-random emigration pattern of metamorphs from the RANA pond is the use of olfactory cues as an orientation mechanism to 'track' the paths of other amphibians to suitable terrestrial sites. Hayward et al. (2000) found that young great crested newts (*Triturus cristatus*) can detect and follow chemical cues left by adults and postulated that tracking is an important mechanism for young amphibians to locate suitable terrestrial habitat. Although I did not track the movement patterns of adults in this study, I feel it is unlikely that *Rana pipiens* metamorphs were following adults, because most adults leave the pond immediately following breeding to forage further from the site (Merrell 1977; Hine et al. 1981; pers obs). Any scent trails left by adults would have to persist in the environment for at least two months until after tadpoles metamorphose to be used in metamorph orientation. Alternatively, metamorphs might follow cues left by earlier emigrants (Malmgren 2002), although this is not likely an effective strategy, as later individuals would have to follow equally naïve individuals into unknown territory. Further experimentation is required to determine the scent trailing ability of *R. pipiens* and whether it is used for orientation.

The mean orientation direction at the RANA pond changed somewhat from 2005 to 2006. As previously mentioned, the results may stem from differences in metamorph numbers caught each year (i.e. had more metamorphs been caught in 2005, the mean directions between years may have been more similar), but they could also suggest that cues associated with metamorph movements differ from year to year. Jenkins et al. (2006) found that emigration direction of metamorph marbled salamanders (*Ambystoma opacum)* differed between years, and attributed this to changes in the physical environment between years. As previously mentioned, there were no obvious physical changes to the environment between years at the RANA pond, but general environmental conditions in 2005 were colder and wetter than in 2006, which may have influenced the sensory abilities of metamorphs. Habitat selection likely occurs hierarchically through the incorporation of cues received by way of olfactory, visual and tactile mechanisms (Rittenhouse et al. 2004) that may vary in their importance. For instance, odours carry further in moist air, making it easier for individuals to orient towards water (Sinsch 1991), and therefore olfaction may play a greater role in orientation under humid conditions compared to other cues under the same conditions. Accordingly, olfaction may have been a more important orienting mechanism for metamorphs in 2005 than in 2006 because conditions were moister in 2005. Likewise, if metamorphs orient according to the sun's position, there might have been a reduced intensity of these cues on cloudy days in 2005 compared to sunnier conditions in 2006 (Semlitsch and Ryan 1999). Other factors that might govern changes in orientation between years include annual changes in metamorph production, prey density, water temperature, and intensity of competition in subareas of the pond (Healy 1975). All of these hypotheses require further testing and additional years of data collection are required to determine if movement patterns are predictable over the long-term. Moreover, even though a significant difference in mean direction between years was detected, emigration was still generally in the same direction (i.e. directionality did not change from north to south among years).

Finally, although a non-random emigration pattern towards the south was observed, not all frogs emigrated southwards. For instance, 77 frogs (12%) were caught in the two northern quadrants in 2006. If the most suitable terrestrial habitat is located south of the pond, then these findings suggest that not all metamorphs select suitable habitat upon emigration. Rittenhouse and Semlitsch (2006) point out that metamorphs often change their direction within 15 m of the breeding pond if they initially moved towards unsuitable habitat. On the other hand, Vasconcelos and Calhoun (2004) found that preference for suitable habitat is evident as soon as metamorph *Rana sylvatica* leave the aquatic environment, and they exhibit nearly straight-line movements up to at least 300 m from ponds. Only 21 marked metamorph *Rana pipiens* were caught in traps on the outside of the fence, in no particular quadrant, in the current study, suggesting that few metamorphs "intended" to change their direction, but they could have returned to the pond to re-hydrate or many still could have changed direction further from the pond. Field work on a broader spatial scale than that used in this study would help determine metamorph movement patterns at greater distances from the pond, and whether immediate emigration direction from the pond is an accurate predictor of long distance orientation.

Distributions of early life stages

Most of the emigration literature investigates only the metamorph life-stage (e.g. Rothermel 2004; Jenkins et al. 2006; Patrick et al. 2007), with little consideration of whether orientation might begin prior to emergence, potentially as early as the egg cluster stage. Hayward et al. (2000) examined *Triturus cristatus* dispersion patterns and determined that larvae associate with oviposition sites, but metamorphs become influenced by orientation mechanisms different from those influencing the larvae. *Rana pipiens* tadpoles did not associate with oviposition sites (Chapters 2 and 3), which contrasts with the findings of Hayward et al. (2000). However, prior to emigration, metamorphs associated with the same quadrant of the RANA pond as that in which most egg clusters were located. Given that no other study has compared these two stages, I can only speculate that these results suggest factors important to the survival of each stage are located in similar areas (e.g. warm, sunny areas of the pond are favourable for both life stages, and potentially more terrestrial prey is also located on the NE side of the pond), and not because metamorphs necessarily associate with egg cluster sites. In agreement with Hayward et al. (2000), however, *R. pipiens* tadpole distribution at the RANA pond was not closely related to metamorph distribution during emigration. Similar to the findings of Miaud et al. (2005), metamorph orientation on the shoreline did not predict emigration direction. Overall, evidence from the RANA pond suggests that, for *R. pipiens*, spatial distributions of early life stages do not entirely predict the distribution of subsequent stages. However, the results do not discount the possibility that factors experienced throughout development may shape the emigration route of metamorphs. To help clarify my results, future work should involve controlled experiments that test the interactions of important environmental factors (e.g. oxygen levels) experienced at different stages of development and how these different conditions influence metamorph movements.

Body size effects

Similar to the findings of Mazerolle and Vos (2006), body size of metamorph *Rana pipiens* did not influence emergence direction, but did differ depending on when frogs emigrate from the RANA pond. Contrary to my predictions, in 2005 early emigrants were larger than later emigrants. Without knowing the timing or the size at metamorphosis in 2005 (I measured the size at emigration), I can only speculate as to whether the size differences resulted from conditions in the aquatic and/or terrestrial stage. Since some studies show that early metamorphosing tadpoles are larger at metamorphosis compared to those that metamorphose later (Altwegg 2002; Altwegg and Reyer 2003), and other studies found no relationship between timing and size at metamorphosis (e.g. Collins 1979; Loman 2002), I cannot determine if size differences at the RANA pond in 2005 are directly related to different times of metamorphosis. However, larger metamorphs can capture a greater size range of prey (Healy 1975), and have a lower desiccation rate (Ray 1958), increased locomotory performance (Goater et al. 1993) and greater dispersal abilities (Vasconcelos and Calhoun 2004) compared to smaller metamorphs. Clearly larger metamorphs are better suited for terrestrial activity and therefore may emigrate as soon as the conditions are appropriate. Boomsma and Arntzen (1985) found that anuran metamorphs spend their first terrestrial weeks in the immediate vicinity of the natal pond, during which time they experience tremendous growth rates. Accordingly, it may be advantageous for smaller metamorphs to remain at the RANA pond in order to increase in size, improving their condition for terrestrial activity further from the pond. However, it is also possible that cues in the environment trigger dispersal regardless of size. Amphibians possess photoreceptors that are used to perceive light for behavioural activities (Adler 1970), which suggests that a decreased number of daylight hours associated with a change of season towards the end of the summer may induce dispersal in amphibians regardless of body size, but this remains to be tested.

Early emigrants were smaller than later emigrants in 2006, in contrast to the results from 2005. The size difference in 2006 is likely not attributable to differences in the timing of metamorphosis because tadpoles metamorphosed in 3 – 4 days (Chapter 3) in the RANA pond, a relatively short time frame to produce differently sized metamorphs compared to the average of 10 days presented in other studies (Collins 1979; Loman 2002). Despite the short time span of metamorphosis, there was a difference of 14 days between when the first and last egg cluster hatched (Chapter 2), resulting in different durations of the larval period, which potentially led to differences in size. Without knowing which metamorphs came from which egg clusters I cannot evaluate whether longer larval periods will lead to a larger size at metamorphosis, especially since the trade-offs associated with either remaining a tadpole or metamorphosing change as tadpoles grow and their environment changes (Smith-Gill and Berven 1979; Semlitsch and Wilbur 1988; Loman 2002). Emigration began nearly a month earlier than in 2005; thus it is less likely that later emigrants were induced to leave the pond because of potential indicators of a change of season later in the summer in 2006. If metamorphs remain at the pond in order to feed and increase in size, this might explain why later emigrants were larger than earlier emigrants. Without knowing the fate of individual frogs after they emigrate from the pond, it is difficult to determine the role size plays in their emigration. Tracking dispersing frogs with radio-transmitters (e.g. Jehle and Arntzen 2000; Bulger et al. 2003; Muths 2003) could be an effective technique to be used in future studies in order to evaluate how size at metamorphosis influences terrestrial movements of frogs.

Year effects

Both the size and number of metamorphs produced at the RANA pond differed between years. Metamorphs caught in 2005 were significantly larger than those caught in 2006, and there were13 times the number of metamorphs emigrating in 2006 compared to in 2005. Yaremko (1994) also found yearly differences in the number of *Rana pipiens* metamorphs between years at similar sites around the Cypress Hills, but did not provide the degree of fluctuation. The number of metamorphs is presumably a function of the number of eggs deposited each year (Collins 1979; Berven 1990). Furthermore, since metamorph size was larger overall in the year with fewer frogs (2005), preliminary results from the RANA pond suggest that the size at metamorphosis is negatively related to the density of tadpoles. Such density-dependence of metamorph size has been demonstrated for European toads (*Bufo bufo*; Goater 1994), spadefoot toads (*Spea hammondii*; Morey and Reznick 2001), moor frogs (*Rana arvalis*; Loman 2002), common frogs (*Rana temporaria*; Loman 2002), wood frogs (*Rana sylvatica*; Berven 1990) and another study of *R. pipiens* (Gromko et al. 1973), and is usually related to increased resource availability (i.e. food) in low density conditions (Healy 1975; Goater 1994; Morey and Reznick 2001). Finally, although I am not aware of any published data on bypass (moving across drift fences without being captured) rates of anurans, it should be recognized that the total number of metamorphs emigrating each year could be underestimated because drift fences might not always attain 100% capture efficiency. This bias, however, is independent of quadrant or trap (i.e. the same likelihood of escape exists around the entire pond).

Another difference between years is that emigration began almost a month earlier in 2006 compared to 2005. If breeding occurred within the same time frame in 2005 as it did in 2006 (late April to the end of May; Chapter 2), tadpoles under higher densities and/or exposed to warmer weather conditions may metamorphose and emigrate more quickly relative to those subjected to lower densities. This might be a means of escaping crowded conditions. Tejedo and Reques (1994) posit that improved growth performance by metamorphs in terrestrial habitats compared to growth of tadpoles in aquatic habitats will favour early metamorphosis of tadpoles. Although I have no data on growth rates in the water or on land, resource availability for tadpoles in the RANA pond was likely much lower in 2006 compared to 2005 for tadpoles because of the difference of at least 600 animals between years.

Weather conditions

Contrary to my predictions and the findings of other amphibian studies (e.g. Dole 1971; Healy 1975; Richter et al. 2001; Vasconcelos and Calhoun 2004; Todd and Winne 2006), the timing of *Rana pipiens* metamorph emigration in this study did not depend on environmental conditions. This could be explained by when the weather data were collected (at every trap check), since environmental conditions were compiled over the previous 24 hr period and therefore weather variables used in the correlation analysis were uncoupled from the exact timing of movements. However, in other studies that examined *R. pipiens* movements and determined that they were not triggered by environmental conditions, metamorphs were either dispersing along streams (Seburn et al. 1997) or movements were directed towards wet, grassy areas (Hine et al. 1981), suggesting that moisture plays an important role in emigration, but not necessarily as rainfall. Metamorphs oriented towards treed areas in 2006, which are often associated with higher moisture content than open areas (Rothermel and Semlitsch 2002), but further research is required to determine if moisture gradients vary surrounding the RANA pond on different days, and the extent to which metamorphs are able to detect them. An alternative possibility is that emigration is triggered by developmental changes associated with metamorphosis, food shortage, predation, or population pressures, and not by the external environment (Bovbjerg and Bovbjerg 1964; Bovbjerg 1965). Controlled experiments, such as the use of artificially-created breeding ponds, can test these hypotheses and provide more insight into the factors triggering *R. pipiens* emigration.

Persistence of emigration direction and handling effects

I tested whether natural movement patterns are persistent by returning all metamorphs to the pond within 24 hrs of their initial capture. In 2005, only 4 frogs (7.5%) were recaptured, but 133 frogs (20%) were recaptured in 2006. Similar low recapture rates are common in amphibian research (e.g. Rothermel and Semlitsch 2002) and are explained by poor survival, low movement rates or behavioural avoidance of traps (Rittenhouse and Semlitsch 2006). In 2005, all recaptured metamorphs were caught in quadrants adjacent to their first capture, and data from 2006 indicate that metamorphs were more likely to be recaptured in the same quadrant as their first capture than in any other quadrant. These results suggest that metamorph orientation is persistent at the same pond, likely because cues used to orient did not differ between captures. They also suggest that short to medium term (i.e. < 24 hr) capture and handling of young amphibians does not disrupt their natural orientation mechanisms when returned to the site from which they were caught. Whether metamorphs would be able to adapt to conditions different from those at their natal pond at a reintroduction site or whether they retain a movement pattern related to their natal pond remains to be tested.

Possible Sources of Error

There were several limitations and necessary assumptions of this study that should be taken into account in the interpretation of results. Due to time constraints and the BC pond drying prior to metamorphosis in both study years, emigration patterns could only be investigated at a single site. As a result, the emigration findings are only relevant to the RANA pond, stressing the need for more research at other ponds with successful metamorph production. Trapping data are also constrained by the location of fences delineating quadrants. Because of the design, two adjacent traps that are separated by fences between quadrants will be closer to each other than two traps at either extremes of a quadrant, but only the traps in each quadrant were combined for analysis. An example of a limitation of this design is that a recaptured metamorph that was caught in an adjacent quadrant from its first capture might actually have emigrated in a more similar direction to its site of first capture than if it was caught in the same quadrant twice. Not being able to mark animals for individual recognition precluded a more in-depth analysis of movement patterns, but ongoing research is assessing individual metamorph movements using their dorsal spot patterns.

Conservation relevance and future studies

Central to a successful amphibian conservation strategy is the protection of habitat important to the species in question, and studies of amphibian emigration can provide a scientific basis for establishing protected areas around breeding ponds and reintroduction sites (Semlitsch 2002; Johnson 2003). My findings suggest that *R. pipiens* metamorphs orient non-randomly when leaving the RANA pond, but more work is needed to determine if they exhibit a similar movement pattern at other breeding ponds, and whether they orient according to long-distance cues or those in the immediate vicinity of the pond. Such data will prove useful in locating future reintroduction sites for leopard frogs to ensure necessary orienting cues are available for released frogs. Otherwise, if cues are not available metamorphs may need to be "guided" in the appropriate direction in order to maximize the potential for survival.

Reintroductions are often hampered by the assumption that released animals will behave exactly as those reared in natural habitats (Dodd 2005). Although previous reintroductions of *Rana pipiens* metamorphs in Alberta have not shown many signs of success, my findings suggest that the capture and handling procedure alone was likely not enough to alter their natural behaviour and therefore likely not a reasonable explanation on its own for failed reintroductions. This is positive news for conservationists because these results suggest that animals can be exposed to stressful situations, at least over the short-term (24 hr) and will likely not be disoriented. Most animals are driven by natural selection to be able to cope with acute stress because of natural predator-prey interactions (Teixeira et al. 2007). However, a new environment offers new survival challenges for reintroduced animals. Released animals must have the capacity to learn the location of resources within the new environment, but whether the stress of capture followed by movement to an unfamiliar environment could impair their cognitive ability to do so is not well understood (Mendl 1999; Teixeira et al. 2007). Also, free-living animals in this study voluntarily left their natal site before the first and second capture, but metamorphs released in reintroduction efforts have no such choice and are captured and released regardless of their developmental state. Such traits could have played a role in the low success rate of metamorph reintroductions in Alberta.

Preface to the next chapter

Reintroduction of metamorphs is a popular tool in amphibian conservation (Zvirgzds 1998; Dudgeon and Lau 1999). Metamorphs of pond-breeding amphibians are also the main dispersal agent preventing extinction among local populations and are known to undertake significant overland migrations (Sinsch 1997; Funk et al. 2005; Todd and Winne 2006). Surprisingly, no studies have shown that reintroduced metamorphs will exhibit similar behaviour and dispersal capabilities to their counterparts in natural populations. Using the knowledge gained from the current chapter, the next chapter pursues this topic further by comparing the post-release movements and dispersal characteristics of *R. pipiens* between a reintroduced and natural population.

CHAPTER 5: COMPARING THE DISPERSAL BEHAVIOUR OF WILD AND TRANSLOCATED METAMORPH *RANA PIPIENS*

Introduction

The transfer of animals between sites is increasingly being used as a technique in the management of threatened and endangered species (Griffith et al. 1989; Short et al.1992; Sullivan et al. 2004). Such translocations often involve reintroducing animals to their former ranges (e.g. gopher tortoises, *Gopherus polyphemus* in the USA; Tuberville et al. 2005). The success rate for amphibian and reptile reintroductions is low (19% successful; Dodd and Seigel 1991) compared to birds and mammals (44% successful; Griffith et al. 1989). The greatest constraint on amphibian conservation planning is the lack of basic biological information for most species (Dodd 2005) and implementing a conservation program without regard to the specific biological constraints of a given species will have little chance of success (Dodd and Seigel 1991). For example, movement behaviour of released animals is one ecological factor that could lower reintroduction success (Tuberville et al. 2005) if animals do not settle in the new habitat and instead disperse away from the release site to settle in unsuitable habitat (Macdonald and Johnson 2001). Metamorph (recently metamorphosed) anurans are the least understood life-stage in anuran ecology (Marsh and Trenham 2001) and therefore, if the behaviour of reintroduced metamorphs differs significantly from that of their wild counterparts, this could lead to factors, such as poor habitat selection, that could lower reintroduction success.

Anuran dispersal occurs mainly during the metamorph stage (Dole 1971; Breden 1987; Berven and Grudzien 1990; Greenberg and Tanner 2005). Therefore, the genetic structure and connectivity of anuran populations is dependent on the success of metamorph dispersal (Berven 1990; Sinsh 1997; Rothermel 2004). Anuran metamorphs exhibit a wide range of dispersal abilities; some species move less than 400 m from their natal site (Roble 1979; Pilliod et al. 2002), whereas other species move more than 10 km (Smith and Green 2005). Although long dispersal distances can promote pond colonization or prevent populations from becoming extinct (Marsh and Trenham 2001), they can also result in high extinction rates if disperser survival is low in fragmented habitats (Funk et al. 2005). Furthermore, dispersal abilities can vary between populations in different habitats (McPeek 1989; Ficetola and De Bernardi 2005). It is likely that anuran conservation programs that under-estimate the complexity of metamorph dispersal may have a low chance of success if released animals behave unexpectedly in a new environment (Macdonald and Johnson 2001; Storfer 2003; Cushman 2006).

The Alberta Northern Leopard Frog Recovery Team (2005) claims that the biology and ecology of *Rana pipiens* is well understood, making it a suitable candidate for reintroductions. However, with only a few studies on *R. pipiens* metamorph movement behaviour (e.g. Dole 1971; Seburn et al. 1997), landscape characteristics conducive to metamorph movement are not clear (e.g. movement corridors; Dodd and Seigel 1991; Pope et al. 2000; Dodd 2005; Ficetola and De Bernardi 2005). Therefore, managers could be lacking critical knowledge of key factors that could affect reintroduction outcomes. Moreover, reintroductions commonly assume that released animals will behave like wild animals (Dodd 2005), but this has not been confirmed for *R. pipiens* and obviously cannot be tested without knowing exactly what 'natural' behaviour is.

Concerted efforts to reintroduce *R. pipiens* metamorphs in Alberta occurred between 1999 and 2004, with the intent to re-establish populations in their formerly occupied range (Kendell 2001). In the fifth year of this program (2003), the Alberta Conservation Association (ACA) and Alberta Fish and Wildlife collected four *R. pipiens* egg clusters from four different, naturally-occurring ponds in southern Alberta and transported them to the Raven Brood Trout Station, close to Caroline in central Alberta (Kendell 2004). The egg clusters were placed in two artificial outdoor rearing ponds. Silt fencing surrounding each pond prevented metamorphs from leaving the pond area prior to the commencement of reintroduction activities (Kendell 2001). Once tadpoles had metamorphosed, the young frogs were captured using funnel traps and nets and released in varying numbers at three sites: one site was located 75 m south of the rearing ponds, and the other two sites were located near Rocky Mountain House and Red Deer, Alberta (Figure 5.1). These efforts were not well documented and yielded little evidence of success, generating concern that few lessons were learned that could improve future reintroduction programs (Dodd and Seigel 1991; Short et al. 1992; Letty et al. 2000).

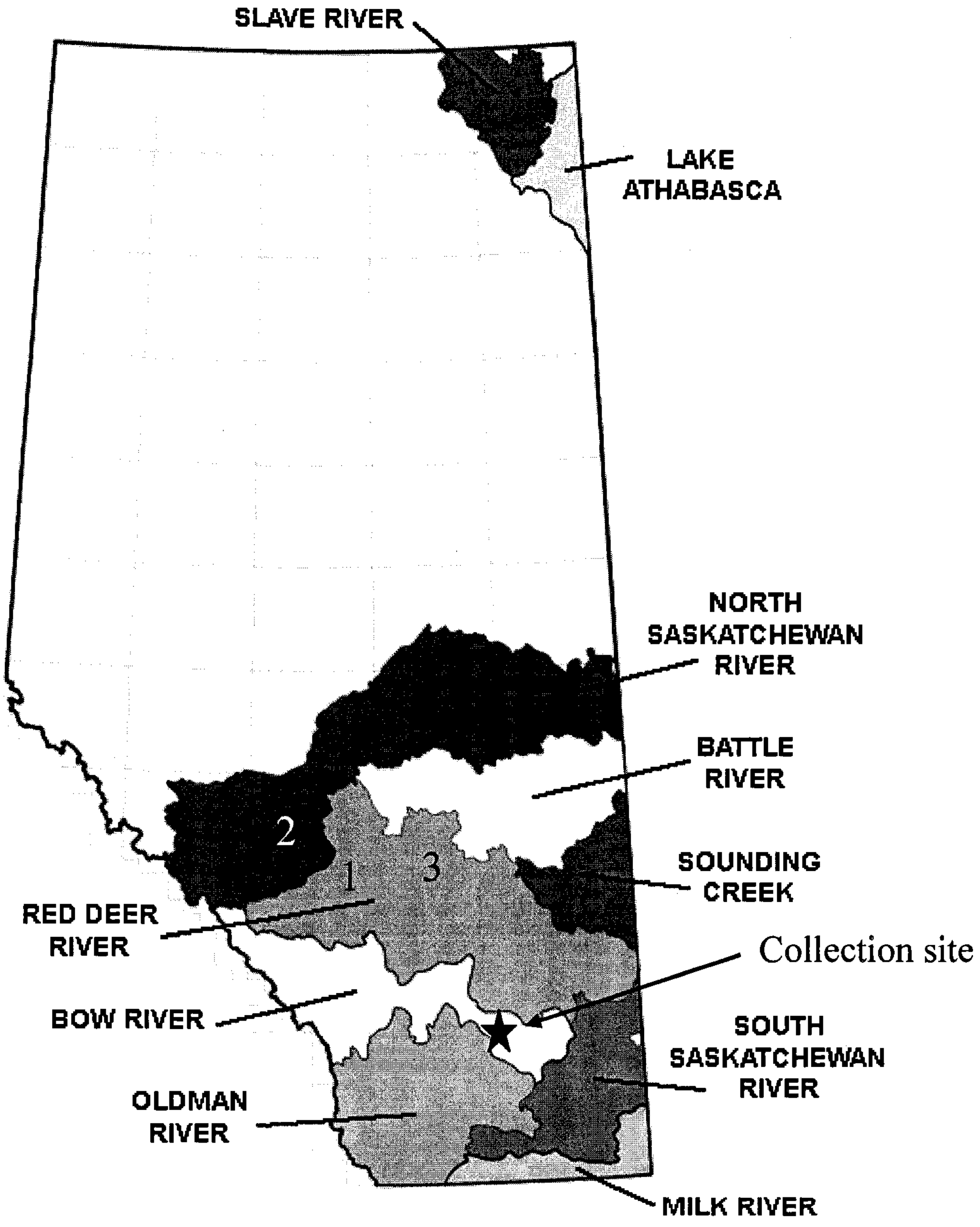


Figure 5.1. Alberta map depicting major drainage systems where *R. pipiens* occur, as well as the area where *R. pipiens* egg clusters were collected from and the release sites near Caroline (1), Rocky Mountain House (2), and Red Deer (3) in 2003 (adapted from Alberta Northern Leopard Frog Recovery Team 2005).

Objectives and Hypotheses

In this chapter I compare *Rana pipiens* dispersal behaviour in a wild population (the RANA pond) with that of cohort of reintroduced metamorphs at the Raven Brood Trout Station. Although post-release movements of reintroduced animals cannot be considered the same as natural dispersal in a wild population, the term 'dispersal' is used in both scenarios in this chapter to minimize word confusion. For the first objective I examine whether the distance travelled following the release of reintroduced individuals is similar to distances moved by metamorphs in unmanipulated circumstances, and whether the mean distance moved changes over time in one or both circumstances. One possible motivation for dispersal from the natal site is to increase the distance between individuals in order to establish a foraging site separate from that of other metamorphs. Dispersing to other sites will also promote gene flow between populations and reduce inbreeding. As a result, I predict that the mean distance travelled will be greater in whichever population has more metamorphs, and the mean distance travelled will increase over time for each population.

For the second objective I address whether the number of recaptures differs between dispersers in a reintroduced population and those of a wild population. Because reintroduced metamorphs were moved a relatively short distance (75 m) between the capture and release site compared to their natural activity range (Dole 1971), I predict that reintroduced metamorphs will resume natural behaviour following capture and release and therefore be recaptured at a similar frequency to their wild counterparts.

Body size influences dispersal ability (Rothermel and Semlitsch 2006), so for the third objective I investigate whether a) the body size of dispersing metamorphs differs between a reintroduced and a wild population, and b) whether body size influences the distance travelled in either population. I predict that reintroduced metamorphs were released at a body size conducive to normal dispersal activity; therefore there will be no difference in body size between recaptures in each population. Finally, since larger frogs have increased locomotory capacities compared to smaller frogs (John-Alder and Morin 1990), I predict that larger metamorphs will travel greater distances in both populations.

Methods

Reintroduction procedures and site descriptions

It was logistically too difficult to conduct this study at all three of the reintroduction sites because they were not located close to each other; therefore this study focuses only on metamorphs released near the Raven Brood Trout Station. The station is located southeast of Caroline, Alberta in the Upper Red Deer River drainage (52° 03' 26.6" N; 114° 41' 16.3" W).

Prior to reintroduction, all metamorphs were captured and marked using Visible Implant Elastomer (VIE; Chapter 4) with a colour that indicated the year of release. Every tenth frog was weighed to the nearest 0.1 g and measured to the nearest 0.1 mm (snout-vent length; SVL; Kendell 2004). Metamorphs were held for no longer than 24 hr before being released. Between August 8 and 26 2003, ACA and Alberta Fish and Wildlife released a total of 1,196 metamorphs at the reintroduction site at the Raven

Brood Trout Station (Kendell 2004). This reintroduction site (hereafter referred to as the 'release pond') was chosen because of its persistence and potential suitability as breeding habitat, and because potential overwintering habitat in the form of creeks was available within 300 m (Kendell 2001). When water level is high, the west end of the release pond connects to Beaver Creek via an approximately 100 m long drainage (Figure 5.2). To the east of the release pond is a series of springs, and a spring fed creek that meanders around the east side of the Raven Brood Trout Station. The release pond is surrounded by a conifer forest to the east and south, and open stands of alders (*Alumus* sp.), aspen (*Populus tremuloides*) and balsam poplar (*Populus balsamifera*) to the north and west (Kendell 2001). Chapter 2 provides a description of the RANA pond used as the wild comparison.

To compare the movements of the reintroduced and wild populations, visual surveys were conducted along the closest creek system to both the release pond and the wild pond. Creeks were chosen for the surveys because metamorph *R. pipiens* have been shown to disperse either along or towards water bodies (Merrell 1977; Yaremko 1994; Seburn et al. 1997). The creeks provided the most comparable habitat feature between sites. The two creek systems are at similar elevations (within 80 m). The springs to the east of the release pond were not surveyed because they were bordered by human-altered landscape (e.g. mowed lawns, buildings), whereas the habitat along Beaver Creek closely resembles habitat found in the Cypress Hills, where the wild population was located (Kendell 2001). Battle Creek is the closest creek system to the RANA pond. In contrast to Beaver Creek, Battle Creek was not directly connected to the RANA pond, but was located 160 m downslope and south of the pond. This is the direction towards which most

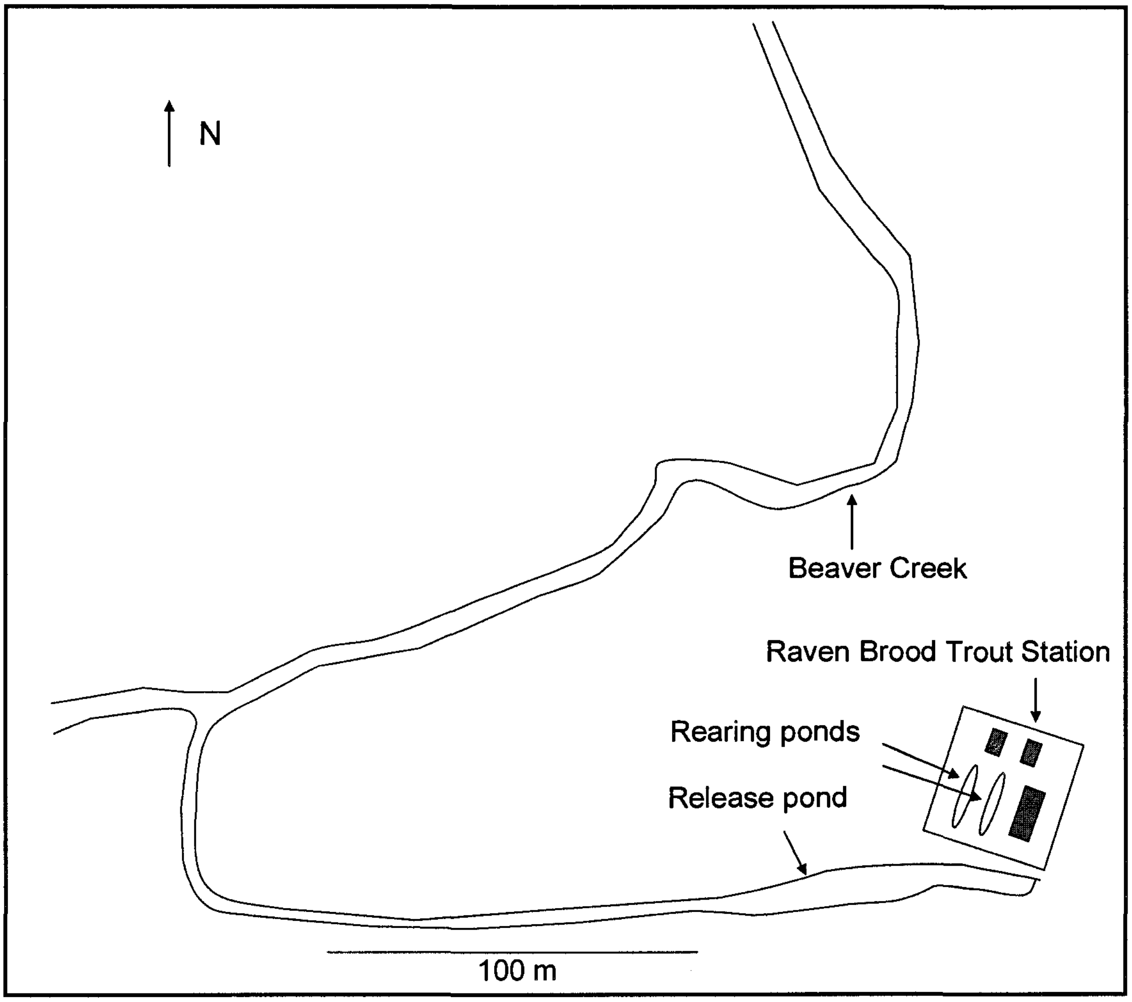


Figure 5.2. Line drawing of the reintroduction site. The Raven Brood Trout Station is not drawn to scale.

metamorph *R. pipiens* had emigrated in two consecutive years, including the year of this study (Chapter 4). The RANA pond was approximately 80 m above Battle Creek. Chapter 4 provides a description of the methods used to capture and mark all metamorphs originating at that site.

Survey methods

Visual encounter surveys (VES) began at the west end of the release pond where it connects to Beaver Creek, and at the closest point on Battle Creek 90° from the RANA pond. For each survey, I walked immediately adjacent to the shoreline of the creek, searching for *Rana pipiens*, with a visual survey span encompassing a 3 m breadth extending equally across the water and adjacent shoreline. At Battle Creek, there was an additional observer who walked 5–10 m from the shoreline and assisted in processing captured individuals. The additional observer at Battle Creek did not double the survey effort because frogs were never located more than approximately 2 m onto land, so the second observer never saw a frog before the observer at the shoreline. It was assumed that every metamorph had the same chance of being observed and captured between sites (Crump and Scott 1994). Surveyors attempted to capture all metamorphs seen and noted any that were seen but not caught. To avoid catching individual frogs more than once during a survey, metamorphs were held in a bucket until the shoreline had been surveyed at least 25 m beyond the capture site, at which point the surveyor returned any captured metamorphs and released them at their point of capture.

A digital photograph (Nikon Coolpix 4300) of the dorsal spots of each metamorph was also taken, to record identifying features so that it could be determined if a metamorph was caught more than once during a survey, or from one survey to the next. Identifying amphibians using digital photographs of their dorsal spot pattern is becoming a popular tool in mark-recapture studies (e.g. Corn and Bury 1991; Kupfer and Kneitz 2000; Bailey et al. 2004; Gamble et al. 2006). Previous work on *R. pipiens* revealed that matching photos is reliable, especially for small samples (i.e. < 50; pers. obs). Spot patterns of every recaptured metamorph were organized hierarchically into the presence or absence of a nose or eye spots, and then the number and shape of spots between the eyes and dorsolateral lines. By attempting to match all digital photographs of marked metamorphs for all survey periods, I was able to determine if any metamorphs were caught more than once. Previous work on metamorph *R. pipiens* indicates that spot patterns would not change over the time frame of this study (L. Fraser, unpublished data).

The following variables were recorded for each metamorph captured: location (recorded using a GPS; Global Positioning System; eTrex Summit ®); VIE marks; snout-vent length (SVL; with dial calipers to the nearest 0.01 mm); and weight to the nearest 0.1 g (with a Pesola spring balance). Since I could not determine the origin of metamorphs that were encountered but not caught, or that were caught but did not have VIE marks, only captured metamorphs with VIE marks were used in the analysis.

Creek surveys began 3–4 weeks after the first day metamorphs were reintroduced (in 2003 at the release pond) or captured in a pitfall trap (in 2006 at the RANA pond; Chapter 4) indicating dispersal had begun. Beaver Creek was only surveyed downstream of the release pond because for the first 150 m (approximately), the only direction available from the release pond was downstream via the drainage. Moreover, once the drainage connected to Beaver Creek, access to property upstream was not permitted. However, surveys at Battle Creek in the Cypress Hills were conducted both upstream and downstream of the pond. Previous studies have indicated that metamorphs do not have a directional preference when homogeneous habitat is available in both directions (e.g. Wahbe et al. 2004). Only the north sides of the creeks were surveyed. For each survey, the observer(s) searched 300 m beyond the location of the last marked metamorph. Three hundred metres was arbitrarily chosen to encompass the majority of metamorphs moving at each site. However, since long-distance dispersers could have travelled beyond this distance, results can only be interpreted to the scale of the study. If no marked metamorphs were captured during an entire survey, the observer(s) stopped searching at 300 m from the start point. Subsequent surveys occurred every three weeks until no metamorphs were encountered in the first 300 m of a survey.

Similar to the approach employed by Doan (2003), the VES searching method used in this study differed from that of others in that I did not use a time-constrained approach. That is, there was not a prescribed time period for searching. Other than for part of one survey, I was the primary observer conducting the surveys at both Beaver and Battle creeks. This minimized intraobserver variation for both the pace used to search for metamorphs and their likelihood of being observed and caught between sites and surveys. However, the total length of time required for each survey depended on the number of metamorphs caught, resulting from the time required for processing metamorphs. Obviously, the more metamorphs that were captured, the longer was the time required for the entire survey. The actual time spent searching (i.e. survey effort), however, did not vary between surveys or sites.

To control for potential differences in amphibian activity patterns at dusk and dawn and under different weather conditions, all surveys were conducted between 0900 and 1600 hr on sunny days. Because surveys were conducted in both the summer and fall, and in different geographic locations, the air temperature varied. Weather conditions at the two sites were compared using data from the nearest Environment Canada weather station. For the reintroduced population, this was located at Sundre, Alberta, at 51° 46.800' N and 114° 40.800' W. The location of the closest weather station to the wild population is described in Chapter 2.

Statistical analyses

Distances travelled

A non-parametric Mann-Whitney U test was used to determine whether the mean distance travelled by metamorphs in the reintroduced and wild populations differed. Non-parametric analyses were used because the data were not normally distributed, even with transformed data. The maximum distances travelled at each site are also reported. Beaver Creek is connected to the release pond; therefore, distances travelled indicate distances from the pond. However, for the wild population, only the distances along the creek relative to the closest point to the RANA pond were used in the analysis. The additional 160 m travelled overland by the wild population is considered in the discussion.

To determine whether the mean distance travelled by metamorphs changed over time at Battle Creek, a non-parametric Kruskal-Wallis test was used and surveys upstream and downstream were combined. Again, normality could not be achieved in order to use parametric tests, even with transformations. If a significant difference in the distance travelled between surveys existed, non-parametric Mann-Whitney U tests comparing each pair of the median distance ranks were conducted, using Bonferroni adjustments for multiple testing. Such an analysis was not possible for the metamorphs at Beaver Creek because no metamorphs were encountered after the first survey.

Recapture comparison

Using the total number of metamorphs that were reintroduced at the release pond and the total number of metamorphs that were released outside the RANA pond at the time the surveys were conducted as the expected number of recaptures, a chi-square analysis was conducted to determine whether the number of metamorph recaptures is independent of the site for each survey period (every 3 weeks). The number of recaptures both upstream and downstream at Battle Creek was combined for the analysis; the fact that Beaver Creek was only surveyed in one direction is considered in the interpretation of results. Wild metamorphs caught at Battle Creek should have had two marks, one on each foot, because after their first capture at the RANA pond they were returned to the pond to be captured a second time (Chapter 4). Only those metamorphs caught twice, and thus having received two marks, were released outside the pond (see Chapter 4 for details). However, several metamorphs were caught at Battle Creek that had only one mark. In such cases it was always on the right foot, which suggests that they were caught upon their first emigration attempt and either lost their second mark or bypassed the drift fence without recapture after replacement back in the pond. Without knowing which the case is, the chi-square analysis was conducted twice. The first analysis assumed a high bypass rate and used the total known number of metamorphs documented at the RANA pond (the total number of first time captures; Chapter 4) as the possible number of metamorphs able to be recaptured at Battle Creek. The second analysis assumed that only those bearing two marks ended up outside the RANA pond, and any metamorphs caught with one mark at Battle Creek had lost their second mark. As a result, the known number of second captures that had been released at the time of the survey was used as the possible number of metamorphs able to be recaptured for the second analysis. In the event that the assumptions could not be met for a chi-square analysis, a Fisher's Exact test was used. The percentage of recaptures was also calculated by dividing the number of recaptures for each survey by the known number of metamorphs available for capture at each site.

Body size comparisons between sites

Two t-tests were used to determine whether the body size of the reintroduced and wild metamorphs differed during their movements. The first t-test determined whether there was a difference in SVL prior to dispersal movements between populations. In the absence of this information, it was not possible to determine if a difference in recapture size between sites is merely because they were of a different size to begin with prior to dispersal, or if the size differences stem from differences during movements between populations. The second t-test determined whether the SVL of metamorphs captured along the creeks differed between sites. Metamorph weights were not considered in the analyses (see Chapter 4 for explanation).

To determine whether there is a linear relationship between body size and distance travelled among metamorphs in the same population, scatter plots between the two variables were constructed and the relationship was tested using a linear regression for each survey.

Results

Incidentally, because of the lay-out of the reintroduction site, I walked by the release pond almost every day. During the first week after release, I observed that hundreds of metamorphs remained at the release pond and none were observed near their natal pond 75 m away. The number of metamorphs observed at the release pond appeared to decrease over time. A family of muskrats (*Ondatra zibethicus*) inhabited the release pond and mink (*Mustela vison*) were often seen in the vicinity. Other amphibian species observed at the reintroduction site were wood frogs (*Rana sylvatica*) and boreal toads (*Bufo boreas*). Although tiger salamanders (*Ambystoma tigrinum*) used the RANA pond as a breeding site (pers. obs), *R. pipiens* was the only amphibian observed at Battle Creek.

Because of time constraints, the first two surveys for the wild population could not be completed in one day (Table 5.1). No metamorphs were encountered during the second survey in the reintroduced population; therefore, surveys were not continued at that site. Examination of the photographs revealed that there were six metamorphs recaptured twice during different survey periods at the wild site. Five metamorphs caught during the first survey at the wild site were recaptured during the second survey, and one

Table 5.1. Dates of surveys in the Reintroduced (2003) and Wild (2006) populations.

Survey	**Reintroduced**	**Wild**	
		Upstream	**Downstream**
1	September 4	August 14	August 7
2	September 24	September 6	September 5
3		September 30	
4		October 24	

metamorph was caught during both the second and third surveys. No metamorphs were captured twice during the same survey period at either site.

Of the six surveys conducted, four took place on sunny days and when the mean daily air temperature was above 12°C. The second survey of the reintroduced population and the fourth survey of the wild population were conducted at mean daily air temperatures of 4.1°C and 2.8°C respectively. Overall, after the first survey at each site, mean daily air temperatures were generally cooler by nearly 10°C at the reintroduction site in comparison to those at the wild site.

Distances travelled

Five and thirty-five metamorphs were caught in the reintroduced and wild populations respectively, which were used to conduct the Mann-Whitney U test (Table 5.2). The mean distance travelled did not differ between sites during the first survey period (Mann-Whitney U test: $U = 82$, $P > 0.05$). However, the greatest distance from the source that a marked metamorph was caught was 221 m in the reintroduced population, and 594 m downstream and 270 m upstream in the wild population (the zero point being the point on the creek closest to the RANA pond, not the RANA pond itself).

The mean distance travelled for each of the first three surveys in the wild population were significantly different (K-W test: $\chi^2 = 7.168$, $df = 2$, $P < 0.05$). With a corrected alpha value (0.017) for multiple comparisons, Mann-Whitney U tests revealed that this difference stems from the data collected in surveys one and two, for which the mean distance traveled after three weeks (mean ± SE; 138.1 ± 26.8 m, $n = 35$) was significantly less than the mean distance traveled after six weeks (227.9 ± 2.6 m, $n = 26$;

Table 5.2. The number of metamorph recaptures at both sites during the first two survey periods. The number of non-recaptured metamorphs is based on the known number of reintroduced metamorphs that were not recaptured, and on metamorphs that were caught at least once at the RANA pond and may have subsequently bypassed the fence evading a second mark. The number in brackets was used for the second analysis and indicates the known number of metamorphs that were not recaptured in the wild population, but received two marks at the RANA pond and had potentially lost one of their marks. The percentage of recaptures is included. The test used to determine whether the number of recaptures is site-dependent is indicated. Where a chi-square test was used, the chi-square value is indicated. df = degrees of freedom.

		Site				
Survey period	**Recapture**	**Reintroduced population**	**Wild population**	**Test**	**df**	***P***
1	Yes	5	35	χ^2 = 48.214 (322.449)	1 (1)	< 0.00 (< 0.00)
	No	1191	624 (79)			
	%	0.42	5.3 (30.7)			
2	Yes	0	26	Fisher's Exact	1 (1)	< 0.00 (< 0.00)
	No	1196	633 (107)			
	%	0	3.9 (19.5)			

U = 288, $P < 0.05$). For the five wild metamorphs caught after nine weeks, the mean distance travelled was 116 ± 23.9 m, and there was no difference in the mean distance traveled when comparing the data for three and nine weeks (Mann-Whitney U test: U = 83, $P > 0.05$) or for six and nine weeks (Mann-Whitney U test: U = 38, $P > 0.05$).

Recapture comparison

For the first two survey periods, there were significantly more recaptures in the wild compared to the reintroduced population for both analyses (Table 5.2). During the first survey, the number of metamorphs encountered but not captured was 10 and 8 in the reintroduced and wild populations respectively. Eleven metamorphs were encountered and not captured in the wild population during the second survey. All five metamorphs encountered were captured during the third survey in the wild population.

Body size comparisons between sites

Prior to release, reintroduced metamorphs were significantly smaller (mean ± SE, SVL = 32.9 ± 0.15 mm, n = 120) than those dispersing from the RANA pond (SVL = 33.8 ± 0.11 mm, n = 659; t-test: t = -4.8, df = 876, $P < 0.05$). Similarly, metamorphs caught in the reintroduced population were significantly smaller (SVL = 31.9 ± 1.2 mm, n = 5) than those caught in the wild population (SVL = 40.8 ± 0.5 mm, n = 35; t-test: t = 6.6, df = 39, $P < 0.05$) during the first survey. Further, linear regressions and scatterplots revealed that the distance traveled by metamorphs did not depend on their size in any survey ($r^2 < 0.5$ in all analyses).

Discussion

Implementing a reintroduction program without complete knowledge of the target species' movement behaviour following release compromises the chance of success (Macdonald and Johnson 2001). One way to gain insight into the behaviour of released animals is to consider that of free-living animals under similar circumstances (Stamps and Swaisgood 2007). To address this, the dispersal movements of *Rana pipiens* metamorphs in a reintroduced and wild population were examined. Although metamorphs in the two populations travelled similar distances, the number of recaptures differed between populations. Metamorphs from the wild population were recorded to have travelled significantly greater distances six weeks after dispersal began compared to three weeks earlier. The distance travelled did not depend on body size in either population. Differences in body size between populations can be explained by already existing differences in body size at the time of reintroduction and start of dispersal. This study is the first to compare dispersal movements between a reintroduced and a wild amphibian population and I consider below the importance of why detailed studies on metamorph dispersal in different habitats are critical for successful anuran conservation strategies.

Comparison of mean distance travelled between populations

Contrary to my predictions, the mean distance travelled during dispersal movements along creeks between populations did not differ. Given that there were nearly twice as many metamorphs in the reintroduced population, one might expect the mean distance travelled to be greater compared to the wild population in order for animals to establish foraging sites. Several factors suggest that this result should be interpreted cautiously. Firstly, surveys were not conducted upstream at Beaver Creek because of permitting limitations, but it is possible that metamorphs travelled in that direction at a similar or different rate compared to their downstream movements, which may have resulted in over- or underestimating the mean distance travelled. Secondly, metamorphs in the wild population travelled at least 160 m overland prior to reaching Battle Creek, and still managed to travel equivalent distances along the creek compared to their reintroduced counterparts, which did not have to undertake a significant migration on land to access Beaver Creek. Compared to dispersal in water, dispersal on land necessitates that anuran metamorphs must traverse a relatively hostile environment because the desiccation risk is much greater (Sinsch 1990; Mazerolle and Desrochers 2005). Therefore, one might expect the reintroduced metamorphs to have travelled significantly farther because of not being constrained to the same extent by the demand of water balance as the wild population. Conversely, metamorphs may mitigate the mortality risk associated with desiccation by travelling more quickly through unfavourable habitats than they would in water (Rosenberg et al. 1998). More intensive sampling, such as the use of pitfall traps at greater distances from waterbodies, would provide insight into metamorph dispersal abilities in different environments.

As expected, the mean distance travelled increased over time in the wild population until at least six weeks after dispersal began; however, the maximum distance travelled was shorter after six weeks than it was after three weeks. This suggests that the spacing dynamics among individuals within this area differs over time, or that there was selective mortality on those that had moved farther. Many amphibian species are site-loyal and do not move far from their natal ponds (Smith and Green 2005), and dispersal tendencies will vary depending on ecological conditions in different sites (McPeek 1989). More data on *Rana pipiens* dispersal characteristics from other sites will clarify these results. The lack of a significant difference in the mean distance travelled after nine weeks compared to earlier surveys, combined with relatively few metamorphs encountered at nine weeks may have been because environmental conditions at the time of the survey likely induced metamorph *R. pipiens* to remain in the creek (Licht 1991).

Other studies have found metamorph *R. pipiens* to disperse over greater distances than those reported in this study (e.g. Dole 1971; Seburn et al. 1997), which suggests that if the creek had been surveyed more than 300 m past the last marked metamorph there may have been more recaptures at greater distances. Although this is possible, the dispersal trajectories of species will differ depending on the habitat quality between patches, and the condition of individuals (Smith and Green 2005). Smith and Green (2005) report that in studies where there are sufficient data, the greatest distance travelled by dispersers is also the greatest possible distance that could have been recorded given the study area. Therefore, results from my study can only be interpreted relative to the scale of investigation, rather than in the context of the overall abilities of the animals (Marsh and Trenham 2001).

Number of recaptures

The small number of recaptures in the reintroduced population was surprising considering metamorphs were not displaced far from their larval pond. Although never tested for metamorph *Rana pipiens*, displaced metamorph and adult red-legged frogs (*Rana draytonii*), adult mountain yellow-legged frogs (*Rana muscosa*) and adult *R. pipiens* are capable of detecting and returning to their natal pond within a few days of being displaced distances from 500 m (*R. draytonii* and *R. muscosa*; Rathbun and Schneider 2001, Matthews 2003) up to 1 km (*R. pipiens*; Dole 1968). This suggests that metamorph *R. pipiens* in this study may have homed towards their larval pond located 75 m away, but surveys in the weeks following reintroduction at the larval pond found no metamorphs with VIE marks, which suggests they were not homing. Metamorphs could have detected Beaver Creek from another direction since it curves and meanders back towards the direction of the release pond 300 m downstream. Survey methods used in this study would not have detected this. Surrounding the release pond with drift fences and pitfall traps would have helped determine the direction in which reintroduced metamorphs headed.

Even if metamorphs did not home towards their larval pond, adapting to conditions at the release site should not have been difficult. Given the short distance between the capture and release sites, they were presumably exposed to similar prey, predators, and local climatic conditions between sites. Reintroduced metamorphs were also at a similar age to that of natural dispersers (Berven 1990), and were released at an appropriate time of year for dispersal (Chapter 4). As a result, metamorphs were provided with many of the necessary stimuli and conditions considered important for reintroduction procedures (Alberts 2007; Stamps and Swaisgood 2007). Further, the previous chapter demonstrated that capture and handling alone is not enough to disorient metamorphs when they are released at the site at which they were captured. Perhaps being released just 75 m from the capture site is sufficient to disorient metamorphs and preclude their ability to behave naturally. If the reintroduction site was an unfamiliar environment for the released metamorphs, the small number of recaptures along the creek may have stemmed from random or long-distance movements away from the release site.

High activity rates of translocated individuals in novel environments are expected (Sullivan et al. 2004), but immediate, long-distance movements away from the release site suggest that the animals perceive themselves to be in unsuitable habitat (Stamps and Swaisgood 2007). Although there is no evidence that reintroduced metamorphs undertook unnatural, long-distance movements following release, it should be considered a possibility because it has been demonstrated in other translocated ectotherms including adult gila monsters (*Heloderma suspectum*; Sullivan et al. 2004), timber rattlesnakes (*Crotalus horridus*; Reinert and Rupert 1999), hognose snakes (*Heterodon platirhinos*; Plummer and Mills 2000), three-toed box turtles (*Terrapene carolina triunguis*; Rittenhouse et al. 2007), and gopher tortoises (*Gopherus polyphemus*; Tuberville et al. 2005). Translocations of younger animals are considered to be more successful than those involving adults because younger animals usually have not yet established a strong affinity for a particular area (Reinert and Rupert 1999; Pedrono and Sarovy 2000). However, as the main dispersal agent of amphibian populations (Berven 1990), the distinction between erratic, long-distance movements and natural dispersal in metamorphs is difficult. A better understanding of the cues used for dispersal in different environments will help predict movements of reintroduced metamorphs. Even if high activity rates are expected following reintroduction, other effects could explain a small number of recaptures in the reintroduced population.

Numerous studies have indicated that mortality of translocated animals due to predation is highest immediately following release (e.g. Short et al. 1992; Miller et al. 1999; van Heezik et al. 1999; Letty et al. 2000; Banks et al. 2002), and is often explained by a lack of anti-predator skills in the released animals (Alberts 2007). Known predators of *Rana pipiens* were seen at the reintroduction site (e.g. mink), but they were also seen close to the natal pond in the Cypress Hills (e.g. wandering garter snakes, *Thamnophis elegans*; Chapter 2) and I observed a garter snake feeding on a *R. pipiens* metamorph at the RANA pond. In addition, reintroduced metamorphs would have been exposed to similar predators in both their larval and release site, which suggests they did not lack anti-predator skills. However, reintroduced animals are moved to a new habitat at the discretion of the program managers, which might not be at a time when they are developmentally ready in a wild population. Indeed, reintroduced metamorphs in this study were smaller than wild dispersers, suggesting they may have been more vulnerable to predation than those in the wild population, even if they had anti-predator skills.

Mortality could also be a result of low food availability due to the presence of other *Rana* species at the reintroduction site. Reintroductions into areas with potential competitors are less successful than reintroductions into areas without competitors (Griffith et al. 1989). Adult and metamorph *R. sylvatica* were observed at the reintroduction site and although the two species (*R. sylvatica* and *R. pipiens*) occur sympatrically over much of North America, there is some habitat segregation between them (Werner 1992). *Rana sylvatica* breed slightly earlier in the spring and inhabit woodland ponds and forested areas, whereas *R. pipiens* is more often located in open, grassier surroundings (DeBenedictis 1974; Guerry and Hunter 2002). Nevertheless, both species can be found breeding in the same ponds (Werner 1992), and larval competition between them has been well documented (e.g. DeBenedictis 1974, Werner 1992). However, competitive interactions post-metamorphosis have not been examined. With an influx of more than 1,000 metamorphs reintroduced within a short time frame (one week) at a site where *R. sylvatica* occurs naturally, one might expect the prey base for both species to be diminished for all individuals if they have the same food habits, or to potentially induce competition for food that might result in *R. pipiens* leaving the site and foraging in other areas. Although these hypotheses are speculative, competition with congenerics affects habitat quality and must be considered when assessing release sites for reintroductions (Griffith et al. 1989).

Evidently many factors could have contributed to the small number of recaptures in the reintroduced population, but this result is most easily explained at the time of the second survey by environmental conditions. Metamorphs were reintroduced nearly a month later compared to when dispersal began in the wild population, and air temperatures were lower at the reintroduction site compared to those at the Cypress Hills. Together, these suggest that preparation for overwintering may have been triggered earlier in the reintroduced population. Indeed, air temperatures at the reintroduction site in late September are known to stimulate *R. pipiens* to remain submerged in water (Emery et al. 1972; Licht 1991). This could explain the lack of reintroduced recaptures during the second survey, because *R. pipiens* individuals are nearly impossible to spot when they are underwater (pers. obs).

Body size characteristics

There was a size difference between reintroduced metamorphs and those emigrating from the RANA pond; therefore, I cannot conclude whether the difference in size of dispersing metamorphs between sites is due to a difference in size prior to release and dispersal, or due to differences in conditions between sites.

There was no relationship between metamorph body size and distance travelled in my results. This differs from results reported by both Pilliod et al. (2002) and Vasconcelos and Calhoun (2004), who noted that larger frogs moved farther than smaller frogs. This disparity could be explained by the habitat in which dispersal was investigated. Both Pilliod et al. (2002) and Vasconcelos and Calhoun (2004) studied terrestrial dispersal, and larger frogs are better equipped to move over land compared to smaller frogs, because the risk of desiccation is lower in larger individuals (Mazerolle 2001). Dispersal was only investigated along creeks in this study, where the risk of desiccation would presumably not be a constraint to movement to the same degree that it is in terrestrial environments. This would explain why small metamorphs moved as far, or potentially farther, than did large metamorphs. Studies that compare dispersal characteristics among different habitat types will help to elucidate whether the importance of body size for dispersal is habitat-dependent.

Although this study provides the first comparison of movement behaviour in a reintroduced and wild amphibian population, the two populations existed under very different conditions and results should be interpreted with caution. For instance, whereas the wild dispersers voluntarily left their natal site at a particular age and a particular time of year, reintroduced metamorphs had no choice but to leave their larval site and were presumably expected to resume natural behaviour in their new environment (Dodd 2005; Stamps and Swaisgood 2007). Further, variations in climatic and ecological conditions between years, sites (Rittenhouse et al. 2007), and even surveys at the same site likely affected movement tendencies and metamorph behaviour (Dole 1965; McPeek 1989). However, since reintroductions involve moving animals into areas from which they have become extirpated (Edgar et al. 2005), one cannot study the behaviour of reintroduced and naturally occurring animals at the same site. Without doubt, more data are needed to determine how other factors, such as time of year of release, and habitat conditions, affect site fidelity and movement patterns of reintroduced animals.

Conservation implications

Reintroduction remains one of the primary tools of choice for *Rana pipiens* conservation in Alberta (Alberta Northern Leopard Frog Recovery Team 2005), even though previous attempts in the province have not all been successful. Such attempts have not been well documented, which limits the possibilities for lessons to be learned (Short et al. 1992). Egg clusters will likely be used in all future reintroduction attempts (Alberta Northern Leopard Frog Recovery Team 2005), a strategy considered to increase the probability of success (Semlitsch 2002). This, however, does not discount the possibility that the source population may be genetically ill-equipped to adapt to a new, but ecologically similar, environment (McPeek 1989; Ficetola and De Bernardi 2005). Studies incorporating a true cross-over design (i.e. reciprocal translocations between study areas; Rittenhouse et al. 2007) will help determine whether differences in *R. pipiens* movements are correlated with landscape differences between sites, or with inherent differences between populations. While it is impossible to know everything there is to know about a species prior to undertaking a reintroduction program, managers should adopt an adaptive management approach which relies on continuous review and refinement of program protocols based on prior experience (Short et al. 1992; Sarrazin and Barbault 1996; Dodd 2005). Although results from this study challenge an underlying assumption of reintroduction programs that released animals will behave similarly to those developing in natural habitats (Dodd 2005), more research is needed to explore the biological constraints acting on this life-stage in different habitats, and ultimately, why previous reintroductions may have failed. Without this, future programs may unwittingly contribute to the decline of populations in the province instead of increasing them. A greater focus on improving and protecting the quality of their current habitat might be an appropriate alternative (Reinert 1991).

CHAPTER SIX: THESIS OVERVIEW, MANAGEMENT CONSIDERATIONS AND AREAS FOR FUTURE RESEARCH

Introduction

This study was conducted in light of practices that were currently being employed to reintroduce northern leopard frogs (*Rana pipiens*) into formerly occupied parts of their historical range in Alberta. My primary goal was to investigate the emigration behaviour of newly metamorphosed frogs in natural populations (Chapter 4), because this was the behavioural characteristic and life-history stage focal to reintroduction efforts. Interestingly, this is the least understood phase in anuran life cycle patterns, possibly because of the logistic problems inherent in its documentation. In order to contextualize the movements of newly-emerged metamorphs I also made observations of earlier life stages (egg cluster and tadpole stages; Chapters 2 and 3). Such background information provided supplementary data to help understand and interpret metamorph movement patterns. The data obtained from the movement patterns in natural populations were employed to retrospectively interpret the outcomes of the attempted reintroductions that triggered the current study (Chapter 5). Information presented in this thesis provides new information pertaining to *R. pipiens* and to anurans in general. Along with information gathered from the literature, it indicates how a better understanding of the ecology and overall life-history of a species can enhance conservation strategies. These findings also provide baseline data from which future studies can be developed. I conclude this thesis with an outline of the next steps that should be taken to further our understanding of movement patterns of metamorph *R. pipiens*, with emphasis on information critical to enhancing the potential success of reintroduction efforts.

Thesis overview

In Chapter 2, I investigated the distribution of *R. pipiens* egg clusters and their hatching rates within three naturally occurring breeding ponds. My results suggested that within ponds, certain areas were more heavily used as deposition sites than were others. Whereas egg clusters took anywhere from 5–20 days to hatch, those laid later in the season exhibited shorter time to hatching than those laid earlier.

In Chapter 3, the distribution of *R. pipiens* tadpoles in the same three ponds was explored, basing observations on the tadpoles that had emerged from the eggs documented in Chapter 2. Several predictor variables were investigated: cardinal direction, study site, Julian day, shade, and water depth and temperature. All of these factors interacted in some way to influence tadpole spatial distribution. In general, tadpoles showed a more positive association with warm, shallow, un-shaded areas of ponds compared to cool, shaded areas, but these effects depended on the cardinal directions within ponds. Two ponds dried resulting in nearly complete tadpole mortality.

In Chapter 4 I used methods similar to those in Chapter 3 to document the shoreline distribution of *Rana pipiens* metamorphs (that had developed from the tadpoles studied in Chapter 3) prior to their emigration at two ponds. Metamorphs were located more often on the north shorelines relative to the south shorelines, which was also where the majority of egg clusters were laid in one of the ponds. In general, they were also located more often in shallow, warm water compared to deep, cool water, and their presence and abundance decreased as the summer progressed.

In Chapter 4 I also documented emigration patterns of *Rana pipiens* metamorphs for two consecutive years at my main study site. Whereas 53 emigrants were caught in 2005, 659 emigrants were caught in 2006. In both years, emigration direction was non-random and oriented towards the south, although the mean direction differed between years. Emigration direction was unrelated to the distribution of previous life stages. The body size of metamorphs depended on the year, with those caught in 2005 being larger than those caught in 2006. Metamorph body size also depended on when they emigrated; in 2005 larger metamorphs emigrated in the first few weeks of emigration and smaller emigrants left the pond later in the season. The reverse occurred in 2006 when smaller metamorphs emigrated earlier than larger metamorphs. The onset of emigration occurred nearly a month earlier in 2006 versus 2005, but the timing of metamorph movements was not weather-dependent in either year. Lastly, the capture and handling of metamorphs did not appear to disrupt their natural orientation behaviour in 2006.

In Chapter 5, I compared the dispersal movements of reintroduced *Rana pipiens* metamorphs to those of wild metamorphs. The distances travelled by metamorphs in the two populations were similar, but more metamorphs were recaptured in the wild population. In the wild population, dispersing metamorphs travelled farther six weeks after dispersal began compared to three weeks earlier. Metamorph body size did not influence the distance travelled in either the reintroduced or wild population; however, body size did differ between populations, which can be explained by already existing differences in their body sizes at the time of reintroduction and start of dispersal.

Contributions to anuran ecology

My study adds new data to both *Rana pipiens* life-history information and the general anuran literature in several ways. A multiple-life-stage study within a natural breeding pond has never been documented for *R. pipiens* prior to my study, and is rare for anurans in general. As the first *R. pipiens* study to investigate terrestrial metamorph emigration in thirty years, my results challenge those from observational studies that concluded emigration direction is random (Bovbjerg and Bovbjerg 1965; Dole 1971). Similarly, my results strengthen those of other anuran studies by suggesting that metamorph orientation is non-random. Also, prior to my study there was no information about the persistence of metamorph directionality from breeding sites for any anuran species. Lastly, my study is the first to compare the dispersal movements between a wild and reintroduced population.

Integration of results and management considerations

Qualitatively, the locations of *Rana pipiens* egg clusters, tadpoles, and metamorphs prior to dispersal did not predict emigration direction. These results suggest that neither tadpoles nor emigrating metamorphs orient towards cues associated with their natal habitat learned early in development. Rather, each life stage orients and distributes itself according to cues associated with that particular stage, likely because factors influencing fitness and survival (e.g. predators) depend on the life-stage. Therefore, visual surveys of early aquatic life stages at breeding sites (either natural sites or those used for reintroduction) will not relay information about emigration direction from the site. More intensive survey techniques, such as the use of pitfall traps around ponds similar to the set-up in this study, or the attachment of radio-transmitters on dispersing frogs will provide more accurate data about terrestrial habitat use.

The information I gathered from natural populations should be simulated in future reintroductions. For instance, if egg clusters are reintroduced, they should be attached to vegetation that is well exposed to sunlight and should be placed in close proximity to conspecific egg clusters (but not touching). Also, tadpoles and metamorphs should have access to warm, unshaded areas of ponds throughout their development, but spatial complexity within ponds may be important (e.g. shaded areas throughout the day may provide hiding spots, so should also be available).

The non-random emigration patterns exhibited by metamorphs in my study suggest that regardless of the life stage employed in reintroduction, if critical factors for successful metamorph orientation are not present at the release site, the metamorphs may wander away randomly and not settle at the chosen site. Testing the orientation behaviour and directionality of metamorph *Rana pipiens* in different environments will shed some light on the cues they use, but until such data are available it may be advisable to "guide" animals towards suitable habitat (i.e. undeveloped, humid sites) at a reintroduction site. My results can also be used to assist in the management of existing *R. pipiens* populations because if terrestrial habitats around breeding ponds are not used equally, it may be possible to conserve critical *R. pipiens* habitat without having to equitably control the entire area around a pond. This could be especially important for populations existing in fragmented habitats, if important migration routes around ponds or larger wetlands can be protected recognizing that other areas around the pond have other uses (e.g. agricultural land, or roads). However, this will only be appropriate if those uses are not adversely affecting the survival of the frog population. Moreover, whether adults use the same migration routes as metamorphs has not been tested, but such routes must also be identified and protected. Non-random emigration could potentially lead metamorphs into ecological traps if cues used to orient do not maximize survival and fitness. This could happen if critical terrestrial habitat for them beyond the pond periphery is destroyed, but immediate cues used for emigration orientation remain. Finally, until emigration data at sites targeted for protection become available, as well as whether preferred directional routes persist at distances further from breeding sites, a more conservative conservation strategy protecting the entire terrestrial area around breeding sites may be appropriate.

Other considerations relating to the reintroduction of *Rana pipiens* stem from my work. If egg clusters or tadpoles are reintroduced, the short-term capture and processing of resulting metamorphs does not appear to significantly influence their behaviour when they are returned to their capture site. Therefore, gathering basic body condition data (e.g. weight) and marking released animals for data collection is thus advocated to monitor the population. Also, a critical decision for reintroduction efforts is where animals to be reintroduced should be obtained from. It is generally recommended that the source population should be the nearest population occurring in the same habitat type as the reintroduction site (Denton et al. 1997), and as genetically similar as possible to animals that previously occurred at the reintroduction site (Semlitsch 2002). However, this is not always possible for threatened species, especially if potential source populations are dwindling themselves and the removal of animals from such sites might have a negative impact on their persistence. My research has identified two *Rana pipiens* breeding sites that have dried at least two years in a row, resulting in a loss of at least 12 egg clusters in one of those years. I recommend that these and similar sites that often dry mid-summer be considered as potential source populations for future reintroductions because larvae at these sites will likely perish otherwise.

The proposition that amphibian populations are declining requires baseline information on natural fluctuations in population numbers (Blaustein et al. 1994). The large difference in the number of metamorphs caught between years at the same site in my study suggests that *Rana pipiens* populations can fluctuate dramatically. Therefore, the status of *R. pipiens* in Alberta should not be based on single year surveys because a relatively small population size one year may reflect natural fluctuation in amphibian numbers, and not actual decline. Single year surveys may estimate rates of decline to be more than twice that of multiple year studies (Skelly et al. 2003). Of equal importance is that, because two of my study ponds dried two years in a row, evidence of breeding activity and larval production that may be detected during a single spring survey at a site could be an unreliable indicator of successful metamorph production. Therefore, sites should also be visited multiple times in a year. In addition, whether the drying of breeding ponds indicates a real threat to *R. pipiens* population persistence or whether drying is a natural phenomenon that keeps population carrying capacities in check is unknown. Clearly, long-term monitoring of sites is necessary in order to accurately assess the status of populations.

Areas for future research

There are many questions in need of answers that will help improve conservation efforts directed towards *Rana pipiens* in Alberta. Although my research suggests that metamorphs do not associate with the locations of earlier life stages in relation to emigration, expanding the temporal and spatial scale of study will help answer questions regarding whether conditions experienced during early life stages influence later habitat selection. For example, do metamorphs return to their natal pond? This has never been tested for *R. pipiens* but is important for reintroduction purposes so that managers know whether reintroduced animals will return to their release site or whether substitute breeding ponds need to be available at a reintroduction site. If metamorphs return to their natal pond as breeding adults, do they deposit eggs in the same location as their parents deposited their egg clusters? Such information will allow managers to assess the long-term effectiveness of management efforts at the release sites. These questions can only be answered by long-term monitoring of populations and will provide important information for understanding levels of philopatry and landscape structure at release sites.

My work identified directional differences in metamorph emigration between years. Whether such directional biases continue to change with additional years of emigration data is unknown. This has important conservation implications because it would mean that targeting areas around breeding sites based on short-term observations may not be effective in the long term. However, if emigration direction is relatively consistent over time, then targeting areas around breeding sites for protection may be part of a viable conservation strategy. Long-term monitoring programs are strongly advocated in order to evaluate terrestrial habitat movements.

My work was conducted in a relatively protected area and there is no information on how different land-use practices, such as agriculture or oil and gas exploration in Alberta, will influence metamorph emigration. Not all amphibian species are adversely affected by agricultural or other major disturbance factors (Bulger et al. 2003; Rothermel 2004); thus, *Rana pipiens*-specific data are necessary. If *R. pipiens* exhibits, for instance, behavioural avoidance of certain disturbances, it may be necessary to maintain direct habitat connectivity (i.e. corridors) between aquatic and terrestrial habitats. However, before implementing such management approaches, additional research is needed to determine movement patterns in different habitat types.

My findings pertain to the orientation patterns of metamorphs emerging from their natal ponds; therefore, I cannot infer movement patterns beyond the pond periphery. Indeed, most amphibian studies focus on breeding habitat characteristics even though nonbreeding terrestrial habitat provides foraging areas, shelter from predators, refuge from desiccation and a landscape through which amphibians can disperse. Because of the variety of nonbreeding habitat uses, it seems possible that directionality after leaving the pond could change at different distances. Although there are few data concerning movements of metamorph *Rana pipiens* at distances further from breeding ponds than those used in this study, managers need both directional and distance habitat data in order to conserve critical terrestrial habitat around wetlands. Data on the size and quality of nonbreeding habitat required to maintain local *R. pipiens* populations should be a priority for future research. Such information may also facilitate population recovery if declines are a result of habitat loss by allowing conservationists to create appropriate new sites for colonization.

In conclusion, my results highlight the need for more research into the cues that metamorphs use in order to detect suitable habitat. If this can be determined it could prove useful for determining the most appropriate age to reintroduce *Rana pipiens* to ensure that the transfer of animals does not disrupt their orienting abilities. It could also help in the design of terrestrial habitat management practices, if orientation towards safe habitat is strongly dependent on certain landscape features.

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