

**The Efficacy of Anti-Predator Behaviour in the Wood Frog Tadpole (*Rana sylvatica*)**

**by**

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## Thesis Abstract

Activity has been suggested as an important behaviour that is tightly linked with predator avoidance in tadpoles. In this thesis I examine predator-prey relationships using wood frog tadpoles (*Rana sylvatica*) as prey and dragonfly larvae (*Anax junius*) and backswimmers (*Notonecta undulata*) as predators. I explore the role of prey activity in predator attack rates, prey response to single and multiple predator introductions, and prey survivorship. The data suggest that *Anax* is the more successful predator, able to capture both active and inactive tadpoles. In contrast, *Notonecta* strike at inactive prey less frequently and are seldom successful when they do. A mesocosm study revealed that the presence of any predator resulted in reduced activity level of tadpoles. Each predator species alone had similar effects on tadpole activity, as did the combined predator treatment. Tadpole survivorship, however, differed significantly among both predator treatments and prey populations. Tadpoles in the combined predator treatment had enhanced risk; survivorship was lower than that expected if the two predators had additive effects. Differences in survivorship among wood frog populations showed that tadpoles from a lake habitat had the lowest survivorship, those from a shallow pond habitat had an intermediate survivorship, and tadpoles from a marsh habitat had the highest survivorship. The frequency of interactions with predators in the native habitat may be driving the population differences observed. In conclusion, results from this study show that complex interactions exist between predators, prey, and the environment, with activity playing a key role in the survival of tadpoles.

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# Chapter 1

## General Introduction

Predator avoidance has a major impact on the behaviour of an organism. The success of an individual depends on its ability to avoid predators and survive to maturity. There are many adaptations that organisms display for evasion of predators, including mimicry, specializing in a niche, unpalatability and/or toxicity, behavioural modification, warning colouration, symbiotic relationships, organs modified for escape, and social organization. Modifications to morphology and behaviour facilitate survival of an individual, but can be costly. The immediate and long-term benefits of these modifications must outweigh the costs if they are to have a positive impact on the success of the organism. The trade-off between the cost of predator avoidance behaviour and its benefits must be balanced to maximize prey fitness. For example, the traits that make an individual a good forager may also lead to a greater risk of predation and therefore the individual must balance foraging requirements with predator avoidance tactics.

In this Chapter, I review survivorship, morphological adaptations, and behavioural responses of tadpoles to predators. I then discuss methods of predator detection and the trade-offs that tadpoles typically face. Finally, I introduce the wood frog *Rana sylvatica*, the species used in this study, give a general overview of its life history, and follow this with the general methods used throughout this thesis.

## *Survivorship*

When an organism is faced with the decision to continue a behaviour which may be beneficial in the long term but detrimental in the short term (e.g. foraging when predation risk is high), the animal is expected to balance the benefits and risks involved in a manner that maximizes fitness. If the risk associated with continuing the behaviour is high and may result in negative consequences, then the individual is expected to cease the behaviour in favour of increasing fitness. However, in a low risk situation, where the long-term benefits of the behaviour will outweigh the associated risks, the individual is expected to continue performing the behaviour. One example of this trade-off is courtship displays that can be continued in a modified form when the environment is risky, as an alternative to maintaining the current risky behaviour or stopping the behaviour entirely. The modified behaviour is a compromise between the two extreme alternatives, allowing the behaviour to continue (maintaining at least some of its benefit) while minimizing the increased risk of predation. For example, females of the common jumping spider (*Jacksonoides queenslandicus*) become less receptive to males when risk of predation is high and males, in turn, reduce courtship displays (Su and Li 2006). Similarly, the wolf spider (*Pardosa milvina*) reacts to increased predation risk by increasing the required length of courtship and decreasing palpal insertion rate; thus mating success is reduced when risk is high (Taylor et al. 2005). While this response likely reduces immediate mating success, the individual that survives will have the chance to mate again. Damselfly larvae provide another example of trade-offs occurring with predator avoidance behaviour. Larval damselflies reduce foraging behaviour in the presence of chemical cues from predators, with further reduction when cues are from predators fed damselflies (an indicator of increased predation risk) (Heads 1985, Koperski 1997, McBean et

al. 2005). However, this dynamic changes with time since last meal; damselflies increase foraging as the period of starvation grows longer, regardless of high risk levels (McBean et al. 2005). Apparently, the risk of starvation eventually overtakes the benefit of predator avoidance behaviour and despite high predation risk, damselflies forage. In these two examples of the trade-off of predation risk with courtship and foraging behaviour, consequences of the inappropriate use of anti-predator behaviour differ. The reduction in fitness of the spiders, although negative, may not be as harmful to the individual as starvation can be to a damselfly. This is at least partially because the larval damselfly system is simplified in that, until maturity, positive fitness depends only on survivorship.

Larval anurans are another system for which non-zero fitness depends solely on survival. This is also a system that has been studied in a variety of contexts. Trade-offs in larval anurans are well understood, especially those concerning predator avoidance strategies (Caldwell et al. 1980, Bridges and Gutzke 1997, Kupferberg 1998, Eklov 2000, van Buskirk and McCollum 2000, Relyea and Auld 2005). In most anurans, the larval stage experiences high mortality rates, with as few as 3.7% of eggs laid surviving to metamorphosis in the wood frog (*Rana sylvatica*) (Herreid and Kinney 1966). Tadpole mortality can be attributed to a number of factors, including fungal infestation, parasites, pond drying, pollution, starvation, cannibalism, and predation. There are various mechanisms that tadpoles use to avoid mortality from one of these factors. For example, most tadpole individuals exhibit phenotypic plasticity and modify both morphology and behaviour to reflect the predation risks present in their environment (Relyea 2002).

After fertilization most anurans spend 7 to 14 days developing inside the egg before hatching out. Immediately after hatching, tadpoles remain fairly inactive and are poor

swimmers as they resorb their yolk and continue to develop. Once the yolk is resorbed (Gosner stage 25, Gosner 1960), tadpoles start to actively feed and are more likely to encounter heterospecifics and predators. It is at this point in time that many complex interactions arise between tadpoles and biotic factors within the environment. Tadpoles must spend sustained time foraging to grow and develop at an appropriate rate to reach metamorphosis. This creates the potential for a trade-off between foraging and predator avoidance. Tadpoles are highly susceptible to predation and have limited defence against predators; morphological plasticity and behavioural avoidance responses have evolved to reduce the risk of predation.

### *Morphological Adaptations*

The morphology of an individual can have a large effect on predator avoidance. Many species of tadpoles have colouration (green, browns and black hues) that helps them to blend in with the substrate (Swart and Taylor 2004). Other tadpoles may change hue seasonally to avoid predators (Wente and Phillips 2003, 2005) or in response to predators (LaFiandra and Babbitt 2004, McIntyre et al. 2004). Colouration has also been shown to play a role in manipulating predator behaviour; bright colouration on the tailfin may attract predators to the tail rather than the body, as seen in *Hyla chrysoscelis*, *Hyla versicolor* and *Hyla femoralis* tadpoles (McCollum and Leimberger 1997, LaFiandra and Babbitt 2004). This benefits the tadpole because tail strikes are far less likely to be lethal than body strikes and the tail colouration attracts the predator's attention away from the body (Wilbur and Semlitsch 1990).

In addition to changes in colouration, some species of tadpoles modify size and shape in the presence of predators (McCollum and van Buskirk 1996, van Buskirk and Relyea 1998,

van Buskirk and McCollum 2000, van Buskirk 2001, 2002, LaFiandra and Babbitt 2004, McIntyre et al. 2004, Relyea 2005a, Wente and Phillips 2005). This includes changes in tail muscle depth and tail length, which presumably aid the individual in evading predators (van Buskirk and Relyea 1998, van Buskirk 2001, 2002, LaFiandra and Babbitt 2004, McIntyre et al. 2004, Relyea and Auld 2005). These changes in morphology can influence swimming speed through changes in musculature (McCollum and Leimberger 1997, van Buskirk and McCollum 2000) and like tail colouration, may also function to deflect predator attacks (McCollum and van Buskirk 1996, van Buskirk et al. 2004).

A variety of studies have shown an increased survival rate in tadpoles that exhibit induced morphology (van Buskirk et al. 1997, Benard 2006, Kraft et al. 2006). Some tadpoles that have changed morphologically in response to a specific predator survive better in the presence of that predator as compared to novel predators (Benard 2006). Also, the more streamlined shape that occurs in some tadpoles developing in the presence of predators may increase survival of these induced morphs (van Buskirk et al. 1997). It has been proposed that intermittent selection on some of these morphological traits has led to their plasticity (van Buskirk et al. 1997). Alvarez and Nicieza (2006) recently found that survival in species exhibiting morphological plasticity also depends on prior experience with predators and that this benefit can precede morphological adaptations.

### *Predator Detection*

The ability to detect a predator is crucial to predator avoidance. Organisms can use any combination of cues to detect predators: sight, sound, scent, and mechanical cues. The method(s) of detection used will depend on a variety of factors, including the habitat, the

potential predators, and any specific adaptations the prey organism may have. For example, some animals have a heightened sense of hearing that functions for early detection of predators, as seen in the classic example of moths that detect the high pitched frequencies emitted by bats (Roeder 1962). Aquatic organisms may use any of the above senses to detect predators. However, vision is often limited by turbid or murky water and dense vegetation, both of which add to the cryptic nature of many predators (Wisenden 2000). Use of sound may also be limited, as sound travels poorly under water. Therefore, the use of mechanical and chemical (scent) cues is typically more useful when assessing predation risk in aquatic systems (Kats and Dill 1998).

Animals may use both direct and indirect cues to detect predators, and anurans use both to assess predation risk. Tadpoles are nearsighted and use chemical cues over visual cues for predator detection (Hoff 1999). Tadpoles rely heavily on chemical cues, showing spatial avoidance of introduced predator chemical cues (Kiesecker et al. 1996, Laurila et al. 1997, Petranka and Hayes 1998, Spieler and Linsenmair 1999, Niecieza 2000). In addition to direct cues from the predator (predator 'scent'), tadpoles can also use predator waste products, dietary cues, and cues from injured conspecifics or heterospecifics as indirect cues (Wilson and Lefcort 1993, Laurila et al. 1997, Adams and Claeson 1998, Kats and Dill 1998).

Many predator-prey studies are carried out using non-lethal predators, restricting predators from consuming prey, but allowing for chemical, mechanical, and visual cues to be detected. This allows for the separation of mortality effects from those of the prey behaviour, but may also reduce the impact on the prey behaviour (Lima 1998). Assuming that non-lethal and lethal predators will have the same effect on behaviour or morphology of tadpoles could be misleading if tadpoles respond less strongly to non-lethal predators. Studies that consider

the effects of both lethal and non-lethal predators are needed if we are to account for differences that may arise between these treatments.

Tadpoles are exposed to a variety of aquatic and semi-aquatic, as well as vertebrate and invertebrate predators. Species of larval anurans found in ponds with fish tend to have morphological and behavioural differences from those found in vernal and semi-permanent ponds (Richardson 2001, 2002). One of the main differences observed in larval anuran species found in permanent ponds with fish is a modified strategy for growth and development. Species breeding in permanent ponds often overwinter as tadpoles (especially in temperate regions), attaining a large tadpole size but taking longer to metamorphose into a frog; this is seen in several ranid species, such as bullfrogs, green frogs, and mink frogs. This strategy of delayed metamorphosis allows tadpoles to outgrow gape-limited fish predators. In contrast to permanent ponds with fish, the species composition of anurans breeding in fishless ponds is quite different. Whether semi-permanent or vernal (drying every year), fishless ponds are dominated by invertebrate predators (Wellborn et al. 1996). Invertebrate predators use a variety of strategies to capture and consume tadpoles. For example, dragonfly larvae (Odonata: Aeshnidae) are “sit-and-wait” predators; individuals perch cryptically on substrate or vegetation and will strike at a passing tadpole with its modified labium, seizing the tadpole and bringing it back to its mouthparts, where it can begin masticating the captured prey item. Tadpoles that can detect dragonfly larvae through chemical cues, emitted either from the predator directly (Kiesecker et al. 1996, Spieler and Linsenmair 1999), or from a combination of predator cues and cues that remain from an eaten conspecific (Adams and Claeson 1998), can increase survivorship through modifications in behaviour (e.g. by becoming less active).



Active predators, such as aquatic beetles and hemipterans, travel through the water in search of food, pausing on the surface film or on aquatic plants. Backswimmers (Hemiptera: Notonectidae) detect prey through both chemical cues and mechanical cues in the water as they move (Murphey and Mendenhall 1973). Some studies have suggested that backswimmers tend to go after free moving prey using the element of surprise, with a swift initial strike at passing prey (Murphey and Mendenhall 1973, Gittelma 1974). Although apparently similar to the predation strategy used by dragonfly larvae, *Notonecta* often chase prey before capture, and are unable to slowly stalk prey as do dragonfly larvae. *Notonecta* use modified forelegs to grasp a captured tadpole and have piercing mouthparts that are inserted into prey injecting poison and digestive juices, paralyzing the prey and allowing the predator to draw out the digested contents (Gittelma 1974). This chase-capture sequence can cause more disturbance than that of a “sit-and-wait” predator, alerting prey of their presence through mechanical cues.

#### *Behavioural Responses of Prey to Predator Presence*

A variety of behavioural tactics are used for predator avoidance. One general response to the threat of a predator is “freezing”, or reduction in activity. Potential prey also commonly use refuges, reducing the likelihood of providing visual cues for the predator to detect and positioning the prey out of reach of the predator. In environments where few or no refuges are available, prey may use a different predator avoidance strategy, such as aggregation behaviour. This anti-predator response is well known in fish, many species of which frequently aggregate, or shoal, to reduce the likelihood of predation. Aggregations can reduce an individual’s risk of predation through the selfish herd effect, the confusion effect, and the

dilution effect, all of which reduce individual probability of predation as aggregation size increases (Hamilton 1971). Finally, some species develop complex behavioural relationships with other organisms to minimize predation risk. For example, clownfish have a mutualistic relationship with sea anemones (Elliot and Mariscal 1997). The clownfish gains refuge from predators due to the stinging tentacles of the anemone, and in turn the territorial clownfish protects the anemone by chasing away fish predators that would otherwise eat the anemone. Thus, many behavioural mechanisms exist to reduce predation risk and the method used by an organism will depend on both the predator and the specific habitat in which prey and predator reside.

Tadpoles exhibit three main predator avoidance behaviours, parallel to those discussed above: aggregation, use of a refugium, and reduction in activity. Tadpoles of the genus *Bufo* are often seen in large conspicuous aggregations. *Bufo* tadpoles contain the chemical bufotoxin, which makes them unpalatable to predators (Krenn and Kopp 1998), and the aggregations help to facilitate predator learning. The presence of fish predators or cues elicits the use of a refugium by some species of tadpoles, reducing the likelihood that a predator will visually detect the tadpole (Bridges and Gutzke 1997, Niecieza 2000, Teplitsky et al. 2003).

Reduction in activity is the main predator avoidance response exhibited by tadpoles when they encounter predators or predator cues (Lawler 1989, Skelly 1994, Anholt et al. 2000, Richardson 2001). A reduction in activity decreases the likelihood that a predator will visually detect the tadpole, allowing the tadpole to evade the predator with minimal energetic investment. However, reduction in activity is only useful for predator avoidance if individuals are able to accurately assess the predation risk.

### *Balancing the Trade-off between Activity and Predator Avoidance*

Activity is required to find food, which is of course, essential for growth. When a predator is detected, tadpoles typically reduce activity. Although the reduction in visual cues arising from decreased activity can benefit the tadpole, there are also negative impacts on foraging and thus growth rates. The tadpoles of some anuran species use an alternative life history strategy of developing faster in the presence of a predator and metamorphosing at a lower body weight to escape the high risk of predation in the larval habitat (Wilbur 1980, Werner and Gilliam 1984, Werner 1986). Tadpoles are able to develop faster in the presence of predators by focusing their energies on development rather than on growth, meaning that they sacrifice weight gain for an increase in development rate (Werner 1986, van Buskirk 1988, Niecieza 2000). This allows them to be released from the intense pressures associated with the larval stage of development, but can have impacts on fecundity and mating success in the adult stage (Wilbur 1980, Werner 1986). Therefore, tadpoles must forage when the threat of predation is low to maximize consumption without increasing the risk of predation (Sih 1987, Lima and Dill 1990, Chivers and Smith 1998). The trade-off between activity and predator avoidance must be balanced such that reduction in activity level correlates to the risk perceived in tadpoles, in order to maximize overall benefit of the behaviour.

### *Wood frogs*

Several life history characteristics of wood frogs (*Rana sylvatica*) make the species an ideal study organism. They are explosive breeders, meaning that all adult frogs congregate at their natal ponds in early spring and breed en masse within a period of days (Wells 1977). Wood frogs are also philopatric; 100% of adults return to the pond in which they first bred,

and 80% are faithful to the pond from which they metamorphosed (Berven and Grudzien 1990). This suggests that populations at different ponds are likely to be reproductively isolated (Berven and Grudzien 1990). Eggs are laid in a large globular mass that contains hundreds of densely packed eggs and is typically attached to emergent vegetation (Figure 1.1). Many females lay egg masses together at the same position in the pond, creating a location with a large number of egg masses in a small area. Wood frogs are one of the few anurans not currently on the decline (Alford and Richards 1999); this provides a species that can be used without permanently impacting natural populations. These factors make wood frog tadpoles an ideal system to study. The results obtained from experiments using these tadpoles can be applied to other threatened and endangered anurans that may have similar life history traits.

In this thesis I outline three sets of experiments that examine the relationship between prey behaviour and predation rate in wood frog tadpoles, including the effect of prey activity on the predator choice of prey (Chapter 2), the effect of different predators on tadpole activity (Chapter 3), and the subsequent mortality resulting from these predators (Chapter 4). I consider this using video recordings of predation events in the lab and estimates of activity in semi-natural ponds to determine the relationships among prey activity, predator choice, and prey mortality.

## **General Methods**

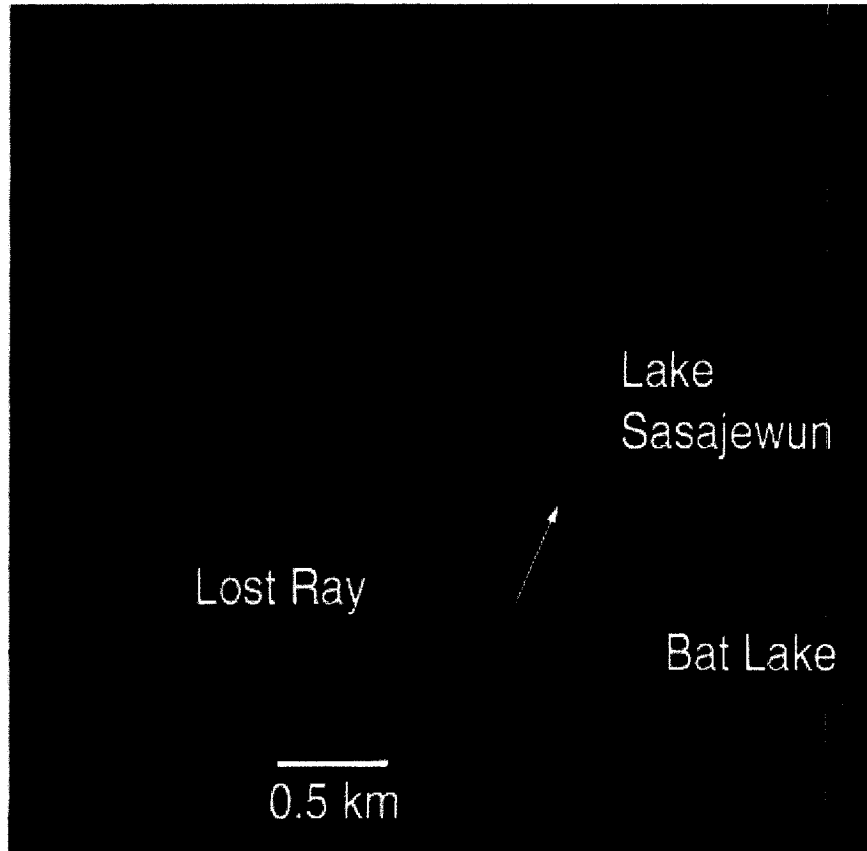
The following methods were common to all studies in this thesis. Egg masses of the species *Rana sylvatica* LeConte were collected by hand in early spring of 2005 and 2006, from three sites located at the Wildlife Research Station in Algonquin Park, Ontario, Canada. The three sites were Bat Lake (45°35.131', 78°31.002'; 3 egg masses in 2005, 10 in 2006),



Figure 1.1: Wood frog egg masses, Bat Lake, Algonquin Park, ON.

Lost Ray (45°35.500', 78°32.299'; 3 in 2005, 10 in 2006), and Lake Sasajewun West (45°35.513', 78°31.354'; 2 in 2005, 5 in 2006). These sites are likely isolated populations (Figure 1.2), and will hereafter be referred to as the three distinct populations studied. Individual egg masses were placed in 38 L Rubbermaid tubs with 20 L of filtered lake water and allowed to hatch. At the time of hatching, hatchlings of egg masses from the same population were combined and placed in clean tubs with fresh lake water. Water used for housing was either UV- treated well water or untreated lake water drawn from Lake Sasajewun. Tadpole housing water was changed when fouled, typically every 3-4 days. All animals were kept in a building on site under natural light and temperature regimes. Tadpoles were fed a combination of tadpole food pellets (Carolina Biological Supply Company, North Carolina) and Wardley® Spirulina Discs (The Hartz Mountain Corporation, St. Thomas) when not in a trial. Tadpoles were used in trials once they resorbed their yolk and started to actively feed (Gosner stage 25, Gosner 1960). Tadpoles were used once and then released back into their native pond.

Collection sites were thoroughly sampled at different times of the day for predators, using dipnets and minnow traps to determine the predator community composition. This resulted in the identification of two prominent predators in the spring when wood frog tadpoles are present: dragonfly larvae (Odonata: Aeshnidae *Anax junius*) and adult backswimmers (Hemiptera: Notonectidae *Notonecta undulata*). All predators used were from Bat Lake. Predators were collected using dipnets and minnow traps, and housed individually in opaque plastic cups (475 mL) with a piece of sphagnum moss (from Bat Lake) or a stick for perching. Only late-instar dragonfly larvae and adult *Notonecta* were used in experiments. Predators were food deprived for a minimum of three days prior to use.



**Figure 1.2:** Map of sample sites in Algonquin Park, Ontario. The arrow at Lake Sasajewun denotes the collection site Sasajewun West.

Predators were fed a variety of aquatic invertebrates (amphipods, dipteran larvae, zooplankton) when not in a trials. I kept track of when the dragonflies moulted to make sure they were not moulting during trials (dragonfly larvae do not feed just prior to moulting). When studies were completed for the season, the predators were released back into Bat Lake. In 2005 there were approximately 25 dragonfly larvae and 75 backswimmers collected. In 2006 approximately 40 dragonfly larvae and 100 backswimmers were collected.

Throughout the thesis, I focus on activity behaviour of tadpoles. Because I was interested in activity as a potential signal to predators that could increase predation risk, I define activity as any movement by the tadpole that displaces its position horizontally or vertically in the water. Throughout this thesis, “activity” should always be taken to have this meaning.



## Chapter 2

### Effect of Prey Activity on Predator Choice of Wood Frog Prey

A predator's choice of prey can shape the evolution of the behaviour and morphology of their prey. Selection pressures from predators can lead to the evolution of fixed action patterns, behavioural modifications, and phenotypic changes. Part of what shapes a predator's choice of prey is the method of capture used by a predator. Predators may be active, moving around in search of prey, or may alternatively use a "sit-and-wait" strategy, waiting for prey to come to them and using the element of surprise in prey capture. The way in which prey react to predators and the predator avoidance strategies used by prey may also influence predator choice of prey.

Predators can detect prey items using visual, mechanical, and chemical cues. The method of detection used by predators influences the predator avoidance strategies used by prey. Predators such as raptorial birds have precise vision and use visual cues to detect prey from above (Perry 2001). The prey of these birds, mainly rodents, reduce activity when a predator is detected and hide in refuges to prevent visual detection (Perry 2001). In contrast, mechanical or tactile cues are used by many invertebrate predators, such as phantom midge larvae, which capture and consume zooplankton they come in contact with while moving through the water (Szulkin et al. 2006). Lastly, scent is a major tool for prey and predator detection and is often used in conjunction with visual and mechanical cues. A number of animals use scent when tracking food and have evolved heightened olfactory sense for this purpose (e.g. species from the Order Carnivora). To avoid being detected by predators, potential prey may reduce or disguise their scent cues. For example, beavers normally use

scent markings to distinguish territories but will not mark over intruder's markings when a predator's scent is also present (Rosell and Sanda 2006).

In an aquatic system mechanical and chemical cues are typically more reliable for locating a predator or prey item, due to lack of visibility underwater. The use of chemical cues in the detection of predators or prey has been extensively studied in aquatic systems, providing valuable information toward the understanding of predator-prey relationships (Chivers and Smith 1998, Wisenden 2000, Rajchard 2006). This includes the use of chemical cues to assess the risk of predation and respond accordingly or alternatively to find prey (Wisenden 2000).

Tests of predator foraging choice typically focus on the different species of prey that are available, rather than the prey behaviour (Downes 2002, Langlois et al. 2006, Moreby et al. 2006). In this study I examine a single prey species and focus on how the activity level of an individual may influence predator choice. Skelly (1994) examined the prey choice of *Anax* between active and inactive tadpoles by anaesthetizing some individuals to render them inactive. Results showed active tadpoles were chosen more often than inactive tadpoles by dragonfly larvae predators, although the methods used (anaesthetic) could have had an effect on the choice (Skelly 1994). To my knowledge, there have been no choice tests examining prey activity effects on predation by backswimmers.

To maximize fitness, predators are expected to forage optimally. Optimal prey choice may depend on handling time, energy intake, and the availability and encounter rate of the prey items (Pyke 1984). More specifically, this may lead to patterns in predator choice of prey with relative prey abundance, quality of prey, prey-specific defence and guild of prey. Relatively little research has been done to determine if the activity of a prey item influences a

predator's choice; the movement of a prey item may influence the likelihood that a predator will strike. Attack on an active individual may generate a less accurate strike, and thus more energy exerted in the chase, or in loss of the prey item entirely. Thus, a predator foraging optimally might be expected to choose the slower or inactive prey to reduce handling time and for ease of capture. Alternatively, if it is the visual stimulus of prey movement that attracts the predator, a predator may selectively prey on moving individuals (Downes 2002, Moreby et al. 2006).

Few studies have focused on the choice and capture of tadpoles by the various invertebrate predators they encounter. Here, I consider whether predators are more likely to choose an immobile tadpole that may be easier to capture, or whether predators tend to choose an active, more visible tadpole that potentially requires a greater energy investment to capture. Movement is a stimulus for dragonfly naiads, attracting them to possible prey (Pritchard 1965), suggesting these predators will be more attracted to active tadpoles. Folsom and Collins (1984) examined the relationship between prey species, prey activity, and the number of tadpoles eaten by the larval dragonfly *Anax junius* and found that *Anax* consume more when more moving prey are present. They also found that this increased consumption plateaus after 25% of the prey are in motion, suggesting that the proportion of prey active may be an important determinant of prey mortality.

Large groups of tadpole prey may also attract predators because capture success increases if more prey items are present. Combining the potential for prey activity rates and prey group size to influence predation risk, the behaviour of neighbouring tadpoles becomes potentially critical to assessment of predator selection on prey. This leads to the question, is an inactive tadpole in close proximity to an active tadpole more likely to be attacked? That is,

do neighbour effects exist? Tadpoles with high activity have poor survivorship in natural settings (Lawler 1989). Tadpoles that use few or rapid movements when in the presence of a dragonfly naiad have greater survivorship (Azevedo-Ramos et al. 1992) and short, sporadic movements may confuse a predator and enable the tadpole to escape (Caldwell et al. 1980). These observations suggest that activity attracts predators and predictability of movement leads to capture.

Here, I address the question of whether prey choice by dragonfly larvae and backswimmers is affected by the activity of prey items. I also examine whether activity of neighbouring tadpoles affects an individual's risk of predation. These questions are addressed by focusing on the predator-prey relationships between wood frog tadpoles and two predators: *Anax junius* (Odonata: Aeshnidae), "sit-and-wait" dragonfly larvae, and adult *Notonecta undulata* (Hemiptera: Notonectidae), a semi-active hemipteran. I quantify the relative frequency of predator strikes on active and inactive tadpoles and consider the effect of movement in nearby tadpoles. Tadpoles from three different populations are considered separately to test for adaptations arising from differences in relative abundance of predators present within any one specific habitat.

## **Methods**

Tadpoles were reared from egg masses collected from three different populations described in Chapter 1 (Bat Lake, Lost Ray, and Sasajewun West). Tadpoles from each population were randomly chosen for use after they started to actively feed (Gosner stage 25, Gosner 1960), and each tadpole was used only once. All trials were conducted in a lab using the set-up pictured in Figure 2.1. A Panasonic Mini DV recorder was positioned on a tripod

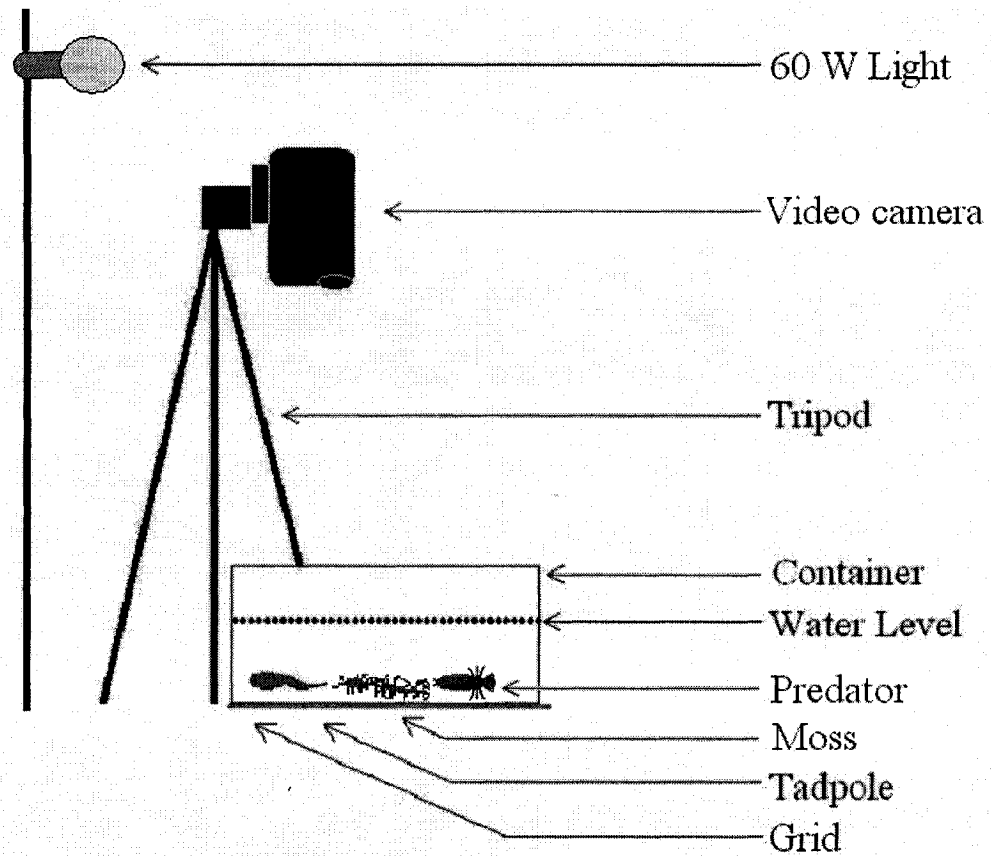


Figure 2.1: The experimental setup. Predators were placed in a square plastic container with sphagnum moss for a 1 hour acclimation period, after which 5 tadpoles were added. A piece of paper with a grid pattern under the container provided visual reference points for determining distances between animals. A 60 watt light was placed approximately 0.5 m above the container. The video camera sat on a tripod and filmed from above. Picture is not to scale.

above a  $23 \times 23 \times 10$  cm semi-opaque plastic container wrapped with a cloth to prevent any outside disturbance. Paper with a grid pattern was placed below the container to aid the observer in noting movement and proximity of tadpoles (Figure 2.1). The container was filled with approximately 500 mL of UV-treated well water and one 5 cm piece of sphagnum moss (collected from Bat Lake) per predator. A 60 watt light, placed approximately 0.5 m above the tadpole container, was turned on at the beginning of the acclimation period. The light remained on for the duration of the trial. Preliminary trials showed predators were more likely to feed in the evening, after 9 pm, although previous studies have found no difference in the activity of tadpoles or predators in the light versus the dark (Zalom 1978, Richardson 1999). Therefore, trials were randomized to start at either 9 pm or 10 pm, allowing two replicates to be run each night.

Two predator treatments were considered; the first had one late instar *Anax junius* larva present per trial and the second had three adult *Notonecta undulata* per trial. The order in which the predator treatments were completed was randomized. Predators were starved for a minimum of 3 days prior to use. Predators were placed in the filming container and allowed to acclimate for one hour before the addition of five tadpoles. Each trial lasted one hour or until all five tadpoles were consumed; the latter occurred only twice and as both of these trials lasted more than 50 minutes, these trials were analysed as identical to the others. Individuals of each predator species were used only once in this experiment. In each trial, the number of strikes and captures were recorded, as was tadpole behaviour (moving versus not moving) and proximity to other individuals. All possible strike-activity-proximity combinations are shown in Figure 2.2. Ten replicates were performed using tadpoles from the Bat Lake population and five from each of the Lost Ray and Sasajewun West populations for each predator, for a total

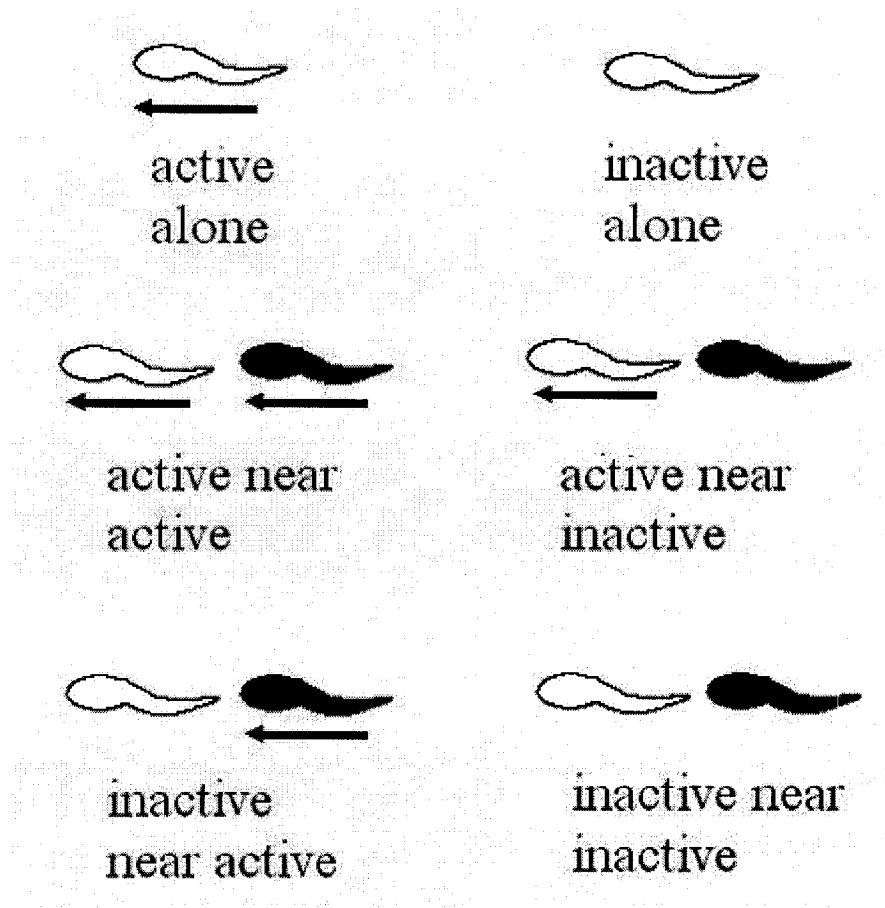


Figure 2.2: A cartoon of all six possible strike activity-proximity combinations.

White symbols denote the tadpole being struck, and black denotes a neighbouring tadpole (within one body length of the tadpole being struck). An arrow below the tadpole signifies that the tadpole was active at the time of the strike.

of 20 trials for each predator species. This protocol was approved by the Brock University Committee on Animal Care and Use (AUPP 05-07-01).

In the *Anax* treatment, a **strike** was defined as the rapid extension of the labium in the direction of a tadpole. In *Notonecta*, a **strike** was defined as the individual propelling itself towards a tadpole, extending its beak slightly, and displaying a characteristic grasping motion with its modified forelegs that was distinguishable from its normal movement using the oar-like hind legs. **Activity** in a tadpole was defined as any movement, horizontal or vertical, through the water. A tadpole was deemed to have a **neighbour** when it was within one body length (from snout to start of tail musculature) of another tadpole. Note that there was never more than one tadpole neighbouring another when a strike occurred. **Capture** was defined as a successful strike, when at least a portion of the tadpole was consumed by the predator.

### *Statistical Analysis*

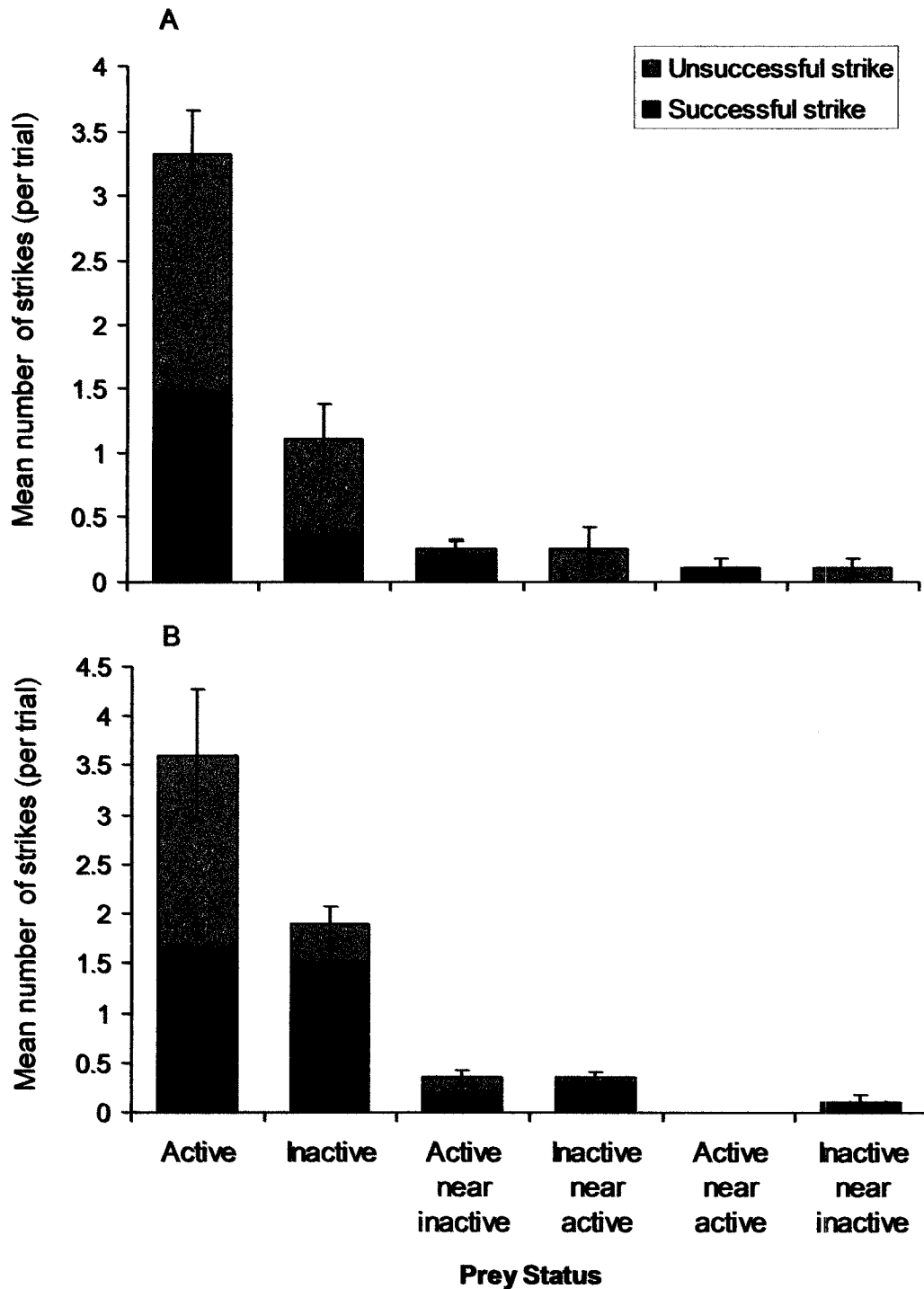
Data were analyzed using SAS 9.1 (SAS Institute Inc., 2003). There were few data points when a tadpole was neighbouring another and therefore data were combined into two categories (active or inactive tadpoles) based only on the tadpole targeted by the predator's strike for further analysis. A log-linear analysis of variance was done using the categorical model procedure in SAS with the number of unsuccessful and successful strikes as the response variable and status of the tadpole (active or inactive), predator treatment (*Anax* or *Notonecta*), and prey population (Bat Lake, Lost Ray, and Sasajewun West) as explanatory variables. There was no effect of the time the trial occurred (either 9 or 10 pm start); therefore it was removed from the model reported here.



## Results

*Notonecta* were approximately 2.5 times more likely to strike and almost 5 times more likely to capture an active versus an inactive tadpole. *Anax* were 1.7 times more likely to strike an active tadpole, but were equally likely to capture an active or inactive tadpole. Very few tadpoles were near another tadpole when attacked: 16 of 126 strike attempts (successful and unsuccessful) in *Anax* trials and 16 of 101 strike attempts in *Notonecta* trials (Figure 2.3). These low frequencies were insufficient for analysis to determine if neighbour effects exist. Instead data were pooled to consider only the effects of activity level in the focal tadpole.

Both predators were equally likely to strike at an active tadpole; however, *Anax* struck and captured inactive tadpoles marginally more often than *Notonecta*. Both predators were equally successful when the prey are active, but when inactive, *Anax* were much more successful than *Notonecta*, with more than double the success rate (Figure 2.4; log-linear ANOVA, predator  $\times$  tadpole activity status term,  $X^2_1 = 7.23$ ,  $p = 0.0072$ ). A predator by population effect existed, whereby the number of successful strikes was similar among populations, but for unsuccessful strikes *Anax* made many more strike attempts on tadpoles from the Sasajewun West population and *Notonecta* made many more unsuccessful strikes on the Bat Lake and Lost Ray populations (Figure 2.5; log-linear ANOVA, predator  $\times$  population term,  $X^2_2 = 15.13$ ,  $p = 0.0005$ ).



**Figure 2.3:** The mean number of successful and unsuccessful strikes per 60 minute trial ( $\pm$  SE) by predators on wood frog tadpoles for each possible activity-proximity combination (prey status) for: A –*Notonecta* as predator and B –*Anax* as a predator ( $n = 20$ ). Five tadpoles were present per replicate and successful strikes were therefore limited to  $\leq 5$ .

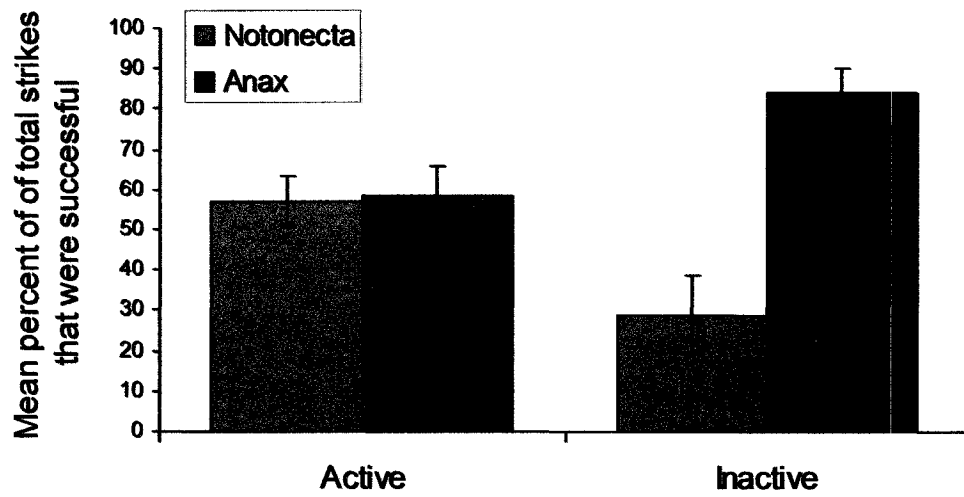


Figure 2.4: Mean percent success of strikes ( $\pm$  SE) on active or inactive tadpoles for each predator (log-linear ANOVA, predator  $\times$  activity,  $X^2_1 = 7.23$ ,  $p = 0.0072$ ,  $n = 20$ )

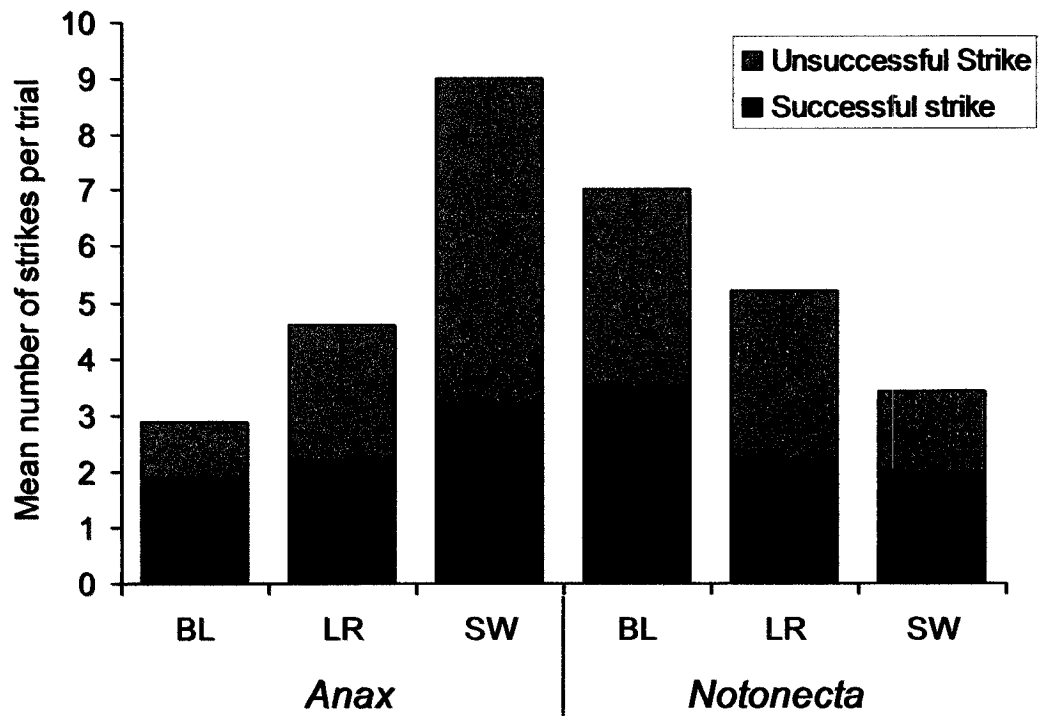


Figure 2.5: Mean number of successful and unsuccessful strikes (- SE) made on each population (BL- Bat Lake population,  $n = 10$ ; LR – Lost Ray population,  $n = 5$ ; SW – Sasajewun West Population,  $n = 5$ ) by each predator during a 60 minute trial (log-linear ANOVA, predator  $\times$  population  $X^2_2 = 15.13, p = 0.0005$ ).

## Discussion

*Anax* are visual predators and may have a better chance of detecting an inactive tadpole (Folsom and Collins 1984), which could explain the increase in strike attempts by *Anax* on inactive tadpoles, allowing them to detect the inactive tadpoles more easily than *Notonecta* which use mechanical or tactile cues more often. Both *Anax* and *Notonecta* were equally likely to capture an active tadpole. Though this may be true, active prey were struck at more frequently than inactive prey by *Anax*. *Anax* larvae were equally successful at capturing active and inactive tadpoles, suggesting that it may be profitable to go after inactive tadpoles when encountered because they may require less time investment in the capture of prey. Past research has shown that tadpoles are more susceptible to predation by dragonfly larvae (Skelly 1994) and fish (Lawler 1989) when they have higher activity rates. Skelly (1994) found that tadpoles were four times more likely to be captured by dragonfly larvae when active. Differences between that study and this are likely due to different methodologies used. Skelly (1994) rendered tadpoles inactive through the use of an anaesthetic, which may have made the tadpoles unappealing to the predator in some way.

The method that predators use for prey capture may also affect predator success rate. *Anax* tend to perch and wait for a tadpole to move closer, or to slowly stalk prey before striking. In most trials the *Anax* could be observed watching the movements of tadpoles, as demonstrated by the direction of head movements in the *Anax*. The method of capture in *Anax*, striking and grasping with the modified labium, also causes less turbulence in the environment compared to *Notonecta*. Fewer tadpoles were able to escape from the grasp of *Anax*, and when they did, it was because the *Anax* struck at the tail rather than the body of the

tadpole. This agrees with previous research that has shown tadpoles are less likely to escape from an attack to the body than one to the tail (Johnson and Eidietis 2005). It has also been shown that the tissues in a tadpole's tail are fragile, tearing easily and allowing escape from predators with minor damage (Doherty et al. 1998).

In contrast, *Notonecta* have specialized mechanosensilla on their legs to detect movement and rarely use vision (Murphey and Mendenhall 1973). This may explain why they are more likely to attack active tadpoles as they would detect movement in the water as opposed to visually identifying an inactive tadpole as prey. Although *Notonecta* have been described as "sit and wait" predators, they appeared to be actively chasing tadpoles during these trials, disturbing inactive tadpoles then striking at them numerous times. *Notonecta* are far more erratic in their movements than *Anax*, and were observed roaming during the trials, striking at tadpoles after disturbing them, suggesting that *Notonecta* do not use visual cues when hunting. *Notonecta* often had to strike more than once and chase a tadpole before successfully capturing it. The ability of *Notonecta* to feed on tadpoles decreases as tadpole size increases (Cronin and Travis 1986), but tadpoles used in this experiment were small and approximately the same size as each other, so this should not have affected predator choice of prey.

Predators had different success rates on tadpoles from different populations (Figure 2.5). It is possible that slight differences in prey behaviour which were not accounted for by the active/inactive scoring could have resulted in these differences. The Bat Lake tadpole population lives in a small, but deep, fishless lake with many invertebrate predators. These tadpoles were able to successfully evade *Anax*, eliciting the lowest number of total strikes and successful captures; however, *Notonecta* had the largest number of total and successful strikes

on tadpoles from Bat Lake. It is likely that this population lives in closer proximity to *Anax* and encounters free swimming *Notonecta* much less frequently, which has been suggested of deeper lakes (Sih 1982). While relatively small in area, Bat Lake is deep. Tadpoles from Bat Lake may therefore have been under greater selection to detect and avoid *Anax*. In contrast, the Sasajewun West population is found in a shallow marshy area and may have more contact with *Notonecta*, accounting for the fact that *Notonecta* attacked tadpoles in this population less than *Anax*, and also had the lowest success rate when attacking this population. The Sasajewun West tadpoles received the most attacks of the three populations when in the presence of *Anax*, but had a relatively low capture rate. Lost Ray is a shallow lake and both predators had an intermediate strike and success rate with tadpoles from this population. This suggests that the Lost Ray tadpole population consists of generalists that are adapted to avoid both predators equally, likely because they encounter both regularly. It has also been shown that backswimmer populations are dependent on the habitat structure and quality (Briers and Warren 2000), both of which a variable throughout the spring and summer due to changing water levels in the Lost Ray and Sasajewun West sites. Although there were no observable differences between populations, it is possible that there are subtle behaviours that exist, creating this difference between populations.

Unexpectedly, a number of instances of prey sharing were observed in *Notonecta* trials; I found no record of prey sharing in this species in the literature. In one instance, a tadpole was captured by a *Notonecta* and injected with digestive juices, and was then subsequently pierced and fed on by a second *Notonecta* perched on the other end of the tadpole. On five other occasions the tadpole was dropped and a second *Notonecta* picked it up and inserted its own beak, presumably to suck the remaining juices from the tadpole. The

energy expenditure for a *Notonecta* to attack is likely high in comparison to *Anax*; there is often a chase with more than one strike occurring before the capture of a tadpole. Therefore, the sharing of prey could be beneficial if this behaviour is reciprocated. Prey sharing in *Notonecta* is an area that requires more study.

Unfortunately there were not enough data to determine whether the activity of neighbouring tadpoles affected the likelihood of strikes on them, due to the infrequent occurrence in this experiment. Therefore further study is needed to determine if this effect exists in this species of tadpole, and if it is relevant to predation. Neighbour effects may be more prominent in anurans such as the genus *Bufo*, which are commonly found in large aggregations, rather than the more solitary ranids.

In conclusion, the activity level of a tadpole affects a predator's choice of prey. Total number of strikes and the success of predators differed depending on prey behaviour and the prey population. The observed differences may be due to the habitat of the populations, and the frequency of encounters with these predators in their natural setting. Further study on predator choice of prey is recommended, with focus on behaviour in a more natural setting providing a more accurate picture of how often tadpoles encounter these invertebrate predators in the wild, and the factors that influence how they choose their prey. Taking these findings into account may explain differences in survivorship and morphological traits in the presence of these predators. In addition, prey sharing in *Notonecta* is a novel occurrence, and is an aspect of this predator-prey system that requires more study.



## Chapter 3

### Effects of Single and Multiple Predators on Tadpole Activity

Predators can have an immense impact on communities, affecting species richness and diversity, with cascading effects that filter down through the food chain. An entire body of research is focused on the complexities of predator-prey relationships. In nature, most systems consist of multiple predators with complex predator-prey interactions, however multi-predator systems are seldom studied (Relyea 2003, Griffen 2006). Focusing on specific predator-prey pairs simplifies relationships, reducing the number of interactions that can occur within these systems. Recently there has been a push to examine more natural relationships within multiple predator systems, because the mortality and subsequent effects of predation in these systems cannot be predicted using the information gathered from single predator studies (Sih et al. 1998, Relyea 2003, Griffen 2006).

When multiple predator species are present, a number of outcomes are possible: additive predation, risk enhancement, and risk reduction (Relyea 2003). If there is no interference occurring between predator species, the competition for prey can lead to additive risk or enhanced risk, both of which can greatly affect the impact on prey population dynamics (Sih et al. 1998). If predator effects are additive, the combined effects of predators can be predicted in a straightforward manner from effects shown by single predators (van Buskirk 1988). In predators of larval treehole mosquitoes (*Onchlerotatus triseriatus*), for example, each predator species independently predicts the overall mortality when added (Griswold and Lounibos 2006). Enhanced risk occurs when the first predator causes a reaction in prey that results in prey having increased vulnerability to a second predator (Sih et al. 1998). Thus,

enhanced risk leads to greater predation risk when both predators are present than the summed individual predator effects. This effect has been observed in snails, which take refuge under rocks in the presence of fish, and in doing so increase their chance of capture by crayfish (Turner 1996, DeWitt and Langerhans 2003). Risk reduction occurs when there is interference between predators (Eklov and Werner 2000) or when one predator feeds on the other (Sih et al. 1985). In the same treehole mosquito system mentioned above, predators of the same size interact, competing for food and causing interference, which leads to reduced predation risk for the mosquito larvae relative to the predicted additive risk (Griswold and Lounibos 2006). A similar result to the mosquito system has been obtained using the bacteria-eating ciliates *Colpidium* and *Paramecium*, which exhibit either risk reduction or risk enhancement depending on the food source present (Jiang and Krumins 2006).

Contradictory results have been reported when examining survivorship in multiple predator systems with tadpoles as the prey item. In some cases prey individuals react to combined predators in the same way that they would react to the most dangerous predator, in terms of morphological plasticity (Relyea 2003, Teplitsky et al. 2004, Griffen 2006) and survivorship (Griffen 2006). Eklov and Werner (2000) demonstrated risk that reduction for bullfrog (*Rana catesbeiana*) and green frog (*Rana clamitans*) tadpoles occurs when both fish and dragonfly predators are present. The combination of predators allowed for decreased predation in two ways: fish reduce the activity of the dragonflies, which in turn lowers the risk of predation by dragonflies for the tadpoles; and dragonfly larvae elicited a reduction in activity of tadpoles, which led to decreased mortality by the fish (Eklov and Werner 2000). Eklov (2000) examined changes in growth and behaviour of bullfrog tadpoles in response to multiple predators and found an increase in survivorship in the presence of multiple predators

relative to any single predator or additive predator effect. Differences in response to multiple predators requires further study to determine if there are common trends that can be used to predict responses.

As seen in Chapter 2, activity has a major impact on predator attack rates and success of strikes. Wood frog tadpoles tend to have a higher baseline activity level than other larval anurans, possibly because they live in temporary fishless ponds with many invertebrate predators (Richardson 2001). The data from Chapter 2 suggest that altering activity as a predator avoidance response will be more effective against *Notonecta*, as they were less successful and made fewer attacks on inactive tadpoles compared to the dragonfly larvae *Anax*.

To build further on the results from Chapter 2, I examine wood frog tadpole behaviour in response to single and multiple non-lethal predators using a substitutive design (no predator, predator A, predator B, [density of A/2 + density of B/2]; compared to an additive design in which the combined treatment would be predator [A + B]), to determine the effect of predator presence on prey activity in a semi-natural setting over a longer period of time. In addition, I address the legitimacy of using scan samples as a surrogate measure of activity in tadpoles.

## **Methods**

To determine how the natural predators *Anax junius* and *Notonecta undulata* affect the activity level of wood frog tadpoles, a semi-natural pond setup was used. Animals were obtained and housed according to the methods described in Chapter 1. Tadpoles were used when they started actively feeding (Gosner stage 25, Gosner 1960). This protocol was

approved by the Brock University Research Committee on Animal Care and Use (AUPP 05-07-01).

### *Effect of Predators on Activity*

Each trial consisted of blocked treatments of a control (no predator) and a non-lethal predator run simultaneously. These treatments were crossed with three predator treatments: *Anax junius*, *Notonecta undulata*, and a combined treatment with both predators present (Figure 3.1). In the non-lethal treatment, predators were confined within two cages. Cages were 500 mL plastic containers with holes to allow water flow but prevent tadpoles or predators from getting in or out and covered with fibreglass window screening held on with an elastic band. Each cage contained a small piece of sphagnum moss as a perch. The control treatment had only empty cages present in the ponds. Predator treatments had two cages with one *Anax* in each, two cages with three *Notonecta* in each, or three *Notonecta* in the first cage and one *Anax* in the second cage. The semi-natural pond habitats were set up outdoors with exposure to natural light and temperature regimes. The ponds consisted of blue rigid plastic wading pools, 0.914 meters in diameter, filled with 20 L of lake water, and a container (~500 mL) of loosely packed sphagnum moss (from Bat Lake) spread around the pool to provide cover. Each pool was considered one replicate.

Tadpoles were fed a combination of algae discs and tadpole food during the afternoon prior to the trial, however there was no food placed in the semi-natural ponds during the trials. Ten replicates were done of each treatment combination (*Anax*, *Notonecta*, and combined predators crossed with non-lethal predator and control) for tadpoles from each of the three

collection sites (Bat Lake, Lost Ray and Sasajewun West ) (Figure 3.1). Ten wood frog tadpoles were placed in each pool at the start of each trial.

*Timetable for Each Experimental Replicate* (Figure 3.2)

**Day 1** – In the afternoon, ponds were set up with fresh lake water and moss. Ten tadpoles were placed in the pools at 8 pm (0 hours). Pools were then covered with aluminium screening and chicken wire staked into the ground with metal pegs to prevent other animals from getting into to the ponds during trials. Tadpoles were left to acclimate to the semi-natural pond for 12 hours.

**Day 2** - At 8 am (12 hours) the wire and screening was carefully removed to minimize disturbance to experimental animals. Pools were left for 1 hour to allow tadpoles to recover from any disturbance caused by removal of the screening. At 9 am (13 hours) I measured activity rates by standing approximately 0.5 meters away from the pond and scoring the number of tadpoles that were active, hereafter referred to as a scan sample. Each pond was scanned this way once every 10 minutes, until six measures were taken for each pool. After observations, water and air temperatures were recorded. Predators and cages were then added to the ponds, and ponds were once again covered with screening and wire (14 hours). The ponds were then left undisturbed for 22 hours to allow for sufficient time for predation to occur (in lethal treatment, to be discussed in Chapter 4).

**Day 3** - At 8 am (36 hours) the covers were again removed, and at 9 am (37 hours), six more scores of activity were taken in the same manner as on day 2. Temperatures and general observations were also taken at this time. Tadpoles, predators and moss were removed from the ponds and the pools were emptied in preparation for the next trial.

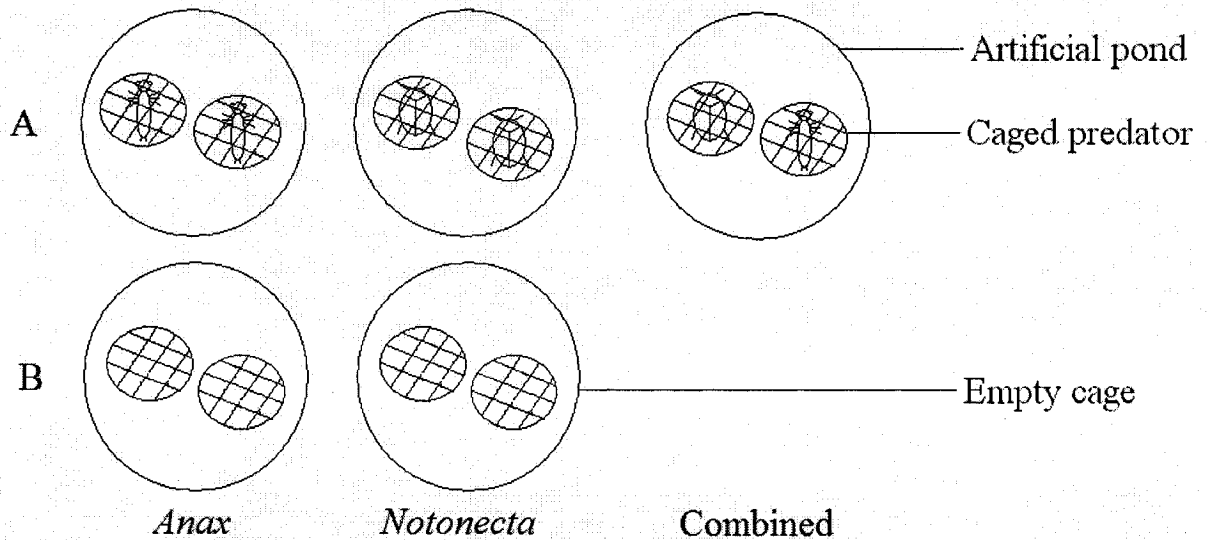


Figure 3.1: Cartoon showing blocking of experimental treatments used: A – non-lethal predator treatments (for *Anax*, *Notonecta*, and combined treatments) and B – control in which no predator is present. Each of these three treatments was replicated ( $n = 10$ ) for each of the 3 populations.

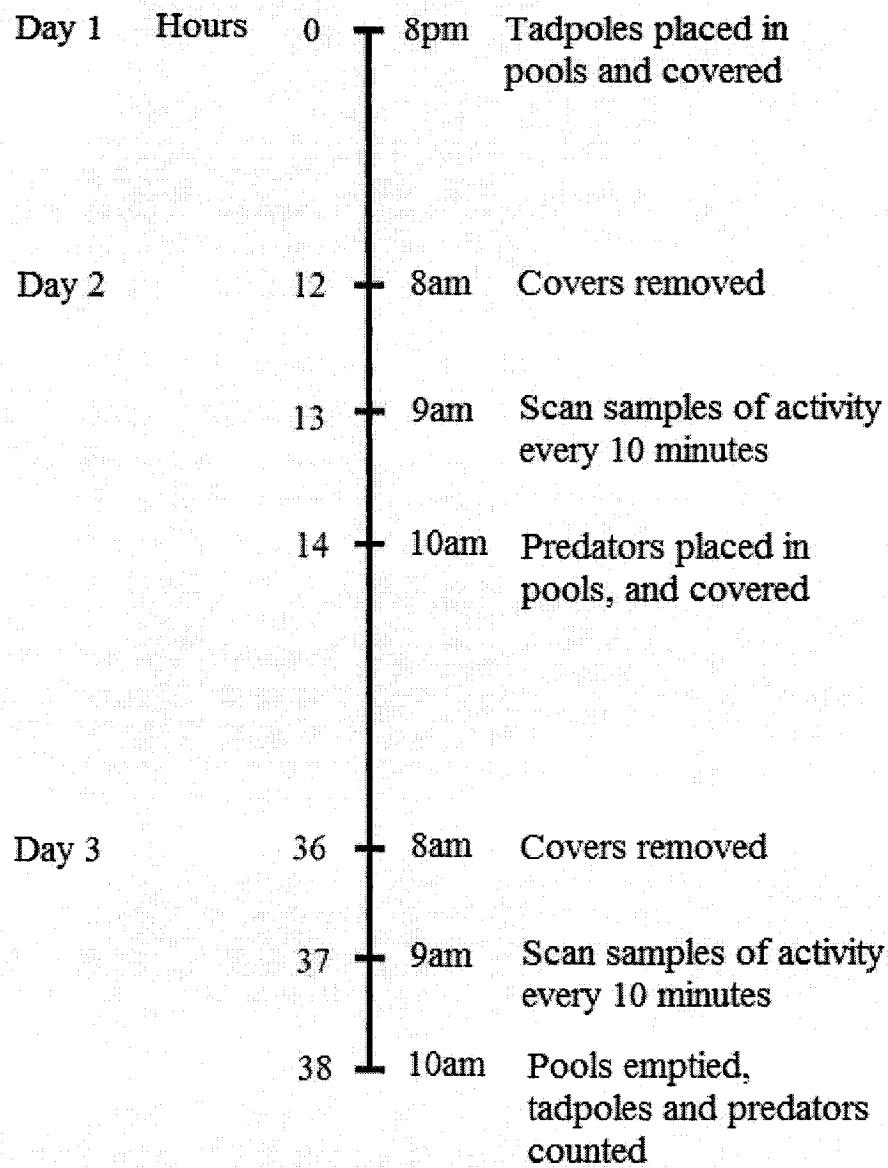


Figure 3.2: Timeline for an experimental replicate. At 8 pm trials commenced with tadpoles being placed into the pools. At 8 am the covers were removed from the pools. Scan samples were done every 10 minutes for an hour at 9 am. After this, predators and cages were added (10 am). Pools were then covered and left undisturbed until 8 am the next day. Covers were again removed and scan samples were taken for an hour (starting at 9 am). Pools were then emptied.

Pools were blocked by both space and time, each randomly assigned. Trials were conducted in May and June of 2005 and 2006. In 2005, nine pools were used at a time, with three sets of blocks run simultaneously. In 2005, Bat Lake tadpoles had six replicates completed for each predator, Lost Ray tadpoles had four *Anax* and three *Notonecta* replicates, and Sasajewun West tadpoles had three *Anax* and four *Notonecta* replicates completed. In 2006, there were 15 pools, with five sets of blocks run simultaneously. In 2006, all combined predator trials were completed (10 replicates for each population), in addition to four replicates of each single predator treatment for Bat Lake, six *Anax* and seven *Notonecta* replicates for Lost Ray, and seven *Anax* and six *Notonecta* replicates for the Sasajewun West population. Different numbers of replicates between years were due to the number of tadpoles available in the first season.

#### *Scan Sample as a Measure of Activity*

To assess the use of scan samples, a separate experiment was done using the same semi-natural ponds used in the above experiment. Trials were done in the morning, afternoon, or the evening to include a range of temperatures because temperature may influence the activity of tadpoles. Five tadpoles were placed in the pond and allowed to acclimate for one hour. After this, tadpoles were observed from approximately 30 cm away from the pond for 60 minutes. A scan sample of activity was taken every 10 minutes for a total of six samples. Each scan sample was a count of how many tadpoles were active within the pool at that point in time. Simultaneously, the total amount of time a randomly chosen focal tadpole was active (activity was any displacement of the body in any direction) was recorded using a stopwatch, as was the total time any one of the five tadpoles was active (i.e. the second stopwatch was



stopped only when zero tadpoles were moving). In addition, starts and stops were considered by recording the number of times the focal tadpole began moving from an inactive state (referred to hereafter as a “start”). As with time active, this number was also recorded for all five tadpoles, to provide a measure of total number of starts made by all tadpoles combined. Ten replicates were done in June 2006, using randomly chosen tadpoles from the Lake Sasajewun population. Tadpoles were used only once in this experiment and released.

### *Statistical Analysis*

To test for difference in tadpoles activity in response to predator presence, I used the difference in mean number of tadpoles before (scan samples, day 2) and after predator addition (scan samples, day 3), i.e. [tadpoles active after]-[tadpoles active before]. Using differences controlled for random variation in activity levels between replicates. A full model, three-factor ANOVA was performed on the data using SAS 9.1 (SAS Institute Inc., 2003) to consider change in activity; after running the full model non-significant interactions were removed and only the reduced model is reported in the results. Independent variables were predator treatment (*Anax*, *Notonecta*, both, and control), year (2005, 2006) and population (Bat Lake, Lost Ray, and Sasajewun West). Pool position was included initially, but subsequently removed as position within the block was not statistically significant. A Tukey post-hoc analysis was then performed on significant terms. Microsoft Excel was used to perform a correlation on the scan sample data with the total time active and number of starts.

## Results

### *Scan Samples*

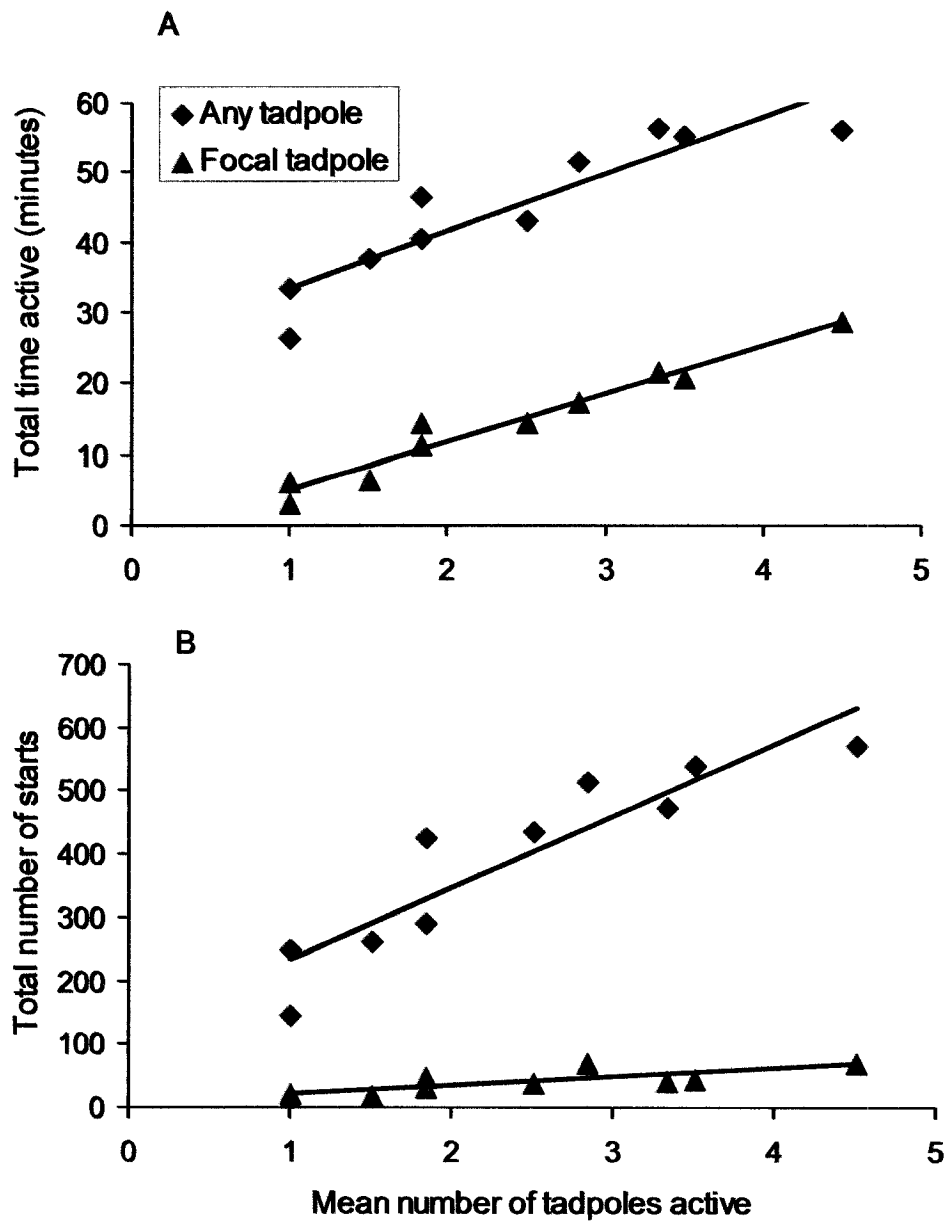
The mean number of tadpoles active (based on scan samples) was strongly correlated with the total time active for both the focal tadpole (Figure 3.3A; correlation,  $r = 0.9771$ ,  $n = 10$ ,  $p < 0.001$ ) and any tadpole (Figure 3.3 A, correlation,  $r = 0.9150$ ,  $n = 10$ ,  $p < 0.001$ ). Mean number of tadpoles active (scan samples) were also correlated to the number of starts made by the focal tadpole (Figure 3.3 B; correlation,  $r = 0.8223$ ,  $n = 10$ ,  $p < 0.001$ ), as was number of starts for all tadpoles in a pool (Figure 3.3 B; correlation,  $r = 0.9138$ ,  $n = 10$ ,  $p < 0.001$ ).

### *Non-lethal predators versus no predators*

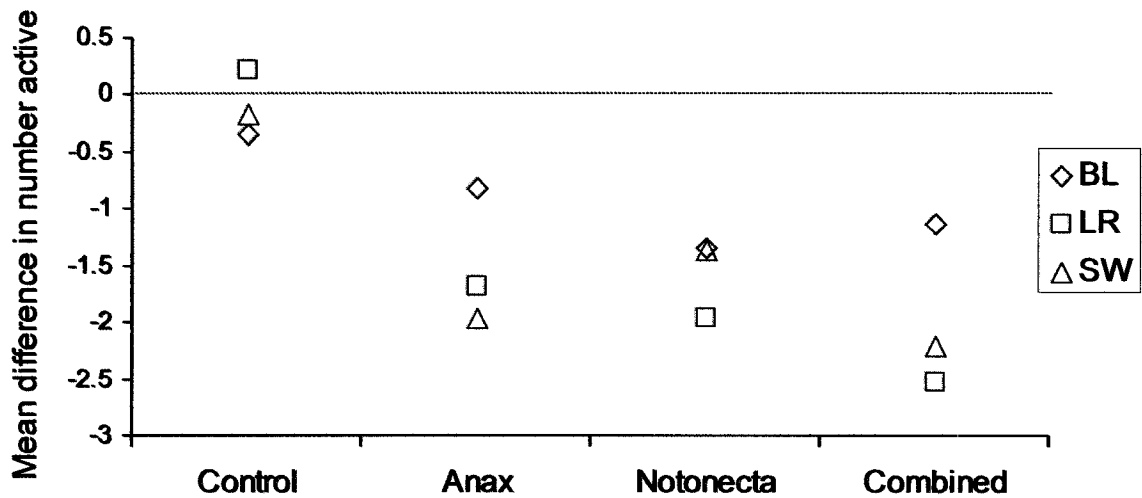
Change in activity was strongly affected by the presence of any predator (Figure 3.4; Table 3.1; ANOVA, predator main effect,  $F_{3, 129} = 10.83$ ,  $p < 0.0001$ ). The *Anax*, *Notonecta*, and combined treatments all showed a reduction in the number of tadpoles active with the addition of a non-lethal predator compared to that of the control, but predator treatments were not different from one another (Tukey post-hoc). Difference in activity did not differ by population (Figure 3.4; Table 3.1).

## Discussion

The reduction in activity observed is consistent with that of prior studies on wood frog tadpoles (Lawler 1989, Richardson 2001). This response to single predators is not novel; however, it is interesting that tadpoles behaved in the same way when different predator types and the combination of these two predators were present. Relyea (2003) showed a similar



**Figure 3.3:** Correlation between the mean number of tadpoles active (out of 5) at a given point in time (scan sample) and A – the total time spent active by a focal tadpole and any tadpole and B – the total number of starts made by a focal tadpole and any tadpole in the pool during a 60 minute trial. All correlations are highly significant.



**Figure 3.4:** Mean difference in the number of tadpoles active (scan sample) [mean number of tadpoles active after the addition of predators – mean number of tadpoles active before addition of predators] across the non-lethal predator and control treatments for each population: BL, Bat Lake; LR, Lost Ray, and SW, Sasajewun West. The dotted line indicates no change in activity. Change in activity differed between the control and predator treatments (ANOVA, predator term,  $F_{3, 129} = 10.83, p < 0.001$ ), but there was no difference among populations. Error bars were excluded for clarity.

Table 3.1: ANOVA summary table for the difference in mean number of tadpoles active [mean number of tadpoles active after the addition of predators] – [mean number of tadpoles active before addition of predators] for predator (*Anax*, *Notonecta*, both or control), and population (Bat Lake, Lost Ray, and Sasajewun West) treatments. Year denotes the year that the trials were completed.

Source	DF	Type III	F value	P
		Sum of Squares		
Predator treatment	3	80.0154	10.83	<0.0001
Population	2	10.3065	2.09	0.1277
Year	1	2.6992	1.10	0.2972
Predator treatment × Population	6	16.8375	1.14	0.3435
Predator treatment × Year	2	2.8485	0.58	0.5624
Population × Year	2	2.7554	0.56	0.5731
Predator × Population × Year	4	11.0263	1.12	0.3505

trend in wood frog tadpoles when examining morphological plasticity in response to single and multiple predators. However, there were no differences in the behavioural responses to different predators in my study, whereas Relyea (2003) found that tadpoles discriminated between predators, both alone and in combination. Tadpoles responded to combined predators in the same manner that they responded to the most risky predator, both in behaviour and morphology (Relyea 2003). The lack of difference in change in activity observed among the three predator-present treatments in my study suggests that perceived threat stayed constant even when there are different predators present. In addition, keeping the different predators in their own cage could have eliminated any interaction that may occur between them in the wild. Alternatively, there could be one fixed response to the presence of any predator, regardless of the capture technique. Eklov (2000) found that activity of bullfrog tadpoles was intermediate when multiple predators were present, compared to the low activity when *Anax* was present and the high activity in the presence of fish. The differences in my results are likely owing to the predators used. There would likely be different interactions occurring between fish and *Anax*, because dragonfly larvae would not be able to prey upon fish in the same way that they can prey on *Notonecta*. *Anax* will forage on *Notonecta* when they are hungry and vice versa. This interaction may result in the threat to tadpoles remaining constant when both predators are present and this may be the reason why tadpoles did not appear to distinguish between the predators.

The predator species used in this experiment co-occur naturally at all sites, however these predators can inhabit different areas within a site. *Anax* are more often found in the littoral zone, perching on vegetation. *Notonecta* are free swimming and are often seen in open water, but can also be found perching on vegetation or on top of the water in the same areas as

*Anax* (C. Kerling, personal observation). Although these predators share overlapping habitats, it is difficult to determine the amount of time they spend in close proximity, or to know how often they encounter one another in the wild. The natural encounter rate between wood frog tadpoles and these predators, and the habitats in which predators are encountered likely contribute to the responses observed. A larger, more variable habitat may elicit different responses in these tadpoles.

The cues from starved predators may not have the same effect on prey as those fed conspecifics or heterospecifics. Chivers and Mirza (2001) found a greater reduction in activity in response to predators (*Anax* and fish) fed tadpoles. However, Petranka and Hayes (1998) showed that there is no difference in tadpole response (a reduction in activity) to a starved *Anax* or an *Anax* which recently consumed a tadpole. Although it is possible that the use of food deprived predators could have affected the behavioural response of the tadpoles in my results, it is unlikely because the diet of predators included wood frog tadpoles when in other trials.

The non-lethal predators used in this study represented an intermediate risk to tadpoles; cages allowed for chemical and visual cues to pass freely. Therefore, I predicted that if tadpoles could accurately assess the risk they should show a decrease in activity, but not to the extent of that predicted if lethal predators were present. It is possible that the use of non-lethal predators in this study reduced the behavioural response in such a way that the differences among predator types present were minimized to a point where they were undetectable. The problem with comparing a free-roaming predator and a non-lethal predator is that mortality can confound the results (Relyea 2003, Griffen 2006). The conspecific cue released when a tadpole is consumed has a greater impact on the activity of other tadpoles

(Adams and Claeson 1998); in addition, this may attract more predators to the area causing increased risk. The next chapter will examine the behavioural response of wood frog tadpoles to lethal predators and will address this issue in more detail.

Activity was assessed in this study using scan samples as a surrogate measure for total time active and the total number of starts (a measure of disturbance caused by tadpoles). Scan samples, where the investigator does a “scan” to see how many tadpoles are active at a specific point in time, have been used in other studies of tadpole activity (Altwegg 2003). Scans are often used when there are many tadpoles being observed at the same time, in larger areas, and it also has the benefit that it can be done from a slight distance. A second common measure of activity is to measure the total time active for single, or multiple, randomly chosen focal tadpoles. While more time-consuming than scan sampling, this method is less time-consuming than examining the total time active of every tadpole present. A third measure frequently used is the number of times a tadpole crosses a line, with a grid pattern, or line as a point of reference (Kiesecker et al. 1996, Chivers and Mirza 2001). This method is slightly easier to measure than the total time active, although it would be difficult to use this method in a larger, more natural setting. Although, these surrogates are often used there is little evidence of which is the most accurate way to measure activity while still remaining feasible. Results of this study suggest that scan samples are an accurate measure of activity, correlated both with total time active and with the number of starts (movements that occur from inactive), both of which are thought to attract the attention of predators (Lawler 1989, Azevedo-Ramos et al. 1992, Skelly 1994). The differences between the slopes of the total number of starts by a focal tadpole versus any tadpole may exist because the activity of one tadpole may induce another tadpole to start moving as well. This could explain the higher slope between number



of starts when examining any tadpole (as opposed to a single focal tadpole) and the mean number of tadpoles active in scan samples. Although all tadpoles used in the scan sample study were from one population, it is reasonable to expect that tadpoles from other populations would show the same correlations when there is no threat of a predator. Therefore, scan samples can be used as a surrogate for total time active, saving time and allowing for data on activity to be collected for more tadpoles simultaneously.

Although the behavioural response to predators agrees with what has been reported in the literature, that all predator treatments elicited the same response suggests that there is no change in the risk to the tadpoles with the different predator treatments, or that a general response to different predators is occurring. The examination of survivorship may shed light on the complex relationships that exist between these aquatic organisms.

## Chapter 4

### Does Activity Affect Survivorship?

The manner in which organisms increase survivorship is an important aspect of the study of animal behaviour, and improving probability of survivorship is the cause underlying many commonly observed behaviours. Survivorship is tightly linked with fitness and it is for this reason that predator avoidance behaviours are both important and well studied. Behavioural modification can allow animals to gain protection against predators. Responding with the correct behaviour in the presence of a predator can help a weaker individual to survive.

Relationships among the environment, foraging behaviour, and parasites can all modify the appropriate behavioural response of an animal to maximize survivorship (Lima and Dill 1990, Bridges and Gutzke 1997, Barber et al. 2000, Gustine et al. 2006, Relyea 2006). Environmental factors related to human impact can also affect survivorship of animals. For example, a study on the escape behaviour of frogs in the presence of cars showed that some species of frogs tend to reduce activity when they detect an approaching car and that this leads to increased mortality rates (Mazerolle et al. 2005). Survivorship of animals in natural settings has also been considered by examining the presence of natural predators and how they affect survivorship. For example, Alaskan moose alter foraging and habitat use when with their calves, maximizing their fitness by protecting their calf when predation risk is high (White and Berger 2001).

Many animals learn anti-predator behaviours through experience rather than using a reactionary response (innate behaviour) to evade predators. Learning to respond to predators

in the correct manner is a steep learning curve – the individual must determine how to detect predators and which behaviours are effective (Chivers and Smith 1998, Kats and Dill 1998, Turner et al. 2006). Data suggest that tadpoles learn from prior experience: their membranous tails allow for the chance to escape from predators, taking away experiences that may make them less likely to be attacked the next time (Doherty et al. 1998). The results in Chapter 2 show that a large number of failed attempts at capture. These failed attempts by predators may benefit tadpoles in their next encounter with a predator.

Wood frog tadpoles have less than a 4% survival rate to metamorphosis (Herreid and Kinney 1966), therefore, predator avoidance is expected to be under strong natural selection at this stage in life. Common examples of predator avoidance behaviours observed in tadpoles include a reduction in activity level, use of a refuge, and aggregation. In addition, morphological plasticity in response to predator presence has been suggested to be effective in increasing survivorship of tadpoles (McCollum and van Buskirk 1996, McCollum and Leimberger 1997, van Buskirk and McCollum 2000, van Buskirk 2002, LaFiandra and Babbitt 2004, McIntyre et al. 2004, van Buskirk et al. 2004, Relyea and Auld 2005).

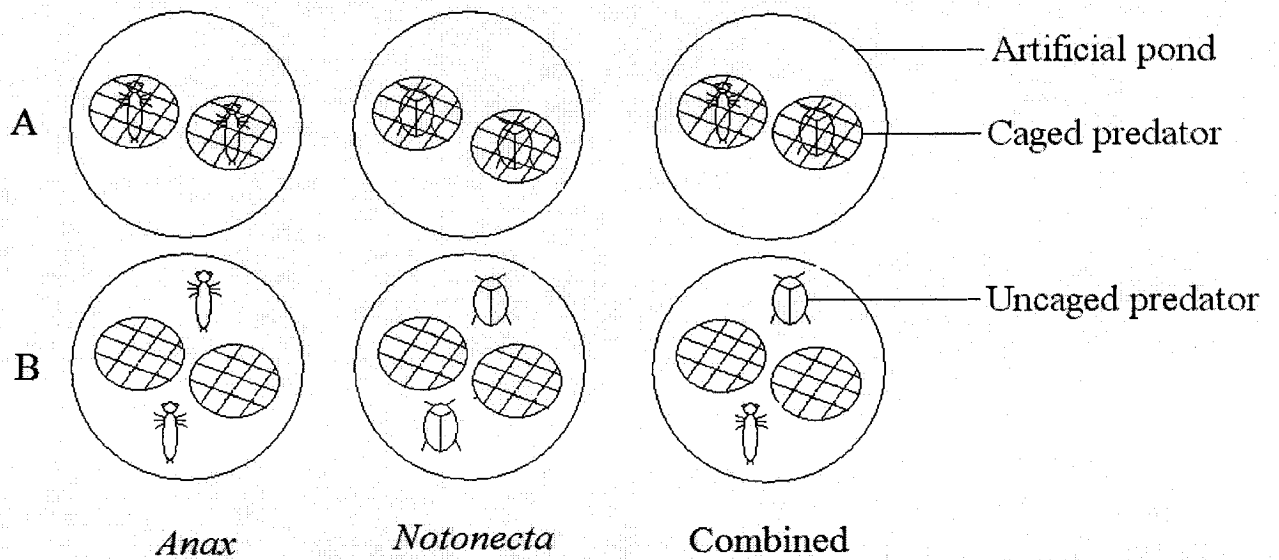
For maximal fitness, the activity of a tadpole is expected to be correlated to the risk perceived; therefore we might predict that tadpoles will increase anti-predator behaviour (e.g. reduce activity more) when a lethal predator is present in comparison to the behaviour observed with the non-lethal predator (Chapter 3). Tadpoles in multiple predator systems have been shown to react in a hierarchical response in terms of morphological characteristics, showing the greatest morphological change in response to the most risky predator whether the predator is alone or in combination with another predator type (Relyea 2003, Teplitsky et al. 2004). However there has been little study of behaviour and its relationship to survivorship in

these systems (Relyea and Werner 1999, Relyea 2000, Teplitsky et al. 2004). In Chapter 3 wood frog tadpoles were seen to respond to natural predators, both single species and multiple species, with a reduction in activity. For the response to be adaptive it must be effective against lethal predators and in this chapter I consider whether reducing activity is effective in reducing mortality when lethal predators are present.

There has been study on the survivorship of tadpoles over time (Herreid and Kinney 1966), in the presence of pesticides (Relyea 2004, Storrs and Kiesecker 2004, Relyea 2005b, Relyea et al. 2005, Boone and Bridges-Britton 2006), and in different environments (Anholt et al. 2003, Olsson and Uller 2003, Govindarajulu and Anholt 2006, Relyea 2006), but few studies examine the efficacy of the various predator avoidance behaviours. Here, I examine how decreased activity levels affect the survivorship of tadpoles using semi-natural ponds for three different prey populations and two abundant, coexisting predators, both alone and in combination.

## **Methods**

Wood frog tadpoles (*R. sylvatica*) and their natural predators (*Anax junius* and *Notonecta undulata*) were collected in the spring of 2005 and 2006 from Algonquin Provincial Park using the methods described in Chapter 1. Semi-natural ponds were used with the protocol described in Chapter 3. A lethal predator treatment was used in this study, examining the behaviour of tadpoles in the presence of free-roaming predators. The lethal predator treatment was run in conjunction with the non-lethal and control treatments described in Chapter 3 (Figure 4.1), on the timeline described in Chapter 3 (Figure 3.1). At the completion of the trial, all pools were carefully sorted through to account for all predators, and to



**Figure 4.1:** Cartoon showing blocking of experimental treatments used: A – non-lethal predator treatments (for *Anax*, *Notonecta*, and combined treatments) and B – lethal predator treatment when predators were free-roaming. Each of these three treatments was replicated ( $n = 10$ ) for each of the 3 populations.

determine the number of tadpoles surviving. In 2005, Bat Lake tadpoles had six replicates completed for each predator, Lost Ray tadpoles had four *Anax* and three *Notonecta* replicates, and Sasajewun West tadpoles had three *Anax* and four *Notonecta*, replicates completed. In 2006, there were 15 pools, with five sets of blocks run simultaneously. In 2006, all combined predator trials were completed (10 replicates for each population), in addition to four replicates of each single predator treatment for Bat Lake, six *Anax* and seven *Notonecta* replicates for Lost Ray, and seven *Anax* and six *Notonecta* replicates for the Sasajewun West population.

The lethal treatment had free roaming predators present in the same ratio as the non-lethal treatment (two *Anax junius*, six *Notonecta undulata*, and the combined treatment with one *Anax* and three *Notonecta* present). There were also two empty cages present in each pool. Measures of activity were taken using the scan sample methods and the timeline described in Chapter 3 (Figure 3.2). At the conclusion of the trial the pools were carefully searched and drained to account for all predators and tadpoles; missing animals were assumed to have been consumed by predators.

The predicted additive effect was calculated using the mean predation rate alone as seen in equations 1 and 2, where the mean mortality due to a particular predator is denoted by  $M_{\text{predator}}$  (Sih et al. 1998). Values were divided by 2 to account for the differences in the density of predators present in the combined treatment compared to that of the single predator treatments.

$$\text{Predicted additive mortality} = \frac{M_{\text{Anax}}}{2} + \frac{M_{\text{Notonecta}}}{2} \quad (1)$$

$$\text{Predicted additive survivorship} = 10 - \text{predicted additive mortality} \quad (2)$$

This protocol was approved by the Brock University Research Committee on Animal Care and Use (AUPP 05-07-01).

### *Statistical Analysis*

All statistical analyses were done using SAS 9.1 (SAS Institute Inc., 2003).

Differences in the survivorship of tadpoles were examined using a logistic regression, with the number of survivors as the response variable, predator treatment (*Anax*, *Notonecta*, both) and population (Bat Lake, Lost Ray, Sasajewun West) as the class variables, and activity before predators added as a covariate to control for possible differences in baseline activity level among replicates.

To test for differences in the activity of tadpoles in the presence of a lethal versus a non-lethal predator, the difference in proportion of tadpoles active [mean number active after the addition of predators/10] – [mean number active before the addition of predators/ number of surviving tadpoles] was analysed using an ANOVA. The full model with all interactions was considered, with predator treatment, population, and risk treatment (lethal or non-lethal) as factors.

## **Results**

When the activity of tadpoles in the lethal predator treatment was compared to that of the non-lethal treatment (Chapter 3), the change in activity differed both by risk treatment

(Figure 4.2; ANOVA,  $F_{1, 150} = 36.35, p < 0.0001$ ) and by population (Figure 4.2; ANOVA,  $F_{2, 150} = 9.09, p = 0.0002$ ).

Analysis of survivorship data revealed that the effect of activity level on survivorship of tadpoles differed among populations (Figure 4.3; logistic regression, activity  $\times$  population  $X^2_2 = 11.5703, p = 0.0031$ ). Bat Lake tadpoles were the least active before predators were added, but did not show any effects of activity level on survivorship. The activity of the Lost Ray population also had a minimal effect of baseline activity on survivorship; however Sasajewun West population exhibited a negative relationship between baseline activity and survival. Survivorship of tadpoles from the Sasajewun West population was highest (45%), Lost Ray had an intermediate survivorship of 38%, and Bat Lake had the lowest survivorship of 34% (Figure 4.3; logistic regression, population main effect,  $X^2_2 = 17.6764, p < 0.0001$ ). There was also a nearly significant interaction between predator treatment and the covariate of activity before (Figure 4.4, Table 4.1; logistic regression, activity  $\times$  predator treatment,  $X^2_2 = 5.6710, p = 0.0587$ ), driven by the strong negative relationship between activity and survivorship in the presence of *Anax*. Tadpoles had the highest survivorship in the presence of *Notonecta*, whereas equal numbers of tadpoles survived when *Anax* or a combination of predators were present (Table 4.2). Although there is slight effect of predator treatment by activity, this effect cannot be partitioned from the effect of treatment on survivorship alone. Thus, the presence of each predator was examined excluding baseline activity. From this comparison it was seen that the combined treatment had a survivorship lower than that predicted if predators had additive effects, showing risk enhancement in the presence of both predators (Table 4.2). All other factors and interactions were not significant (Table 4.1).



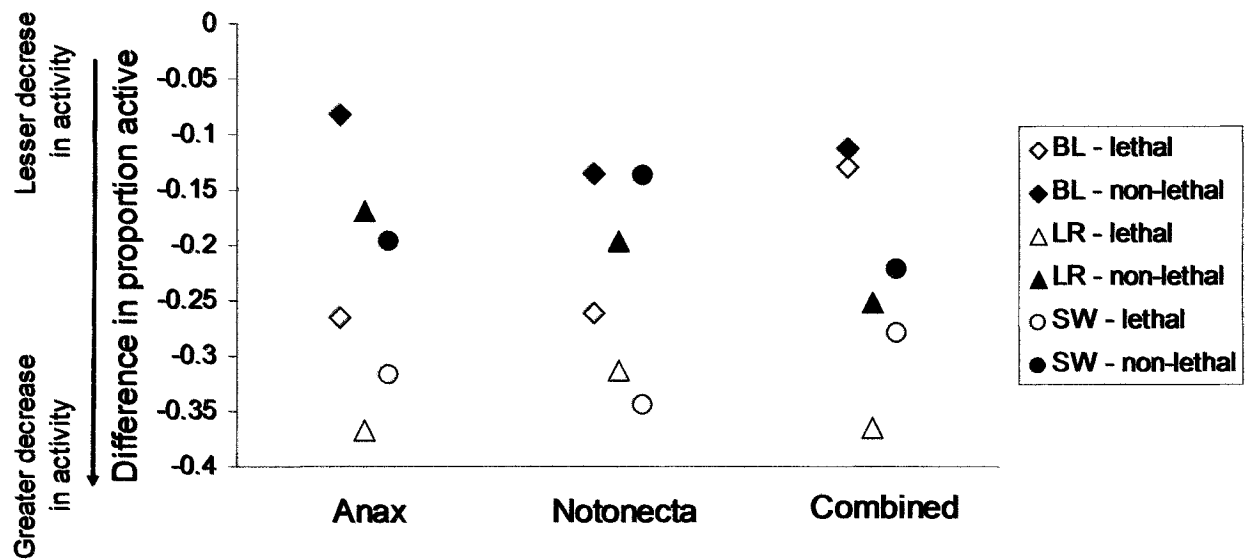
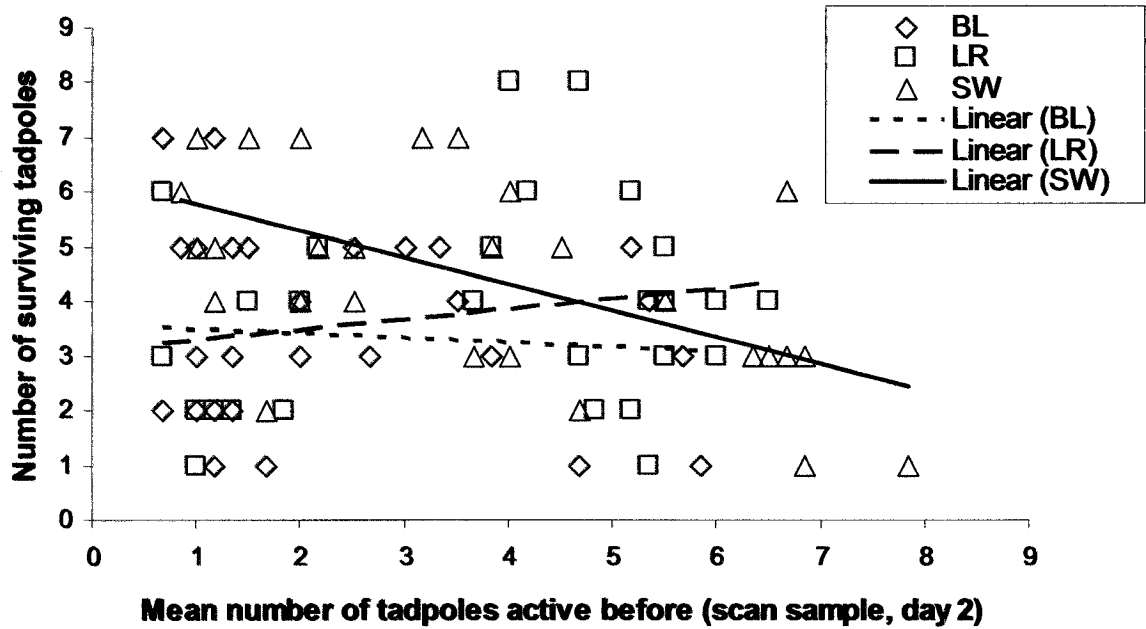


Figure 4.2: Mean difference in activity of tadpoles (scan samples) [after the addition of predators - before addition of predators] for both lethal (empty symbols) and non-lethal predator treatments (closed symbols) for each population (risk treatment  $F_{1, 150} = 36.35, p < 0.0001$ ; population  $F_{2, 150} = 9.09, p = 0.0002$ ). Abbreviations used denote the different populations: BL, Bat Lake, LR, Lost Ray, SW, Sasajewun West ( $n = 10$ ).



**Figure 4.3:** Survivorship of tadpoles (out of 10 tadpoles) by population plotted against the mean number of tadpoles active before the addition of predators (scan sample, day 2) as a covariate (logistic regression, activity  $\times$  population,  $X^2_2 = 11.5703, p = 0.0031$ ). Letter combinations represent populations: BL, Bat Lake; LR, Lost Ray, and SW, Sasajewun West.

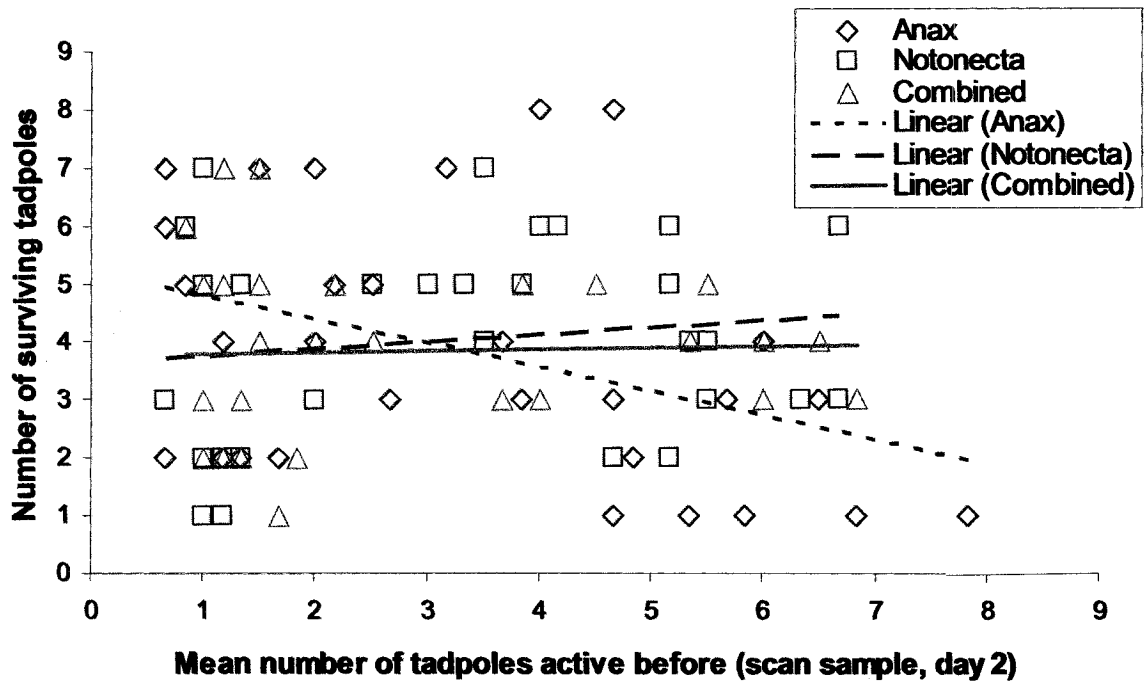


Figure 4.4: Survivorship of tadpoles in each predator treatment (out of 10 tadpoles) with the mean number of tadpoles active before (scan sample, day 2) a predator as the covariate (logistic regression, activity  $\times$  predator  $X^2_2 = 5.6710, p = 0.0587$ ).

Table 4.1: Logistic regression summary table for the survivorship of tadpoles in the lethal predator treatments. Compares the three predator treatments (*Anax*, *Notonecta*, or both) and populations (Bat Lake, Lost Ray, and Sasajewun West), with activity before the addition of predators as a covariate (scan sample, day 2).

Source	DF	Wald $X^2$	<i>P</i>
Predator treatment	2	3.0516	0.2174
Population	2	17.6764	0.0001
Activity	1	2.1825	0.1396
Predator treatment × Activity	2	5.6710	0.0587
Population × Activity	2	11.5703	0.0031

Table 4.2: Summary of survivorship values, and the predicted additive survivorship based on the single predator trials, with all populations combined ( $n = 30$ ). Predicted additive survivorship was calculated using equation 1 and 2. Total number of tadpoles in each trial was 10.

Predator	Mean number of survivors
<i>Anax</i>	3.8
<i>Notonecta</i>	4.1
Both	3.8
Predicted Additive Survivorship	3.95

## Discussion

Tadpoles from all sites exhibited a relationship between activity level and predator treatment, reducing activity or remaining immobile in the presence of predators, regardless of the predator treatment. Inactivity is a known predator avoidance response in tadpoles, and has been reported for wood frog tadpoles previously (Lawler 1989, Skelly 1994, Anholt et al. 2000, Richardson 2001). The ability to accurately assess predation risk is vital for potential prey and previous studies have suggested that wood frog tadpoles are able to accurately interpret this risk (Anholt and Werner 1998). My results agree with this, as the number of tadpoles active decreased in the presence of any predator, and activity was further reduced in the presence of a lethal predator compared to a non-lethal predator, showing that wood frog tadpoles can accurately assess and respond to different levels of predation risk.

There was a nearly significant interaction between the predator treatment and baseline activity on tadpole survivorship. The combined predator treatment and the *Anax* treatment both had a survivorship of 38%, which was lower than the 41% survival rate in the presence of *Notonecta*. This suggests that *Anax* is the top predator in this system, which makes sense knowing that these tadpoles face a greater risk from *Anax*, as seen from the higher success rate of *Anax* when attacking (Chapter 2). Previous studies that have focused on multiple predator effects on survivorship in tadpoles have shown a number of responses, including additive effects and reduced effects (Eklov 2000, Eklov and Werner 2000, Relyea 2003, Teplitsky et al. 2004, Gunzburger and Travis 2005). An additive model predicts that mortality when the predators are combined would be equal to that of the sum of mortality for each predator when alone (equations 1 and 2). However, survivorship was lower than would be predicted by this value, suggesting risk enhancement is occurring. This result conflicts with prior multiple

predator studies on tadpoles that have shown a risk reduction when multiple predators are present (Eklov and Werner 2000). Differences in the survivorship between my results and those of Eklov and Werner (2000) are likely due to the predators used. Fish and dragonfly larvae were used by Eklov and Werner (2000), and interactions between this vertebrate and invertebrate predator likely differ compared to a system with only invertebrate predators. In particular, the two invertebrate predators could each potentially successfully prey on the other, whereas dragonfly larvae would not have been able to consume a sunfish predator.

In addition, hungry predators can overcome prey defences, as an increased need for food makes them more likely to search out prey items that would otherwise be inaccessible due to predator avoidance behaviour. Previous work found that survival rate of tadpoles in the presence of satiated predators (*Anax imperiator*) was 70-90%, compared to 50-60% in the presence of a starved predator (Altwegg 2003). Both of these survivorship estimates are far greater than the survivorship in my study. Difference in time of exposure to lethal predators likely accounts for the differences in survivorship between my values and those of Altwegg (4 hours compared to 25 hours), as well as differences between the predator species used. The longer exposure time seems likely to give more accurate results, as it gives predators the chance to hunt both at night and during the day, which was found to be important when examining the frequency of strike of predators in Chapter 2.

Differences observed between populations are likely due to adaptations to specific predator regimes in each habitat. Although sites were thoroughly sampled and the most prominent predators used, other factors may act in the natural environment to generate selection for different traits. Bat Lake is a location where many wood frogs breed. It is a unique system in that it is a deep lake in which fish might exist, but it is not inhabited by fish

because it is naturally acidic water. There were approximately 500 wood frog egg masses laid there each year studied. It is possible that due to this large influx of prey for the predators studied, predators are often satiated and therefore many tadpoles survive due to random chance in this system, allowing “bad” genes to be passed on and reducing the impact of natural selection. This would explain the decreased survivorship of the individuals from Bat Lake. Sasajewun West showed the highest survivorship, which is odd because this is likely a non-viable population; very few egg masses are laid at this site per year, there is dense vegetation present, and site drying occurs early. Lost Ray had an intermediate survival, and the habitat lies somewhere between the deep open water of Bat Lake and the marsh-like environment of the Sasajewun West population.

In conclusion, predation risk in wood frog tadpoles is enhanced when there are multiple predators present, possibly due to slight changes in behaviour causing tadpoles to be more susceptible to one of the predators present. However, the reduction in activity is the same for predators alone and in combination, suggesting that there may be a generalized response to predators. The activity of a tadpole before the introduction of predators influences predation risk, with higher survivorship occurring in cases where initial activity is lower. Survivorship in tadpoles also depends on the specific population. Further study should be done on predation risk in the presence of multiple predators in a variety of habitat types to determine if different predator avoidance strategies are used in each combination of habitat, predator, and population. Tadpole behaviour should also be examined in conjunction with the morphological differences that can occur in some species to get a complete picture of what is occurring in these environments.



## Chapter 5

### General Conclusions

Predator avoidance is imperative for all animals, but is especially critical to juveniles. Tadpoles are no exception and are very vulnerable to predation risk. To reduce the risk of predation wood frog tadpoles can reduce activity level, in some cases staying totally inactive in the presence of a lethal predator (Anholt et al. 2000, Richardson 2001, van Buskirk 2002). Results of this thesis suggest tadpoles can accurately assess and respond to varying levels of predator risk. This agrees with the previous literature showing that tadpoles alter behaviour according to predation risk (Anholt and Werner 1998, van Buskirk and Yurewicz 1998, Anholt et al. 2000, Richardson 2001). Previous literature has found differences in behaviour and morphological plasticity of tadpoles when in the presence of different predators, mainly sunfish and dragonfly larvae (Lawler 1989, Anholt and Werner 1998, Eklov and Werner 2000, Teplitsky et al. 2004, Relyea 2005a, Kraft et al. 2006). However, my results show that the type of predator present (*Anax* and *Notonecta*) did not affect the response in terms of change in activity of tadpoles: tadpoles showed a generalized response to any predator present. Different predators often generate varying levels of risk, leading prey to respond with different behaviours or levels of behaviour. It appears that *Notonecta* are nearly as much of a threat to tadpoles as the more commonly studied *Anax*. This has implications for the study of behaviour and survivorship of tadpoles, as *Notonecta* is often overlooked as a major tadpole predator. Therefore, I suggest that future studies also examine *Notonecta*, in addition to *Anax*, and consider their presence when examining predator avoidance responses of tadpoles.

The two predators used in this study are likely to interact in nature due to the proximity of the predator habitats in their natural environment. That behavioural response to single and multiple predator treatments does not differ suggests that a generalized response to both predators exists, which differs from many of the studies that focus on the morphological changes (Eklov 2000, Eklov and Werner 2000, Relyea 2003, Teplitsky et al. 2004). However, results showed that risk enhancement was occurring when both *Anax* and *Notonecta* were present suggesting that slight differences in the response to one predator may make tadpoles more susceptible to predation by the other predator.

The presence of lethal and non-lethal predators elicited a similar response in tadpoles, although the lethal treatment led to a greater reduction in activity than the caged, non-lethal treatment. Therefore, this fact should be taken into consideration through the use of both lethal and non-lethal predator treatments when examining predator avoidance behaviours, because behaviour in the presence of non-lethal predators may not accurately reflect behaviour in the field. This should also be kept in mind when examining morphological responses to predators, as different results may be obtained when free-roaming predators are present.

Data on predator choice of prey in this study suggest that *Anax* are much better predators, able to successfully capture both active and inactive tadpoles more frequently than *Notonecta*, which strike at inactive prey much less frequently and with lower success rates. Failure of *Notonecta* to strike at inactive prey may reflect difficulties in detection and method of capture, but further study is required to determine if *Notonecta* actually choose the more active prey. The slightly higher survivorship seen in tadpoles tested with lethal *Notonecta* could be attributed to the lower success rates when striking; if *Notonecta* are unable to detect inactive tadpoles, then inactive tadpoles are likely to evade backswimmer predation, leading to

greater survivorship. The body of research focused on *Anax* has shown that these dragonflies will go after active tadpoles rather than their inactive counterparts (Skelly 1994, Laurila et al. 1997, Eklov 2000). These results suggest that dragonflies are more successful when attacking inactive tadpoles, which should be taken into consideration when examining survivorship of tadpoles. This has implications for other studies that have assumed that active tadpoles are at much greater risk from predators.

Although there appear to be minor differences in activity level between populations, it is likely that this is due to the specific habitat and vegetation structure at the different sites. Populations found in more similar habitats may show less variance in activity level and survivorship, as they would have similar encounter rates with the predators. In the future it would be beneficial to compare populations and determine if there is a genetic basis for these differences and to determine if any interbreeding occurs among these populations, which is feasible although previous research has suggested that adults are faithful to the pond from which they metamorphosed.

The importance of using a more natural setting when examining predator-prey relationships is key, since in a larger space there is more room for natural variation in the encounter rates of tadpoles and their predators. Results obtained from using smaller habitats and smaller groups of tadpoles and predators can lead to false interpretations of the relationships existing in natural settings. A variety of studies examining predator-prey relationships have used much smaller and less natural settings to determine behaviour and morphological effects on tadpoles (Skelly 1994, LaFiandra and Babbitt 2004, Teplitsky et al. 2004, Richardson 2006). The results obtained in these settings appear to be different in terms

of intensity of response from those reported in this study and it should therefore be noted that they may not be the most reliable indicator of what is occurring naturally.

The studies described in this thesis reveal a strong relationship between activity and survivorship of wood frog tadpoles that also depends on the predator(s) present. Examination of activity under varying levels of predation risk has shown that tadpoles will alter behaviour in accordance to the level of risk. Results suggest that there may be a genetic basis to the initial baseline activity level of tadpoles, as populations differed in initial activity levels. The frequency of strikes and predator's choice of prey likely differs due to differences in methods of detection and capture by different predators, with *Anax* showing a much higher success rate when attacking an inactive prey item. Complex relationships exist between predator, prey and the environment, and future work should focus on examining these relationships in a more natural setting.

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