

**The Effects Of Predation Risk On Phenotypic And Hatching Time
Variation In Fathead Minnow (*Pimephales Promelas*) Embryos**

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Abstract

Predation has long been recognized as a strong selective force influencing the behaviour, morphology and life history traits of prey species. Some prey species have the ability to alter their phenotypes in response to predation threat, to decrease their chances of being detected, caught and/or consumed by predators. It has also been demonstrated that some species have control over the timing of transition between one specific life stage and the next. This ability gives the prey control over how much time it spends in a risky stage. A particularly vulnerable stage for many prey fishes is that of the embryo, as they are a major prey item for many invertebrate and vertebrate predators. As such, strong selection pressure should exist for the development of anti-predator defenses specific for this time period. In a series of four experiments I assessed the ability of fathead minnow embryos to alter their hatching time and/or phenotype in response to various predation threats. In the first three experiments injured embryo cue was used to simulate a predation threat, as it has been shown to represent a general predation risk for many aquatic animals. In the fourth experiment predator odour was used in conjunction with injured embryo cues. Results of a power analysis conducted on the first three experiments determined with 95% confidence that fathead minnow embryos do not

alter their hatch time in response to injured embryo cues. However, the embryos in the predation treatment did hatch with an altered phenotype; fry were significantly smaller (total body length) when exposed to predation cues. In the fourth experiment the embryos hatched with the same altered morphology in response to injured embryo cues combined with predator cues. Moreover, in this experiment the embryos hatched faster in the predation treatment than the control treatment. This is the first empirical evidence that fish can alter their hatching time in response to predation.

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Table of Contents

| | |
|---|-----|
| Permission to Use..... | i |
| Abstract..... | ii |
| Acknowledgements..... | iv |
| Table of Contents..... | v |
| List of Figures..... | vii |
| | |
| Chapter 1: Introduction..... | 1 |
| 1.1 Background Information..... | 1 |
| 1.2 Objective..... | 12 |
| 1.3 Significance of Thesis Work..... | 14 |
| Chapter 2: Methods..... | 15 |
| 2.1 Basic Experimental Protocol..... | 15 |
| 2.2 Experiment One: Hatching time and hatchling phenotype of fathead minnows exposed to different predation cues during embryonic development (in late spring)..... | 17 |
| 2.3 Experiment Two: Hatching time and hatchling phenotype of fathead minnows exposed to different concentrations of predation cue during embryonic development..... | 18 |
| 2.4 Experiment Three: Hatching time and hatchling phenotype of fathead minnows exposed to different predation cues during embryonic development (in late summer)..... | 20 |
| 2.5 Experiment Four: Hatching time and hatchling phenotype of fathead minnows exposed to crayfish predation cues during embryonic development..... | 20 |
| 2.6 Statistical Analysis..... | 21 |
| Chapter 3: Results..... | 23 |
| 3.1 Experiment One: Hatching time and hatchling phenotype of fathead minnows exposed to different predation cues during embryonic development (in late spring)..... | 23 |
| 3.2 Experiment Two: Hatching time and hatchling phenotype of fathead minnows exposed to different concentrations of predation cue during embryonic development | 27 |
| 3.3 Experiment Three: Hatching time and hatchling phenotype of fathead minnows exposed to different predation cues during embryonic development (in late summer)..... | 32 |

| | | |
|-----------------------|--|----|
| 3.4 | Power analysis of distilled water versus injured egg predation cue..... | 36 |
| 3.5 | Experiment Four: Hatching time and hatchling phenotype of fathead minnows exposed to crayfish predation cues during embryonic development..... | 38 |
| Chapter 4: | Discussion..... | 45 |
| Literature Cited..... | | 55 |

List of Figures

| | | |
|-------------|---|----|
| Figure 3.1 | Percent of fathead minnow embryos that hatched during the night when exposed to different predation cues during embryonic development in experiment one..... | 24 |
| Figure 3.2 | Median (\pm quartiles) hatch time (hrs) of fathead minnows exposed to different predation cues during embryonic development in experiment one..... | 25 |
| Figure 3.3 | Cumulative proportion hatch distributions of fathead minnows exposed to different predation cues for experiment one..... | 26 |
| Figure 3.4 | Mean (\pm SE) total length (mm) of fathead minnow hatchlings exposed to different predation cues during embryonic development in experiment one..... | 27 |
| Figure 3.5 | Percent of fathead minnow embryos that survived to hatch when exposed to different predation cues during embryonic development in experiment one..... | 28 |
| Figure 3.6 | Percent of fathead minnow embryos that hatched during the night when exposed to different concentrations of predation cue during embryonic development in experiment two..... | 29 |
| Figure 3.7 | Median (\pm quartiles) hatch time (hrs) of fathead minnows exposed to different concentration predation cues during embryonic development in experiment two..... | 30 |
| Figure 3.8 | Cumulative proportion hatch distributions of fathead minnows exposed to different concentrations of predation cues for experiment two..... | 31 |
| Figure 3.9 | Mean (\pm SE) total length (mm) of fathead minnow hatchlings exposed to different concentrations of predation cue during embryonic development in experiment two..... | 32 |
| Figure 3.10 | Percent of fathead minnow embryos that hatched during the night when exposed to different predation cue during embryonic development in experiment three..... | 33 |
| Figure 3.11 | Median (\pm quartiles) hatch time (hrs) of fathead minnows exposed to different predation cues | |

| | | |
|-------------|---|----|
| | during embryonic development in experiment three..... | 34 |
| Figure 3.12 | Cumulative percent hatch distributions of fathead minnows exposed to different predation cues during embryonic development in experiment three..... | 35 |
| Figure 3.13 | Mean (\pm SE) total length (mm) of fathead minnow hatchlings exposed to different predation cues during embryonic development in experiment three..... | 36 |
| Figure 3.14 | Percent of fathead minnow embryos that hatched during the night when exposed to different crayfish predation cues during embryonic development in experiment four..... | 39 |
| Figure 3.15 | Median (\pm quartiles) hatch time (hrs) of fathead minnows exposed to different crayfish predation cues during embryonic development in experiment four..... | 40 |
| Figure 3.16 | Cumulative percent hatch distributions of fathead minnows exposed to different crayfish predation cues during embryonic development in experiment four..... | 41 |
| Figure 3.17 | Mean (\pm SE) total length (mm) of fathead minnow hatchlings exposed to different crayfish predation cues during embryonic development in experiment four..... | 42 |
| Figure 3.18 | Total length (mm) of hatchling versus hatching time for fathead minnows exposed to different crayfish predation cues during embryonic development in experiment four..... | 44 |

Chapter 1: Introduction

1.1 Background Information

Ecology is the study of the interrelationships between/among organisms and all aspects (living and nonliving) of their environments. An important interrelationship category that is prevalent in shaping the lives of all organisms is that of predation. One can look at the concept of a food web from two perspectives, either as representing the challenge of securing food or as attempting to avoid becoming it. The co-evolution of predator and prey was described first as an evolutionary arms race by Edmunds (1974) in his book Defence in Animals. This concept is based on the existence of selection pressure on the prey to perfect their anti-predator defenses and on the predator to overcome these defences. The existence of these selective pressures in both groups is very apparent. Predators have evolved an array of very effective methods of capturing their particular prey. An interesting example is that of the female bolas spiders (*Mastophora hutchinsoni*), which mimics the female sex pheromone of two specific moth species (*Lacinipolia renigera* and *Tetanolita mynesalis*) in order to attract male moths. Once the prey is within range and has been detected by its wing vibrations the female spider constructs a bolas (sticky globule at the end of a silk thread), which she uses to capture her meal (Haynes et al. 2001).

The pressure to obtain food is fundamental in the predator's life. In turn predation has the same capacity to influence prey directly through modulating the density and size structure of the population (Brönmark et al. 1995) and/or indirectly by altering the population by causing changes in growth, survival and fecundity rates (Fraser and Gilliam 1992). As a result, prey species have evolved a number of defense mechanisms to minimize their risk of being detected, caught and/or consumed by predators (Sih 1987, Lima and Dill 1990, Chivers and Smith 1998, Kats and Dill 1998). An example of a predator specific anti-predator defense is that of increasing tetrodotoxin toxicity in populations of newts (*Taricha granulosa*) that coexist with more resistant snake (*Thamnophis sirtalis*) populations (Hanifin et al. 1999). Any adaptation which reduces the chances of a successful attack by a predator on a prey item can be defined as a defensive adaptation or mechanism. In general prey species have a variety of predators and most predators can eat a variety of prey species, so the defensive adaptations of animals are usually directed at several different predators. There is an optimal ability of anti-predator defense expression for each response option, where predation avoidance is high, but other parameters such as health or reproduction are not greatly compromised. In other words, fitness is maximized. This translates into a higher proportion of the next generation obtaining this optimal anti-predator ability. Areas in which anti-predator

adaptation ability have been demonstrated to occur are behaviour, morphology and life history (Chivers and Smith 1998, Chivers and Mirza 2001).

Evolutionary pressure on prey to survive has resulted in organisms having at their disposal an entire defensive repertoire with different components concerned with predator detection, predator avoidance and predator escape. Fathead minnows (*Pimephales promelas*), for example, are a well studied prey species and have been demonstrated to have a diverse defense repertoire. They are a species of focus because they possess a damage released alarm substance called “Schreckstoff” (Smith 1992). This alarm substance is released from epidermal club cells when mechanical damage occurs to the skin. Exposure to conspecific alarm substance is a general indication of predation to other individuals in the vicinity and results in a typical anti-predator response in fathead minnows. This “typical response” could mean an increase in shelter use, an increase in shoaling behaviour, a decrease in activity, freezing, dashing and/or area avoidance (Mathis and Smith 1993b). Which component of the repertoire is utilized when, is context dependent (Chivers et al. 1995). One response variable may be more effective over another depending on which predator has been encountered. Furthermore, there could be other factors determining the effectiveness of each response variable. For example, it is logical to suggest that freezing would be a more effective mechanism when the environment is more conducive to crypsis or if visibility is reduced (Houtman and

Dill 1995). Dashing (irratc movements), however, may be the preferred mechanism when visibility is high. Being able to perceive, recognize and respond accordingly to alarm cues has been demonstrated to increase the survival time of fathead minnows during encounters with one of their biologically relevant predators, the northern pike (*Esox lucius*) (Mathis and Smith 1993b).

The benefit of anti-predator defense, although not specifically defined, intuitively is a reduction in the probability of mortality. The cost of anti-predator defense is just as difficult to pin down, but there is an apparent energy and time budget trade-off. The time and energy allocated to anti-predator defense could have been funneled into other necessary activities, such as foraging and reproduction. If accurate predation threat assessment is possible prey could greatly reduce the cost of their anti-predator response repertoires and take steps to maximize their fitness. Individuals would be less likely to respond inappropriately, by either responding when there is no need or by responding more intensely than necessary, thus wasting their limited resources. There should then exist a strong selection pressure for the accurate assessment of predation, as predation often varies temporally and spatially. Predation risk can vary seasonally, diurnally and from moment to moment. If information from the environment can be used to reliably determine the present risk level there is the possibility for response mediation. Furthermore, the ability to accurately assess the risk of predation would be beneficial as each anti-predator

defense has its own innate cost to the user and the effectiveness of each response option is dependent on the context of the encounter and the specific predator. This mediation of response to match the threat perceived is called threat-sensitive predator avoidance (Helfman 1989).

Fathead minnows have been shown to exhibit threat-sensitive predator avoidance as they respond more intensely to the odour of pike as the concentration was increased. Moreover, it was demonstrated that the subjects responded more intensely overall to the scent of small pike in comparison to the scent of large pike (Kusch et al. 2004). The minnows perceived the smaller predator cue as a greater threat than the larger predator cue, even though the concentration of the small pike odour would have been lower. This cue discrimination would suggest that small pike and large pike must smell different. Small pike in the system examined are likely a greater threat than large pike to the minnows due to an increase in encounter rate (small pike are more often found in the shallows cohabitating with schools of minnow) and due to a smaller size ratio between prey and predator.

Anti-predator adaptations are often mediated or induced by chemical cues (Kats & Dill 1998), especially in aquatic systems where visual cues are limited (Smith 1992). Chemical cues may provide more reliable information under circumstances when other cues are hindered, such as at night, in a highly complex habitat, in turbid water or when visual or other sensory systems are under

developed. Chemical cues function well in an aquatic medium as a large number of compounds can dissolve in water allowing for the production of a great number of possible signals (Hara 1994). Another possible advantage is the potential for cue persistence due to a slower transmission rate in this medium, which may make detection easier (Kleerekoper 1969). Persistence could possibly translate into a disadvantage, however, due to a lack of temporal information being conveyed in the cue. In other words, when a predator has left the area there are no visual cues left to indicate its presence, but there may still be chemical cues present after the fish leaves the area. Research, in the past decade, has indicated that the assessment of these chemical cues has been underestimated in the past, and is in fact highly sophisticated (reviews Chivers & Mirza 2001). Behavioural responses by fathead minnows to the predation threat simulated by chemical cues have been well studied and teased apart, whereas morphological and life history adaptation responses have not. Inducible defenses, such as morphological and life history switch point alteration, are believed to be adaptive responses to spatially and temporally variable predation risk (Van Buskirk and Relyea 1998). The fact that these adaptations are highly effective maintains them in a population, the fact that they are costly ensure that they are only expressed when sufficient predation pressure is present.

The ecological theory of life history trait adaptation dictates that the timing of the transition between one life stage and the next will vary with the costs and benefits associated with each stage (Werner 1986). If the mortality to growth rate ratio is lower in the succeeding stage early transition will be exhibited and if it is higher then delayed transition should occur (Chivers et al. 2001). For example, when raised in the presence of chemical alarm cues from injured conspecifics, western toad (*Bufo boreas*) tadpoles metamorphose earlier than those raised in the absence of such predation cues (Chivers et al. 1999). In that study tadpoles were raised in the presence of either predatory backswimmers (*Notonecta* spp) fed tadpoles, nonpredatory water boatman (Corixidae) or conspecific chemical alarm cue. Those embryos reared in the presence of backswimmers fed conspecifics or injured conspecific cue alone metamorphosed in significantly shorter time than those raised in the control treatment. Reducing the time spent as a tadpole would translate into a decrease in one's exposure time to aquatic predators.

Several other studies have shown that many species of amphibians possess the ability to facultatively adjust the timing of life history switch points (Moore et al. 1996, Laurila et al. 1998, 2002, Chivers et al. 1999, 2001, Warkentin 1995, Warkentin 2000, Warkentin et al. 2001, Kiesecker et al. 2002, Altwegg 2002, Johnson et al. 2003). For example, Sih and Moore (1993) showed that simulated larval predation threat, both flatworm (*Phagocotus gracilis*) and flatworm chemical

cue exposure, induced delayed hatching in salamander (*Ambystoma barbouri*) embryos when compared to embryos exposed to a control of adult isopods (*Lirceus fontinalis*). Embryos that hatched later resulted in heavier, longer and more developed hatchlings. Phenotype alteration and life history adaptation are frequently demonstrated together. In the case of early hatching this association is often perceived as an indication of a cost-benefit trade-off. Warkentin (1995) demonstrated that red-eyed treefrog (*Agalychnis callidryas*) embryos will exhibit induced hatching in response to the physical presence of a cat-eyed snake (*Leptodeira septentrionalis*). Live predation trials showed that embryos that hatched at a less developed stage to escape the terrestrial predator were more vulnerable to aquatic predators that they would likely encounter in the next life stage (i.e. shrimp). This study indicates that phenotypic change, in this case, is not an anti-predator response in itself, but a side effect of a shortened developmental period, which is the exhibited anti-predator response. Furthermore, this side effect is costly in the next life stage.

Phenotypic and life history responses, however, are not always coupled. Laurila et al. (2001) showed that common frog (*Rana temporaria*) embryos did not alter hatching time in response to the presence of a predatory diving beetle (*Dytiscus marginalis*), but that the hatchlings in the predator treatment displayed an altered phenotype. The hatchling group had shorter body lengths and deeper tail fins

relative to those in the control group. In this incident we would have a clear case where phenotypic anti-predator alteration was the target response. Several studies have shown that prey have the ability to alter their phenotypes in response to predation threat (Brönmark and Miner 1992, Trussel 1996, Reimer and Tedengren 1996, Weber and Declerck 1997, Van-Buskirk and Schmidt 2000,). These responses appear to be highly predator-specific. Brönmark and Miner (1992) conducted laboratory experiments to quantify body morphology in crucian carp (*Carassius carassius*) as a function of both food level and predation (northern pike, *Esox Lucius*) presence. Carp that were in the high food (no predator) treatment were marginally deeper in the body than those in the low food (no predator) treatment. The presence of predators, however, caused a significant increase in body depth when compared to both predator absent treatments (low and high food). The biologically significant predator chosen was gape-limited. Such phenotypic variation has important ecological consequences as these changes alter the interactions among the species present in the community. In this case, the body depth change results in a size refuge, i.e. reduced predation threat on those individuals with the phenotypic alteration. The change has additional indirect consequences, the increase in body depth also appears to result in a reduced swimming ability and escape speed. This hindrance may have other costly side effects such as reducing foraging ability or the ability to avoid other ecologically

significant predators. These costs could be the driving force behind why the gape-limited predator-induced phenotype is only expressed when appropriate. In another study, Van-Buskirk and Schmidt (2000) investigated the actual benefits of such phenotypical adaptations. When exposed to caged predators (*Aeshna*, dragonfly larvae) two species of larval newts (*Triturus alpestris* and *T. helveticus*) developed darker tail fin pigmentation, larger heads, larger tails and spent more time hiding in the leaf litter in comparison with newts in predator-free ponds. The individuals with the predator-induced phenotype survived significantly longer during survival trials, when exposed to free dragonfly larvae, than those without the altered phenotype (Van-Buskirk and Schmidt 2000). This phenotypic alteration obviously is effective against this particular predator.

In the determination of adaptive life history and phenotypic responses studies have demonstrated a high degree of context-dependency and species-cue specificity. Chivers et al. (2001) demonstrated that Pacific treefrog (*Hyla regilla*) embryos hatched earlier and at a less developed stage when exposed to predatory leeches (*Desserobdella picta*), cues from predatory leeches (*Desserobdella picta*) or injured egg extract. Cascade frogs (*Rana cascadae*) embryos also hatched earlier and at a less developed stage when exposed to predatory leeches or predatory leech cues, but not injured egg extract alone. Furthermore, Laurila et al. (2002) illustrated that common frog (*Rana temporaria*) embryos delay hatching in the presence (not

direct contact) of stickleback (*Gasterosteus aculeatus*) (a tadpole predator), but did not adjust their hatching times in the presence of leeches (*Haemopsis sanguisuga*) (an egg predator) or dragonfly larvae (*Aeshna* sp.) (a tadpole predator). In all three predatory treatments the hatchlings did develop shorter tails. The same study demonstrated that moor frog (*Rana arvalis*) embryos do, however, hatch earlier and at a less developmental stage when exposed to any of the three predatory treatments. Moreover, Li (2002) showed that the cue necessary to produce a response does not necessary have to be elicited by the predator directly. Egg-carrying spitting spider (*Scytodes pallida*) embryos hatch earlier due to the presence (not direct contract) of predatory jumping spiders (*Portia labiata*). The induced hatching, in this system, seems to be initiated by the mother spitting spider's response to the predator's presence, as unattended eggs do not respond in predation treatments. It appears that these anti-predator abilities are highly context dependent and seem to exist in several taxa. The ability of predator-induced life history adaptation, however, has not been demonstrated in fish. The ability may be predicted, however, as whitefish (*Coregonus* sp.) embryos were demonstrated to hatch earlier when exposed to chemical cues from eggs infected with bacteria (*Pseudomonas fluorescens*) (Wedekind 2002).

1.2 Objective

The early life stages are often the most vulnerable to predation, therefore, there should exist strong selection pressure for the development of defense mechanisms specifically for this vulnerable stage. Little research has been conducted on the possible defense mechanisms employed by the embryos themselves and how these adaptations are environmentally triggered. As outlined above, Chivers et al. (2001) showed that cascade frog (*Rana cascadae*) embryos hatch sooner and at a less developed stage when exposed to predator leeches and chemical cues from predatory leeches. An alteration was not elicited, however, in this species by injured egg extract alone. In my studies predation was simulated by exposing fathead minnow embryos to injured egg extract alone, predator cue alone or predator odour combined with injured egg extract. Treatments were devised based on research investigating diet-dependent anti-predator responses (Mathis and Smitha 1993, Chivers and Mirza 2001).

My objective was to examine the influence of predation cue exposure on a fish prey species' phenotype and life history. Specifically, what is the effect of injured egg extract and/or crayfish odour exposure during embryonic development on the incubation period and/or fry phenotype of fathead minnows. I hypothesized in the first experiment that fathead minnow embryos exposed to injured egg cues would hatch sooner and at a less developed stage than those embryos exposed to

either an injured shrimp or distilled water control. The injured shrimp control was chosen to represent a generalized response to damaged tissue from an organism in a distant taxonomic group. The injured egg extract was chosen to represent a general indication of predation on conspecifics in the same life stage as the test subjects. In the second experiment, where the concentration of the injured egg cue was addressed, it was hypothesized that there would be a threshold concentration and possibly a graded response among the concentrations above threshold. In other words, threat-sensitive predator avoidance may be a possibility if concentration differences are a reliable indication of predation threat variation. The third experiment was conducted to replicate experiment one later on in the season. In the fourth experiment, it was postulated that embryos exposed to injured egg cues in combination with predator cues would induce hatching when compared to embryos exposed to either predator cues alone or a treated tap water control. The blank control was switched from distilled water to treated tap water in the last experiment, as it was suggested to be more appropriate and healthier for the developing embryos (water chemistry, hardness, etc.). If predator recognition, of the specific predator used, is innate it is expected that the predator cue alone would induce hatching in comparison to the treated tap water control. Choosing a biological relevant predator is fundamental to this experimental design. Research indicates that crayfish (*Orconectes virilis*) are significant egg predators on this species. Matity et al.

(1994) found that breeding male fathead minnows had significantly more scars from crayfish pinches than non-breeding males or females. This was suggested to be due to their involvement in nest defense. Furthermore, crayfish do coexist with the minnow population chosen to be tested.

1.3 Significance of Thesis Work

Egg predation is common, but relatively little research has been conducted on the possible defense mechanisms employed by the embryos themselves and how these adaptations are environmentally triggered. My research attempts to answer some of these questions and to determine whether chemical cues of predation alone are sufficient to elicit a morphological and/or life history response in a fish species.

Chapter 2: Methods

2.1 Basic Experimental Protocol

My experimental protocol consisted of placing individual eggs in separate plastic containers, exposing them to various predation risk cues and following their development through to hatching in order to identify differences in hatching time and hatchling phenotype. Adult fathead minnows in breeding conditions were collected using minnow traps from Briarwood Lake, Saskatoon, Saskatchewan. Briarwood Lake is a man made water body that is approximately 1 hectare in size and, in addition to containing fathead minnows and goldfish (*Carassius auratus*), it contains crayfish (*Orconectes virilis*). Collections were conducted during the summer of 2002 and 2003 and immediately after being brought into the lab fish were artificially spawned. All fish were sacrificed following our animal care protocol by applying a single blow to the head. The number of males and females differed among the experiments, see the specifics experiments for these details. The eggs were removed from the females and placed into a 9x9x2 cm glass container. The testes from the males were then placed in close proximity to the eggs. Room temperature tap water, treated with Aqua Plus (Nutrafin) to remove chlorine and neutralize heavy metals, was added, at which point the testes were shaken to release the sperm. Sperm movement was facilitated by the light agitation of the water with

a feather. After fifteen minutes the fertilization success was assessed by visually examining the embryos for any clouding of the membrane or white spots. If a sufficient number of eggs were fertilized a weak solution of tannic acid in treated tap water (200mg/l) was used to separate the eggs. The embryos were then rinsed and the embryos left to water-harden in room temperature treated tap water for two hours.

Viable eggs were then placed individually into 500 ml plastic cups filled with 250 ml of room temperature treated tap water. The cups were randomly assigned to a treatment category and placed throughout the rearing room prior to fertilization. The photoperiod and light regime were consistent throughout the duration of each experiment. Eggs were treated twice a day and monitored every four hours, except for a six hour period when the lights were turned off. Monitoring occurred at 6:00am, 10:00am, 2:00pm, 6:00pm and 10:00pm, while treating occurred at 8:00am and 4:00pm. All treatments were an equivalent volume of 5 ml to control for disturbance and each batch of stimulus was made just prior to administration using a polytron. Treatments were slowly injected against the side of the cup using a 5 ml pipette. The experiment ended after all eggs had either died or hatched. Hatchlings found during a monitoring session were removed using a pipette, euthanized using a lethal dose of ethyl-m-aminobenzoate methanesulfonate salt (MS222) and stored in a glass vial of 10% buffered formalin for morphological

analysis (total length measurements). For experiments one, two and three the technician was blind to the hatchling treatment when measuring total body length, in attempts to reduce the possibility of a bias.

2.2 Experiment One: Hatching time and hatchling phenotype of fathead minnows exposed to different predation cues during embryonic development (in late spring)

Experiment one was designed to investigate whether minnows alter their hatching time when exposed to predation risk cues. The first collection of minnows occurred in the middle of June, 2002. Fifteen couples were fertilized separately, the standard lengths (mm) of the parents were recorded and each batch of eggs had its own fertilization time. The rearing room temperature was controlled at 25°C and the light cycle was 18:6 hr L:D. There were three treatment conditions: (1) a predation cue of injured egg (N=52), (2) a control of injured shrimp (previously frozen brine shrimp (*Artemia* sp.) (N=53) and (3) a control of distilled water (N=61). The injured egg cue was prepared fresh for each use by homogenizing 450 eggs at the same developmental stage as the test subjects in 1125 ml of distilled water. Each treatment was equivalent to an exposure to two injured eggs (2eggs/5ml), therefore, the subjects experienced cues from four injured eggs a day. The shrimp cue was kept as consistent as possible to the egg cue by homogenizing

an equivalent mass of shrimp (450 eggs weighed 0.236 g) in the same volume of water.

2.3 Experiment Two: Hatching time and hatchling phenotype of fathead minnows exposed to different concentrations of predation cue during embryonic development

The objective of experiment two was to investigate the role of concentration in the mediation of a response and to determine whether or not the inability of experiment one to elicit a hatch time alteration was due to an insufficient cue concentration. The setup and protocol were the same, except 6 males and 7 females were artificially spawned together (at one time) and only 125 ml of water was added to the treatment containers (instead of 250 ml) allowing for the production of high injured egg concentrations later in the season when fewer eggs were available to make stimulus. Minnows for the second experiment were collected in early July, 2002. The four treatment groups were (1) a predation cue twice the concentration of first experiment (4eggs/treatment) (N=149), (2) a predation cue half the concentration of experiment one (1egg/treatment) (N=124), (3) a predation cue equivalent to that in experiment one (2 eggs/treatment) (N=132) and (4) a control of distilled water (N=138).

Cue concentrations were chosen primarily based on previous publications and logistic limitations. Chivers et al. (1999) and Kiesecker et al. (2002) were able to demonstrate induced metamorphosis by western toads (*Bufo boreas*) and red-legged frogs (*Rana auror*), respectively, in response to conspecific alarm cues. The concentration used in their experiment was 0.17 tadpoles homogenized in 10 ml of distilled water three times a week. Chivers et al. (2001) were able to demonstrate induced hatching by Cascade frogs (*Rana cascadae*) in response to injured conspecific eggs, the concentration used was three injured eggs twice a day. In my experiment the large sample size of 150 eggs per treatment made it logistically impossible to create such high treatment concentrations. In order to produce the final concentrations chosen 1100 eggs had to be sacrificed every day for stimulus. The biological significance of these concentrations is unclear, but we may be able to speculate that due to the voracious nature of the predator chosen cues from distant predation events may be more relevant in the mediation of hatch time alterations than if the predator is foraging on the subject in question's clutch.

2.4 Experiment Three: Hatching time and hatchling phenotype of fathead minnows exposed to different predation cues during embryonic development (in late summer)

The objective of experiment three was to repeat experiment one later in the breeding season. I hypothesized that eggs laid later in the season may be less flexible in displaying a phenotypic alteration than those laid early in the season. The setup and protocol were the same as that for experiment two. Minnows were collected at the end of July, 2002. There were three treatment conditions (1) a predation cue of injured egg equivalent to that in experiment one (N=60), (2) a control of injured shrimp (N=63) and (3) a control of distilled water (N=53).

2.5 Experiment Four: Hatching time and hatchling phenotype of fathead minnows exposed to crayfish predation cues during embryonic development

The objective of experiment four was to determine the effect of predator cue exposure during the embryonic stage on hatching time and hatchling phenotype of fathead minnows. Minnows in breeding condition were collected from Briarwood Lake in Saskatchewan on July 3, 2003. The setup and protocol were the same as that for experiments two and three except that 10 males and 15 females were artificially spawned and the rearing room temperature was 23°C. There were three

treatment conditions: (1) crayfish fed minnow eggs combined with injured egg extract (N=85), (2) crayfish fed plant (Tape grass, *Vallisneria americana*) combined with plant extract and (N=76) (3) a treated tap water control (N=82). The crayfish fed egg cue was prepared by housing three crayfish, on a diet of fathead minnow eggs, in a 37 l aquarium for one week. The crayfish fed egg cue was then combined with injured egg extract, in order to increase the stimulus strength. The injured egg cue was prepared fresh for each use by homogenizing 150 eggs in 375 ml of treated tap water, resulting in each subject experiencing (twice a day) the equivalence of a single predation event on one egg. The crayfish fed vegetation cue was prepared by housing three crayfish, on a diet of aquatic plants, in a 37 l aquaria for one week. To keep the concentration of the treatment as equal as possible 375 ml of tank water from the crayfish fed plant aquarium was added to 0.079 g (the weight of 150 eggs) of aquatic plant material homogenized in 375 ml of treated tap water.

2.6 Statistical Analysis

The hatching time data was analyzed using nonparametric tests, as it was not normal (all $P_s < 0.001$). To ensure that there was no difference in survival due to treatment Pearson chi-square tests were conducted. To examine hatching time preference (dark versus light) chi-square tests were used to analyze the binomial response within each treatment. In terms of life history alteration, the predation

treatment could influence the temporal pattern of hatching and/or the median hatch time. Consequently, I employed two techniques to analyze for treatment effects. Kolmogorov-Smirnov tests were conducted to determine if the hatching distributions differed among the treatments. Mann-Whitney U tests were conducted, as well, to determine if the median hatch times differed between the treatments. To analyze the morphological data, which was normal ($P_{\text{exp1}}=0.223$, $P_{\text{exp2}}=0.112$, $P_{\text{exp3}}=0.725$, $P_{\text{exp4}}=0.160$), one-way ANOVA analyses were conducted on the fry total lengths. If a significant difference was detected, Tukey tests were then conducted to determine which treatments were different from each other. A more in depth examination of the relationship between hatchling size and developmental period length was undertaken by conducting Pearson correlations on the morphology data from experiment four. The data from each experiment was analyzed separately.

Chapter 3: Results

3.1 Experiment One: Hatching time and hatchling phenotype of fathead minnows exposed to different predation cues during embryonic development (in late spring)

The survival of embryos was moderate, ranging from 52% to 61%. Pearson chi-square tests conducted on the survival data, however, showed that there was no mortality bias due to treatment (all $P > 0.199$). Moreover, regardless of treatment the great majority of embryos hatched during the night (between 12:00am and 6:00am) (all $P < 0.001$, fig. 3.1).

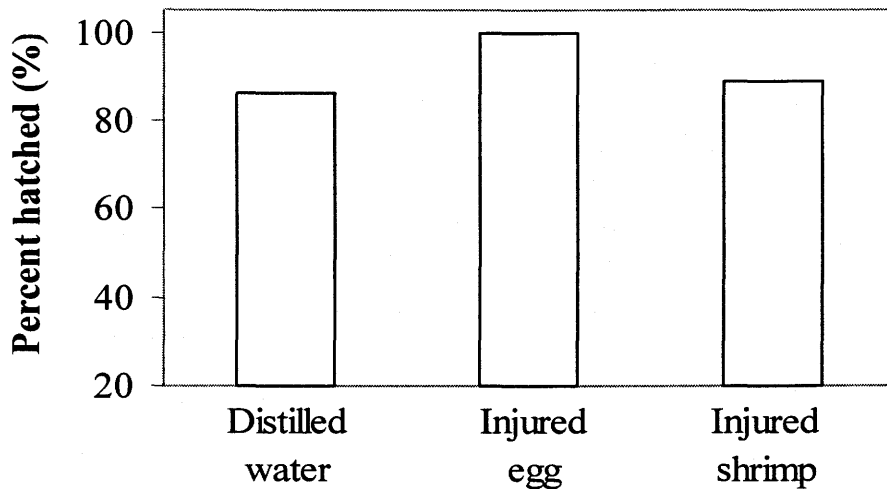


Figure 3.1 Percent of fathead minnow embryos that hatched during the night when exposed to different predation cues during embryonic development in experiment one. At least 86% of hatchlings, regardless of treatment, hatched during the night ($P>0.001$)

Mann-Whitney U tests conducted on the hatching time data revealed that embryos in the injured egg treatment hatched significantly sooner than those in the injured shrimp treatment ($P=0.023$, fig. 3.2). Embryos in the injured egg treatment did not, however, hatch sooner than those in the distilled water control ($P=0.099$, fig. 3.2). The median hatch time of the embryos in the injured shrimp treatment was not significantly different from those in the distilled water control ($P=0.355$, fig.

3.2) Kolmogorov-Smirnov tests conducted on the same data revealed that there were no significant differences between the three treatments in terms of hatching pattern (all $P > 0.113$, fig. 3.3).

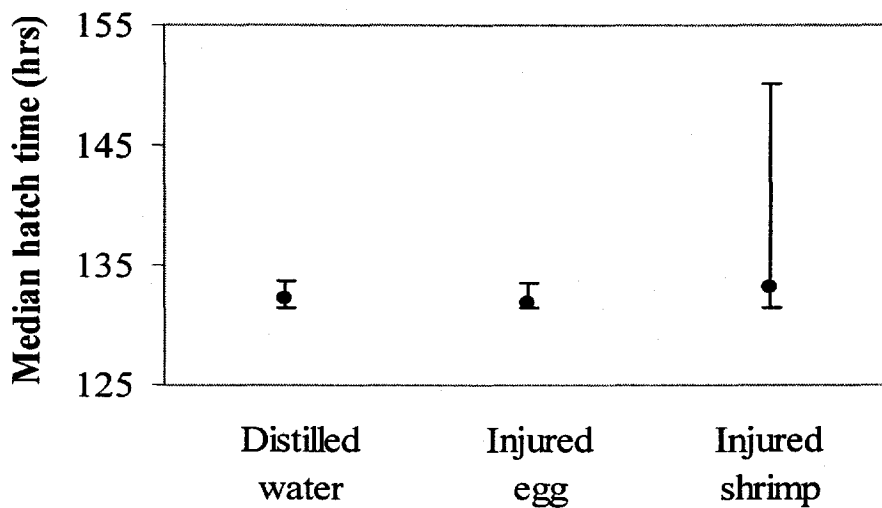


Figure 3.2 Median (\pm quartiles) hatch time (hrs) of fathead minnows exposed to different predation cues during embryonic development in experiment one.

Embryos in the injured egg treatment appear to hatch sooner than those in the injured shrimp treatment ($P=0.023$)

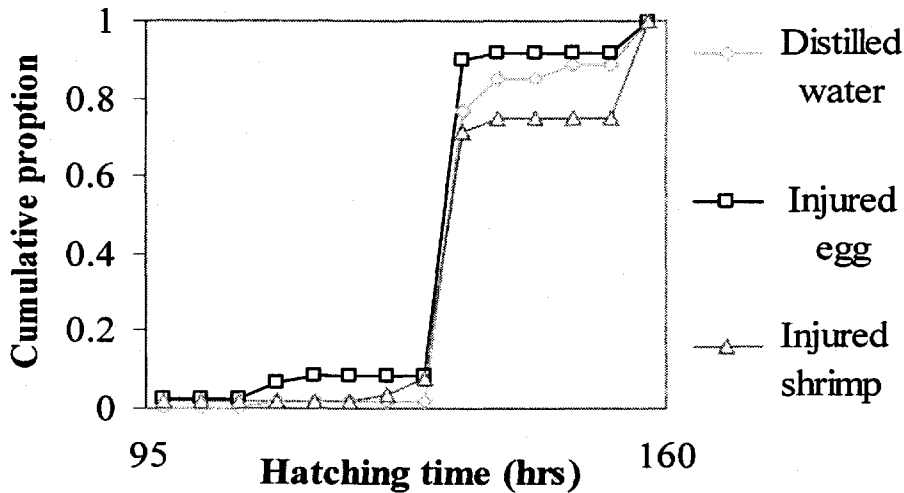


Figure 3.3 Cumulative proportion hatch distributions of fathead minnows exposed to different predation cues during embryonic development in experiment one. There was no effect due to treatment ($P>0.133$).

One-way ANOVA analysis on the phenotype data revealed that there was a significant difference among the three treatments ($P<0.001$). Tukey tests showed that hatchlings in the injured egg treatment were shorter than those reared in the injured shrimp treatment ($P=0.001$, fig. 3.4) and tended to be shorter than those in the distilled water group ($P=0.056$, fig. 3.4). Hatchlings in the distilled water treatment were the same length as those in the injured shrimp treatment ($P=0.94$, fig.3.4).

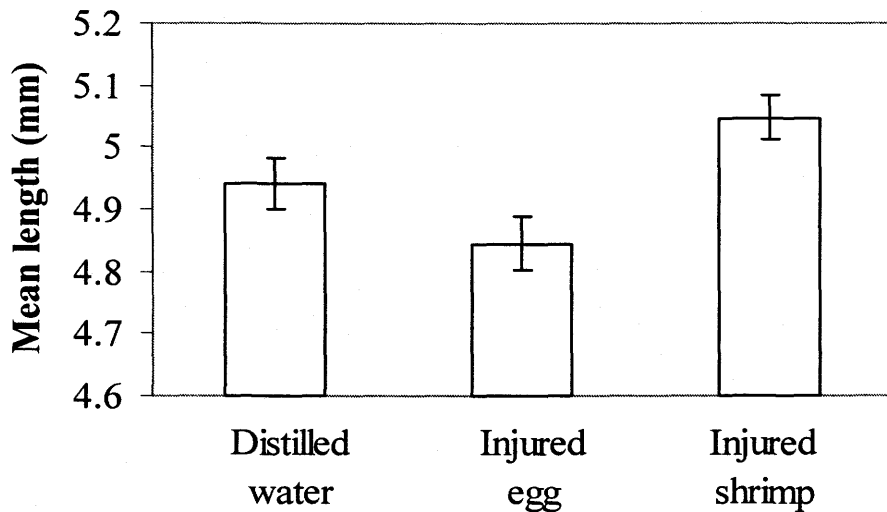


Figure 3.4 Mean (\pm SE) total length (mm) of fathead minnow hatchlings exposed to different predation cues during embryonic development in experiment one.

Hatchlings in the injured egg treatment has shorter total body lengths than those in the controls ($P > 0.056$).

3.2 Experiment Two: Hatching time and hatchling phenotype of fathead minnows exposed to different concentrations of predation cue during embryonic development

Survival of embryos was high, ranging from 83% to 99%. Pearson chi-square tests conducted on the survival data revealed that significantly more eggs survived in the high predation threat treatment in comparison to the low ($P < 0.001$),

medium ($P < 0.001$) and no predation ($P = 0.002$) threat treatments (fig. 3.5).

Significantly more embryos survived in the distilled water control than in the low predation threat treatment ($P = 0.015$, fig. 3.5). Survival in the medium predation threat treatment was the same as that in the low ($P = 0.192$) and no predation threat ($P = 0.248$) treatments. Regardless of treatment, a significantly higher proportion of the embryos hatched during the dark period (all $P < 0.001$, fig. 3.6).

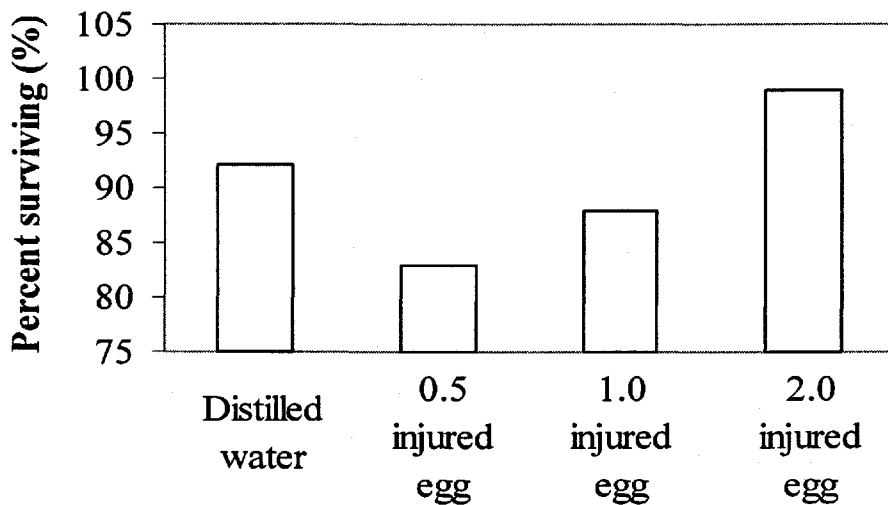


Figure 3.5 Percent of fathead minnow embryos that survived to hatch when exposed to different concentrations of predation cue during embryonic development in experiment two. Embryos in the injured egg treatment appear to have experienced less mortality than the other three treatments ($P < 0.002$).

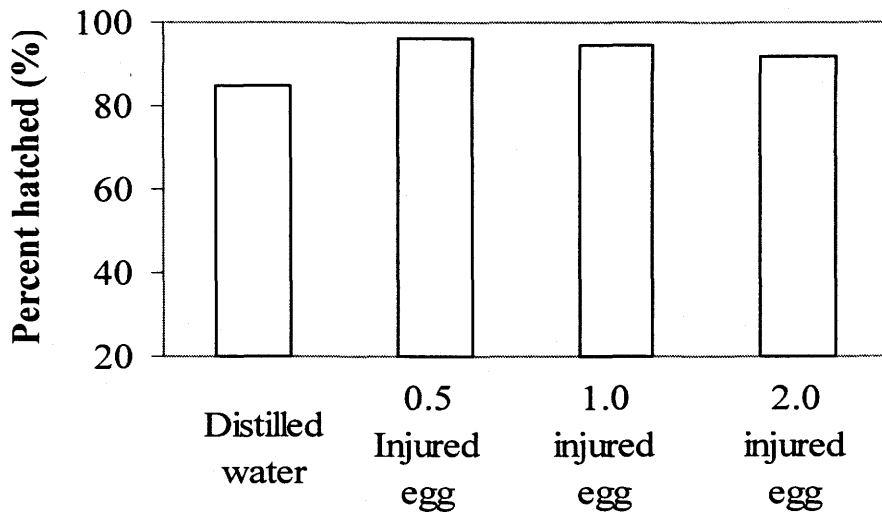


Figure 3.6 Percent of fathead minnow embryos that hatched during the night when exposed to different concentrations of predation cue during embryonic development in experiment two. At least 84% of the hatchlings, regardless of treatment, hatched during the night.

Mann-Whitney U tests showed that there were no significant differences among treatments in median hatch time, as the Bonferroni adjusted alpha value was 0.025 ($P > 0.083$, fig. 3.7). There was a trend, however, for the embryos in the high predation treatment to delay hatching when compared to the control of distilled water ($P = 0.030$). Kolmogorov-Smirnov tests conducted showed that there were no

significant differences due to treatment in temporal pattern of hatching (all $P > 0.122$, fig. 3.8).

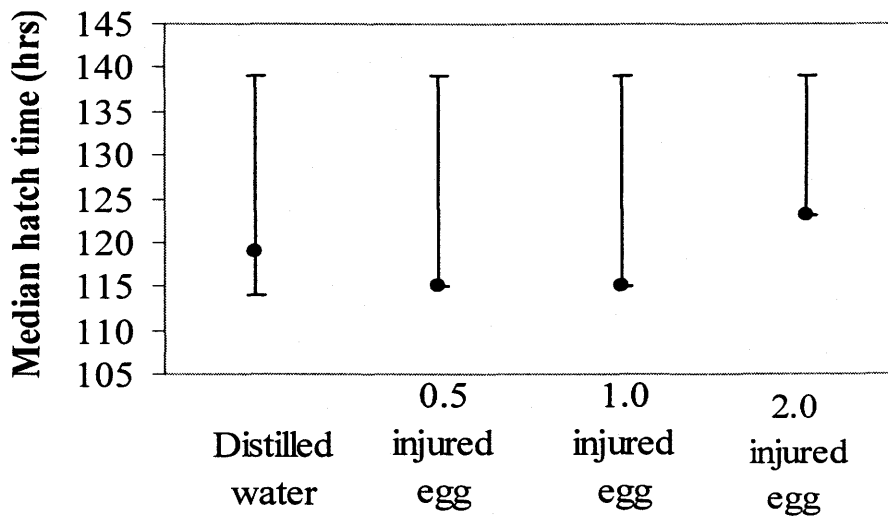


Figure 3.7 Median (\pm quartiles) hatch time (hrs) of fathead minnows exposed to different concentrations of predation cues during embryonic development in experiment two. There was no effect due to treatment.

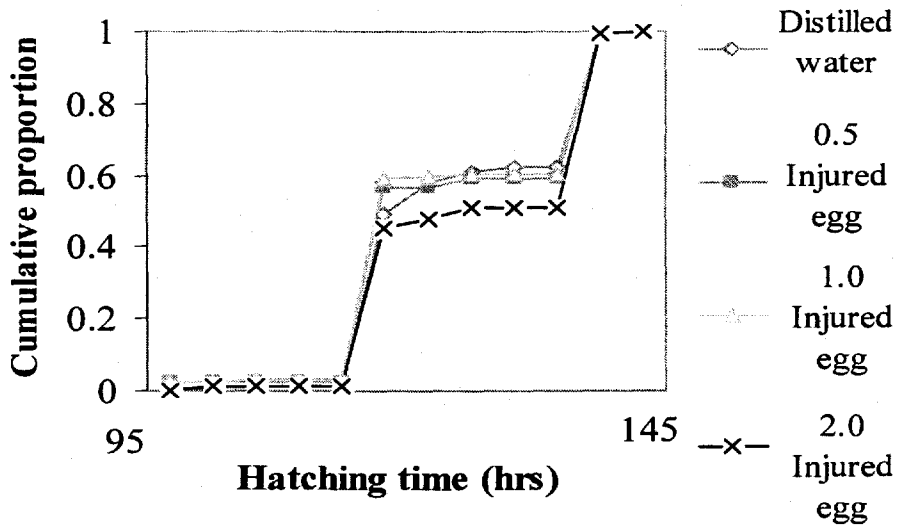


Figure 3.8 Cumulative proportion hatch distributions of fathead minnows exposed to different concentrations of predation cues during embryonic development in experiment two. There was no effect due to treatment.

One-way ANOVA analysis on the phenotype data revealed that there were no significant differences among the four treatments ($P=0.731$, fig 3.9).

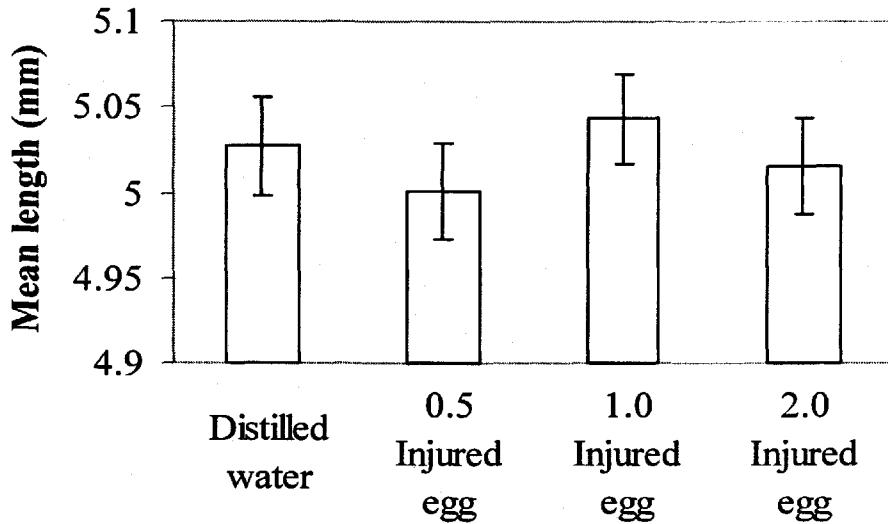


Figure 3.9 Mean (\pm SE) total length (mm) of fathead minnow hatchlings exposed to different concentrations of predation cue during embryonic development in experiment two. There was no effect due to treatment.

3.3 Experiment Three: Hatching time and hatchling phenotype of fathead minnows exposed to different predation cues during embryonic development (in late summer)

Embryo survival was high, ranging from 76% to 90%. Pearson chi-square tests on the survival data revealed that the treatments were statistically the same in terms of mortality ($P > 0.185$), except that embryos in the injured egg treatment tended to survive better than those in the injured shrimp treatment ($P = 0.051$). The

majority of embryos hatched during the night, regardless of treatment (all $P < 0.001$, fig. 3.10).

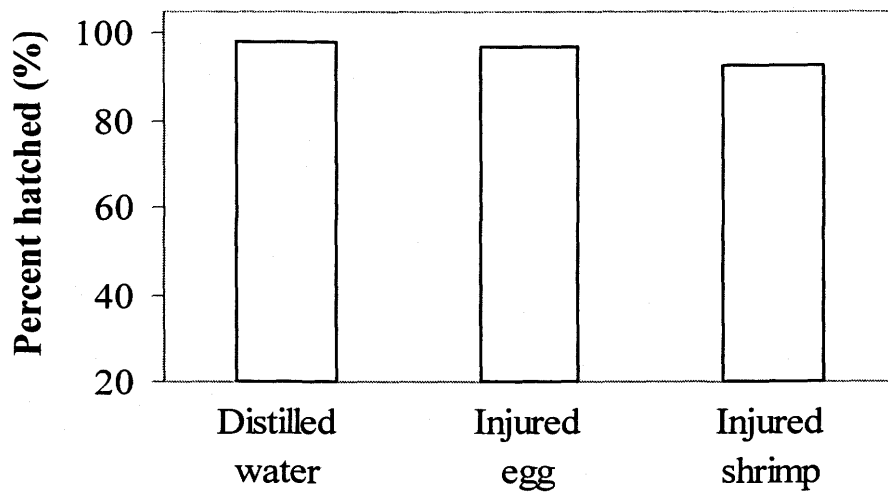


Figure 3.10 Percent of fathead minnow embryos that hatched during the night when exposed to different predation cues during embryonic development in experiment three. At least 92% of hatchlings, regardless of treatment, hatched during the night.

Both the Mann-Whitney U tests (all $P > 0.127$) and Kolmogorov-Smirnov tests (all $P > 0.560$) conducted on the hatching time data showed that there were no significant differences between the three treatments (fig. 3.11 and 3.12).

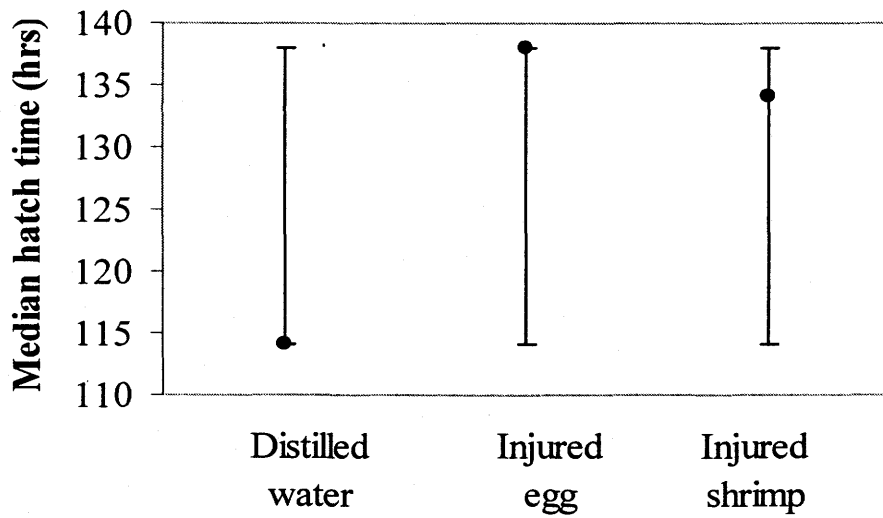


Figure 3.11 Median (\pm quartiles) hatch time (hrs) of fathead minnows exposed to different predation cues during embryonic development in experiment three. There was no effect due to treatment.

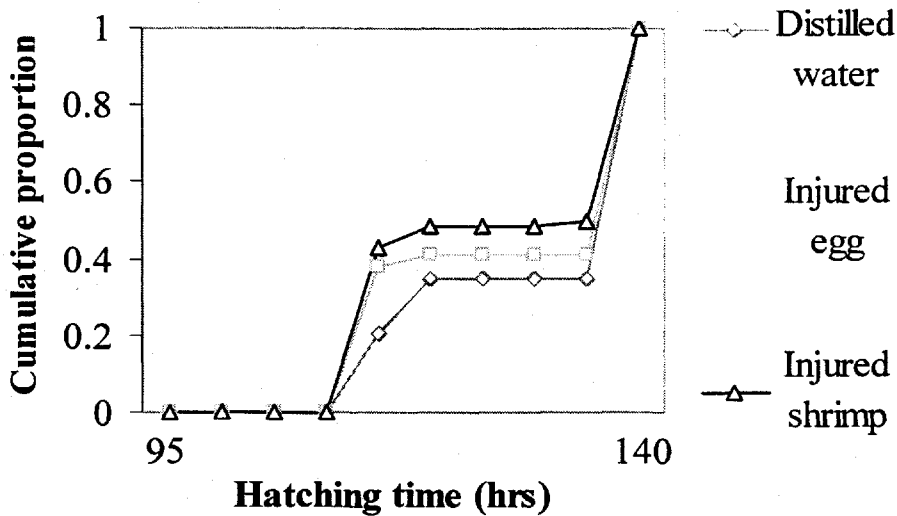


Figure 3.12 Cumulative proportion hatch distributions of fathead minnows exposed to different predation cues during embryonic development in experiment three.

There was no effect due to treatment.

One-way ANOVA analysis on the phenotype data revealed that there was a significant difference among the three treatments ($P=0.010$). Tukey tests conducted showed that hatchlings in the injured egg treatment were shorter than those reared in the injured shrimp treatment ($P=0.047$, fig. 3.13) and those in the distilled water group ($P=0.014$, fig. 3.13). The injured shrimp and distilled water treatments were statistically the same ($P=0.863$, fig. 3.13).

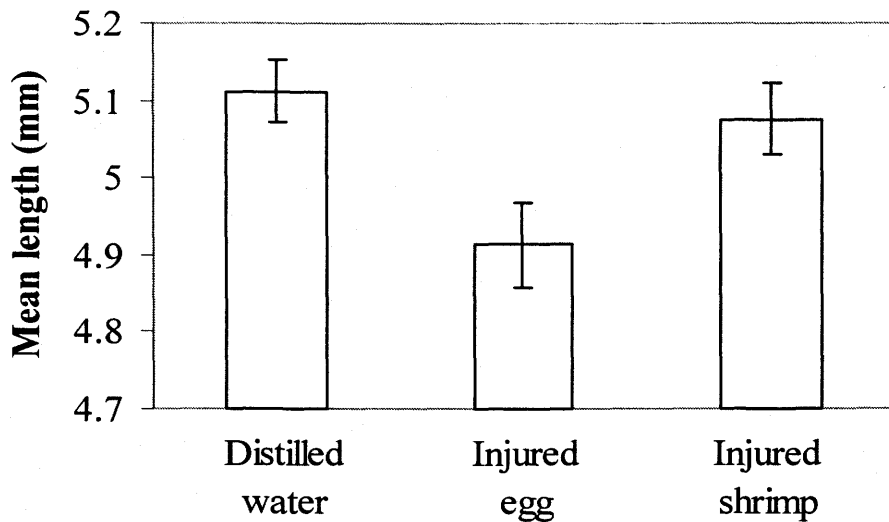


Figure 3.13 Mean (\pm SE) total length (mm) of fathead minnow hatchlings exposed to different predation cues during embryonic development in experiment three. Hatchlings in the injured egg treatment had significantly shorter total body lengths ($P < 0.047$)

3.4 Power analysis of distilled water versus injured egg predation cue

A retrospective power analysis (Thomas 1997) conducted on the comparisons between distilled water and injured egg for all three experiments revealed that with 95% confidence the null hypothesis (no effect) was true for an effect size of 5% or greater, i.e. all three experiments were powerful enough to detect a change of 5% or greater.

In experiment one a 5% effect size would be a difference of 6.76 hrs between the distilled water treatment (mean = 135.26 hrs) and the injured egg treatment. The observed effect was -3.4116 hrs. The 95% confidence interval for the observed effect of injured egg cue exposure was derived as $\pm (SE)(t) = (1.61)(2.01) = \pm 3.25 = -6.67$ to -0.17 . As the 5% effect size does not fall within the observed effect confidence interval we can say with 95% certainty that a 5% effect size or greater was not induced by the predation cue exposure.

In experiment two a 5% effect size would be a difference of 6.23 hrs between the distilled water treatment (mean = 124.54 hrs) and the injured egg treatment. The observed effect was -0.66 hrs. The 95% confidence interval for the observed effect of injured egg cue exposure was derived as $\pm (SE)(t) = (1.12)(1.98) = \pm 2.21 = -2.87$ to 1.55 . As the 5% effect size does not fall within the observed effect confidence interval we can say with 95% that a 5% effect size or greater was not induced by the predation cue exposure. In this experiment the minimum detectable effect size would have been 3% or greater.

In experiment three a 5% effect size would be a difference of 6.25 hrs between the distilled water treatment (mean = 124.94) and the injured egg treatment. The observed effect was 3.28 hrs. The 95% confidence interval for the observed effect of injured egg cue exposure was derived as $\pm (SE)(t) = (1.48)(2.00) = \pm 2.97 = 0.31$ to 4.83 . As the 5% effect size does not fall within the observed effect

confidence interval we can say with 95% that a 5% effect size or greater was not induced by the predation cue exposure.

3.5 Experiment Four: Hatching time and hatchling phenotype of fathead minnows exposed to crayfish predation cues during embryonic development

The survival of embryos was high, ranging from 76% to 85%. Pearson chi-square tests conducted on the survival data revealed that there was no mortality bias due to treatment (all $P < 0.108$). The strong preference for embryos to hatch during the dark in all three experiments conducted in 2002 was no longer evident in 2003. In the fourth experiment's treatment of predator cue alone as many embryos hatched during the light period as during the dark period ($P = 0.829$, fig. 3.14). Furthermore, a significantly higher proportion of hatchlings emerged during the light period than during the dark in both the crayfish fed injured egg treatment ($P = 0.006$, fig. 3.14) and the control of treated tap water ($P < 0.001$, fig. 3.14) treatments.

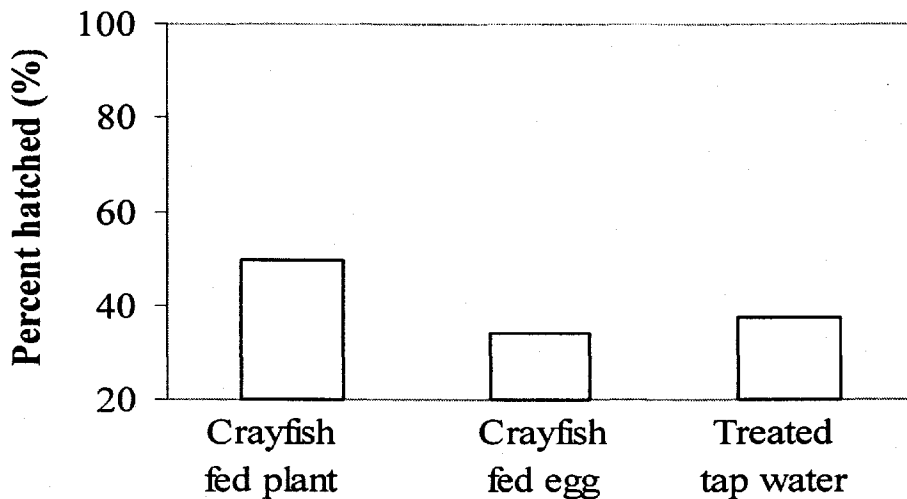


Figure 3.14 Percent of fathead minnow embryos that hatched during the night when exposed to different crayfish predation cues during embryonic development in experiment four. Significantly more embryos hatched during the day than during the night in the crayfish fed egg and treated tap water control ($P < 0.058$).

Mann-Whitney U tests conducted on the hatching time data revealed that embryos in the crayfish fed egg treatment hatched significantly sooner than those in the treated tap water treatment ($P = 0.043$, fig. 3.15). Moreover, there was the trend for embryos in the crayfish fed plant treatment to hatch earlier than those in the treated tap water treatment ($P = 0.058$, fig. 3.15). The median hatch times of embryos in the predator odour alone and the injured egg predator odour treatments

were not significantly different from each other ($P=0.887$). Kolmogorov-Smirnov tests conducted showed that there was also a significant difference between crayfish fed egg treatment and the treated tap water treatment ($P=0.048$), but not the crayfish odour alone treatment ($P=0.137$) in terms of hatching distributions (fig. 3.16).

Furthermore, the hatching pattern did not differ between the predator odour alone and the treated tap water treatments ($P=0.194$).

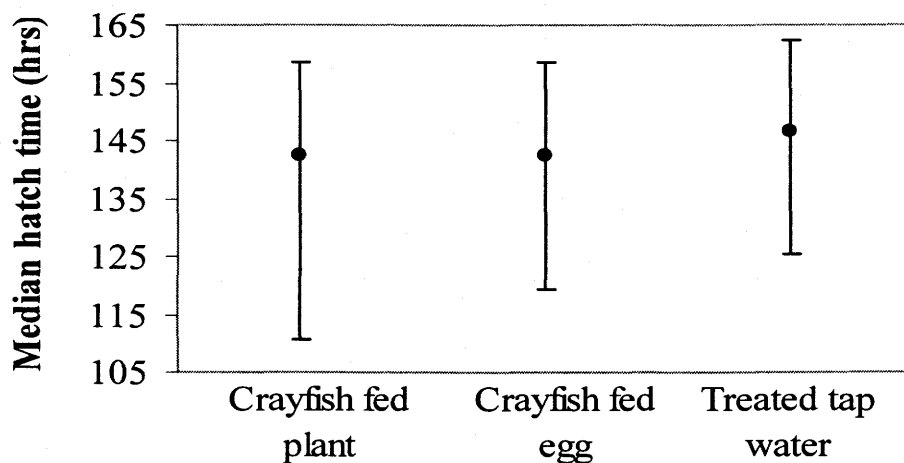


Figure 3.15 Median (\pm quartiles) hatch time (hrs) of fathead minnows exposed to different crayfish predation cues during embryonic development in experiment four.

Embryos in the crayfish fed egg treatment hatched sooner than those in the treated tap water control ($P=0.043$).

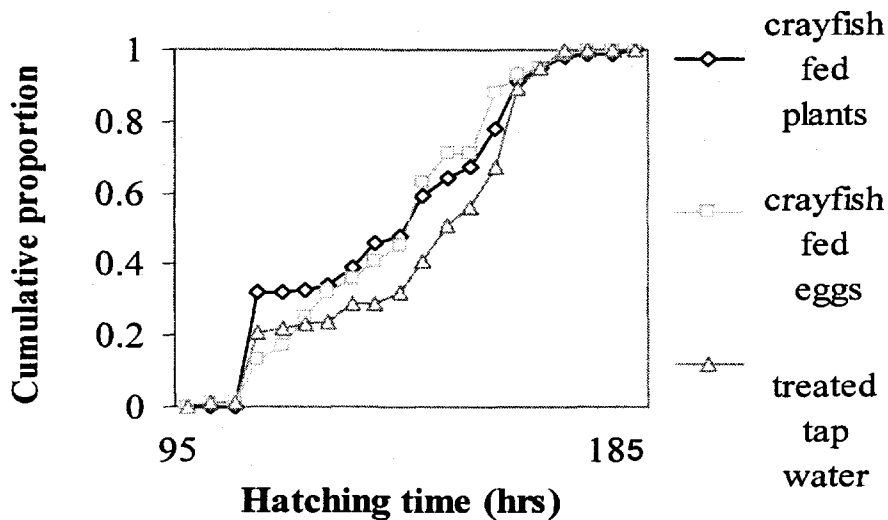


Figure 3.16 Cumulative proportion hatch distributions of fathead minnows exposed to different crayfish predation cues during embryonic development in experiment four. Embryos in the crayfish fed egg treatment hatched sooner than those in the treated tap water control ($P=0.048$).

One-way ANOVA analysis on the phenotype data revealed that there was a significant difference among the three treatments ($P<0.001$). Tukey tests revealed that hatchlings in the crayfish fed egg treatment were shorter than those reared in either the crayfish fed plant ($P=0.001$, fig. 3.17) and the treated tap water treatments ($P<0.001$, fig. 3.17). No difference was detected between the phenotypes of the

hatchlings from the crayfish odour alone and the control treatments ($P=0.987$, fig. 3.17).

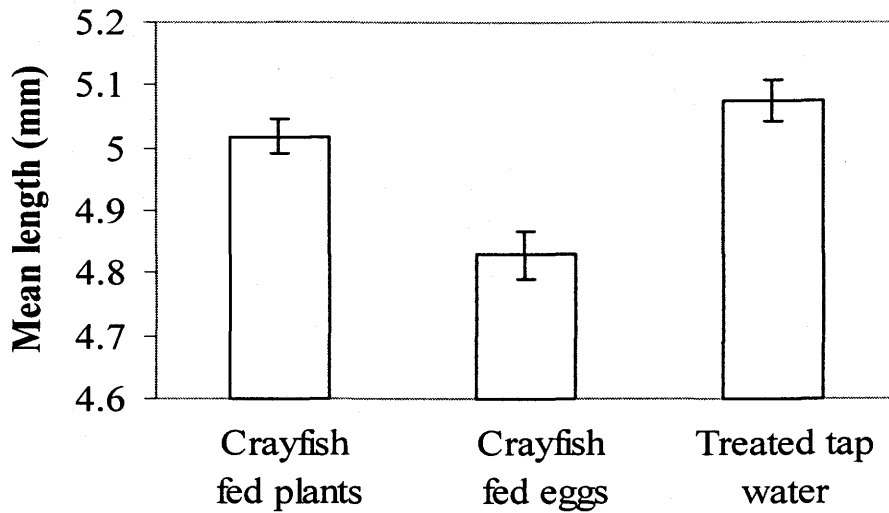


Figure 3.17 Mean (\pm SE) total length (mm) of fathead minnow hatchlings exposed to different crayfish predation cues during embryonic development in experiment four. Hatchlings in the crayfish fed egg treatment had significantly shorter total body lengths than those in crayfish fed plant and treated tap water control ($P<0.001$).

Pearson correlations, conducted to examine the relationship between fry size and length of developmental period, show that there was a significant effect (treatments 1 and 2: $P<0.001$, fig 3.18), except in the high predation threat treatment

(treatment 3: $P=0.489$, fig. 3.18). The coefficient of determination (r^2) is a measure of how much of the total variability in total length is accounted for by variation in developmental time. The r^2 values for the control of treated tap water and predator fed plant material treatments indicate that 23% of the variability in total length is accounted for by the length of the developmental period (treatments 1 and 2 $r=0.485$, $r^2=0.23$), whereas only 0.1% of the variation can be explained by this determining factor when the embryos are reared in the presence of high predation threat cues ($r=0.089$, $r^2=0.01$).

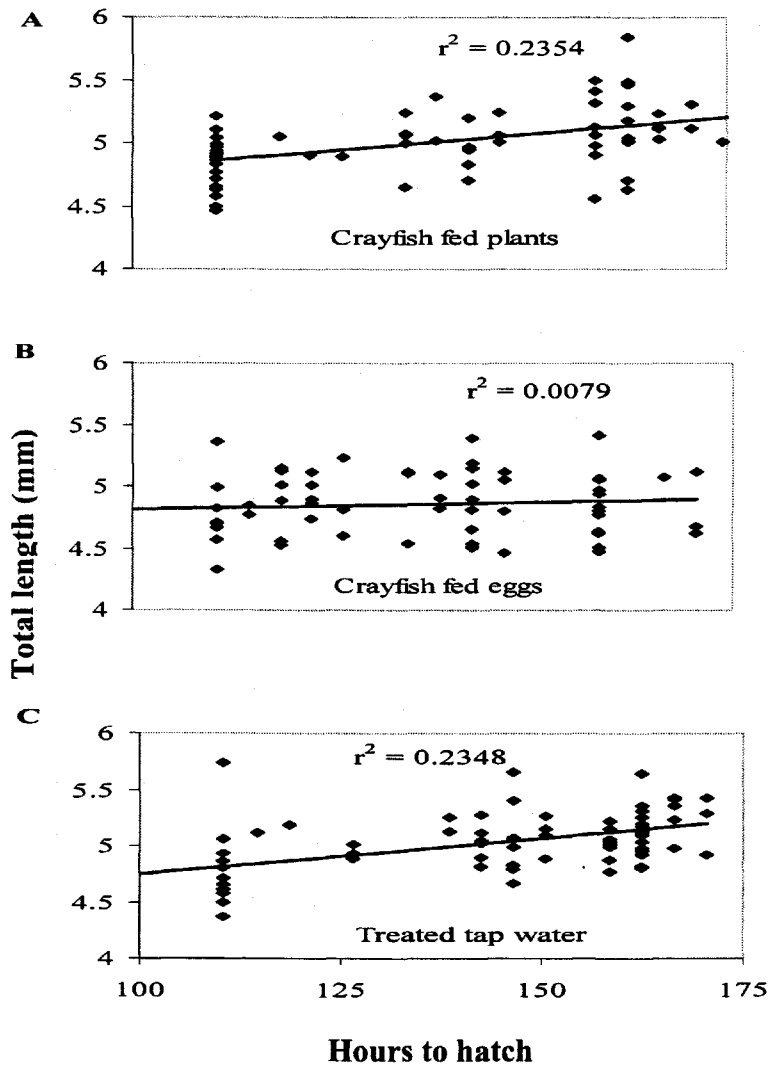


Figure 3.18 Total length (mm) of hatchling versus hatching time for fathead minnows exposed to different crayfish predation cues (A = crayfish fed plants, B = crayfish fed eggs and C = treated tap water) during embryonic development in experiment four.

Chapter 4: Discussion

The results of my research demonstrate that fathead minnow embryos, in general, do not alter their hatching time in response to the predation threat simulated by injured egg extract exposure during embryonic development. The perceived predation threat does, however, result in hatchlings expressing an altered phenotype, specifically they have shorter total body lengths. Laurila et al. (2001) found that the presence of a predator (larval diving beetle, *Dytiscus marginalis*) did not alter hatching time in the common frog (*Rana temporaria*), but that hatchlings reared in the presence of a predator had relatively shorter bodies and deeper tail fins than those reared in the predator absent environment. Facultative adjustment of hatching time may not be possible in some species or under certain circumstances and changes in hatching time are not the only defenses available to embryos. In the three experiments conducted in the summer of 2002 it was determined with 95% confidence that fathead minnow embryos do not alter their hatch time in response to injured egg cue exposure when compared to those reared in the distilled water treatment. In experiment one there was one isolated significant effect due to treatment, when embryos in the injured egg treatment hatched sooner in terms of median hatch time than those in the injured shrimp treatment. This appears to be an artifact of the large hatch time variation seen in experiment one. The preference of

the embryos examined in this experiment to hatch during the dark translated into a high degree of variation in hatch time. If an embryo did not or was not capable of hatching during one dark period it would most likely remain in the embryo stage for another 24 hours. This hatch time preference created some logistic problems as hatching was not observable during the 6 hour period of dark. This preference may have also been overriding any other hatch time factor, such as maternal and paternal investment. Originally, experiment one was designed to investigate a parental effect (pairs spawned separately), but due to the hatch time preference these investigations were in the end neglected. This phenomenon may also have been behind the significant difference observed between the injured shrimp and injured egg treatment in experiment one. There were 13 embryos in the injured shrimp treatment that held on and hatched the following day, while only 7 in the distilled water and 4 in the injured shrimp treatment did so.

The embryos did, however, appear to perceive and respond to the predation threat simulated by the injured egg extract, as they exhibited a predator-induced morphological adaptation. The results, however, were not consistently replicated among the three experiments. In experiment two it is speculated that the gametes may have been environmentally stressed before they were brought into the lab and fertilized, as temperatures were unusually high for three days prior to their collection. The stress was not enough to increase mortality, but may have

contributed to their inability to express an alteration in hatch time. This or some other unknown factor resulted in the embryos having a relatively shorter developmental period. The mean hatch time for experiment one (134.65 hrs), three (127.55 hrs) and four (140.04 hrs) were higher than that of experiment two (124.94 hrs). There was, however, no difference in mean hatchling size among the experiments. It would appear that all the embryos in experiment two hatched quickly, but that this alteration in developmental period length did not affect hatchling phenotype. Hatchlings exposed to predation cues during embryonic development in experiments one and three were significantly shorter [total body length (mm)] than those reared in the control treatments. Such morphological alterations, not obviously the result of a reduced developmental period, are believed to improve the anti-predator capacities of the prey in some manner. Phenotypic changes are highly predator specific and function to decrease the prey's risk of being detected, caught and/or consumed (Brönkmark and Miner 1992). If the threat is temporally variable, the shorter body length may translate into a size refuge from predators that prefer larger prey items. By the time the prey have reached the preferred size class the predator in question may have switched to focusing on another, now seasonally available, prey item. Alternatively, the driving force behind the shorter body length may be that smaller prey animals are not as easily detected. Furthermore, the alteration made to the developmental rate may not have

even been directly employed to change the hatchling's phenotype, but instead was a byproduct of the prey decreasing its metabolic rate and/or activity rate to avoid releasing chemicals that attract predators. It seems logical that an egg batch with a higher metabolic rate would produce more chemical cue than a batch with a lower metabolic rate and have a higher chance of being detected. This possibility, however, remains to be investigated.

Chivers et al. (2001) demonstrated that not all predation cues are equal in triggering life history trait adaptations in all species. Further investigations need to be conducted into the possibility of hatch time plasticity in fathead minnows. In the fourth experiment, conducted in 2003, I showed that fathead minnow embryos can alter their hatching time reducing the amount of time they spend in a high risk state. The embryos in the crayfish fed injured egg treatment hatched earlier when compared to the control of treated tap water and hatched at a less developed stage. The embryos exposed to predator odour, that lacked the conspecific diet cue, also tended to exhibit earlier hatching when compared to the control, they did not, however, differ significantly from the control in morphology. To our knowledge this is the first time that specific predator-induced hatch time plasticity has been clearly demonstrated in a fish species. The embryos raised in the presence of crayfish fed minnow eggs also had an altered phenotype. Phenotypic alteration, being smaller, as a result of a shorter developmental period could be costly, as the

smaller fry may be more vulnerable to predators that actively forage on fry. The reduction in tail muscle length or total length often is correlated with a relative decrease in escape speed (Warkentin 1995). However, as discussed above, phenotypic alterations not induced by an alteration in developmental time should be beneficial to the individual. The data would suggest that the alternate phenotype of a shorter total body length, in fathead minnows, may not be a direct result of a shorter developmental period. This led to speculation that there must be something else driving the expression of the alternate phenotype. Further examination of the relationship between hatchling size and length of developmental period, revealed that there was a significantly positive correlation between the two variables that apparently was muted in the high predation threat treatment. This could be an indication of a lowered metabolic rate and/or growth rate response in this treatment.

The results would also appear to indicate that the recognition of crayfish as an egg predator may be innate and that the predator odour may be interpreted as a relatively more significant threat when paired with a conspecific diet cue. Diet-dependent anti-predator responses are in fact widespread in predator - prey systems (review Chivers and Mirza 2001). For example, in Mathis and Smith (1993a) it was shown, in laboratory and field experiments, that pike-naïve minnows exhibited fright responses when exposed to pike fed nonbreeding fathead minnow odour, but not when exposed to pike fed breeding male fathead minnow (do not have alarm

substance cells at this stage) odour. This would indicate that minnows can recognize an unknown predator as a threat if it labeled by conspecific alarm cue. In another interesting study, Hagen et al. (2002) showed that green sea urchins (*Stongyloocentrotus droebachiensis*) respond more intensely to wolfish (*Anarhichas lupus*) fed conspecific cues than to either undiluted urchin extract or wolfish fed mussels (*Mytilus edulis*) cues. They suggest that the predator was being labeled by a latent chemical cue, that's potency is a function of either activation by or interaction with a substance present in the digestive tract. The results could also be explained if multiple predation risk cues are additive and thus affect the preys' anti-predator response in the same fashion. The difference in morphology between minnows exposed to crayfish fed minnows and crayfish fed plants in my study could also be interpreted as reflecting an additive effect. The crayfish fed plants cue, and injured egg cue used in the first three experiments, may have been recognized as a sufficient threat or an appropriate threat to respond to by altering ones phenotype, but not by changing the timing of one's life history.

Another hatch time preference, besides early hatching in the presence of an egg predator, seemed to become apparent. In the experiments conducted in 2002 there appeared to be an obvious bias for embryos to hatch during the period of dark, as over 80% of hatchlings emerged from 12:00am to 6:00am. In 2003, however, 50% of the embryos in the treated tap water treatment hatched during the day.

Furthermore, a significantly higher proportion of the eggs in the predator treatment hatched during the light period. The bias for hatching during the light period could be explained by the fact that the specific predator used is less active during the day, but why the eggs in the control treatment no longer hatched during the dark is unclear. Besides year effect there were no differences between 2002 and 2003, except for a decrease in overall disturbance due to technician experience, a room temperature decrease of 2°C and the use of treated tap water instead of distilled water as a control. The overall decrease in disturbance might have been a factor that affected the hatching period preference. The disturbances (visual shadows overhead and mechanical vibrations due to the application of treatments) all occurred during the day and may have prompted the eggs to hatch during the night if perceived as simulating predation. In the fourth experiment, due to less crowding and technician experience, the level of disturbance may have been significantly reduced.

Egg defense against predators use to be thought of in terms of parental care only. Due to recent and enlightening research anti-predator defense ability in embryos has been demonstrated in several taxa, including amphibians, spiders and fish (Chivers et al. 2001, Li 2002, Warkentin 2000, Warkentin et al. 2001, Wedekind 2002). The actual mechanism that results in the alteration in hatch time has only been speculated about. Our ability to determine the mechanism behind this adaptation is limited by our knowledge of the mechanisms involved in the process

of hatching itself, especially in aquatic embryos. Perhaps, the cues of a predator feeding on conspecifics in the same life stage triggers the process by initiating the metabolism or release of enzymes involved in the breakdown of the egg membranes. The cues could, on the other hand, result in an increase (or decrease) in metabolic rate, resulting in the acceleration (or delay) of the whole process. The prior suggestion would seem to be supported by our data as the hatchlings that exhibited predator-induced early hatching were shorter than the control hatchlings. In the three previous experiments, however, a shorter body length was also expressed in predation treatments that were not sufficient to induce hatching. The ability to take a number of other morphological measurements would have been very beneficial to us at this point. Perhaps, future work should focus on larger species where tail muscle width, egg yolk weight and hatchling weight measurements would be more feasible. From my data I can only conclude that the hatchlings in the predator fed plants treatment and those in the treated tap water treatment were the not different in terms of total length from each other, but both differed from those in the predator fed eggs treatment. Furthermore, it would have been very advantageous if we could have had some idea of yolk sac absorption rate and if there were difference among the various treatments. If the hatching is induced by an increase in metabolic rate we would expect that the yolk sac absorption would be higher in the predator fed eggs treatment than in the predator

fed plants and the treated tap water treatments. The egg yolk sac absorption rate, however, may not have been altered overall as phenotypic alteration (a reduced size) was also seen in this group. In terms of examining the phenotypic alteration seen in the first and third experiment, yolk sac absorption would also have been very helpful. It could be speculated that the hatchlings with the altered phenotype may have had a relatively larger yolk sac, which might be useful if predation risk could be lessened initially after hatching by not actively foraging for a longer period of time. Due to the size of the hatchlings and equipment available morphological measurements, however, were limited. Furthermore, in conjunction with or the cause of the possible increased metabolic rate there may have been an increase in activity rate. An increase in activity could result in the accelerated breakdown of the egg membranes or it could trigger another undetermined necessary physiological process altogether that brings about hatching. Warkentin (1995) noted that embryos hatch by producing vigorous movements that rupture the egg membrane. This may be supported by anecdotal observations made during my four experiments. It appeared that the fathead minnows became increasingly active around the third day and then on the fifth day seemed to cease activity. This may reflect that the egg membrane had been worn down sufficiently to allow for immediate hatching and the embryos ceased activity until the optimal opportunity is perceived. The exact

mechanisms involved in these inducible anti-predator defenses are still undetermined at this time.

My research has shown that predation plays an important role in the life history of prey animals. Predation is just one of many factors that has a great impact on shaping the biology of prey species. Studies such as this one should not encourage us to become focused on predation, but to recognize the possibility of intricate relationships in the lives of all organisms. For example, perhaps competition induced hatching could be illustrated in response to variation in the timing of a seasonal food source. The possibilities are endless.

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