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Effects of predation on nest-guarding behaviour of male fathead minnows

by

Hilary M. Jones ©

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

Department of Zoology

Edmonton, Alberta
Fall 1995

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled The effects of predation on nest-guarding behaviour of male fathead minnows in partial fulfillment of the requirements for the degree of Master of Science.

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Date June 7, 1995

Abstract

I conducted a three-part study to determine the effect of predation threat from northern pike on territorial and egg-guarding behaviours of the fathead minnow (*Pimephales promelas*). First, I carried out a field experiment evaluating the behavioural response of egg-guarding males to a live pike in a wire cage or to a control. I used time to return to the nest after introduction of a threat as a measure of risk-taking. Males took different amounts of risk based on predation threat; males in the predator treatment took longer to return than males in the control. Risk-taking was not related to the size or age of the clutch but to distance to nearest nesting neighbour; males with close neighbours returned faster than more solitary males. In a second set of field experiments, my objective was to determine if patterns of nest use by fathead minnows in a boreal lake were related to northern pike densities. I sampled northern pike and determined four areas of "high-pike density" and three areas of "low-pike density" in a boreal lake. I censused artificially placed nests and natural nests in these areas for use (presence of a clutch) by male fatheads. Males were found to use nests more frequently in areas with low densities of pike than in areas with high densities of pike. Predation and nest site availability may be an important factors influencing the observed pattern of nest use. Finally, I conducted a laboratory experiment to determine if males altered their territorial behaviours (chases, cleans, circles) in response to combinations of visual and chemical cues from northern pike. I used the presence of a pike as a visual cue and fathead minnow alarm pheromone as a chemical cue. I examined territorial defense, prior to egg-guarding because this was when males spent the greatest time away from the nest, presumably making them vulnerable to visual predators, like northern pike. Control males did not decrease their behaviours when a stimulus was added. Male minnows experiencing visual threat alone significantly reduced their territorial behaviours but within 12 h returned to pre-exposure activity levels. Male minnows subjected to chemical cues alone and to visual and chemical cues combined significantly reduced their behaviours, abandoned their nests, and did not return to pre-exposure levels of activity. I suggest that because risk of predation induces prolonged decreases in territorial defense and courtship behaviours, it may affect competition between nesting males and female mate choice. I conclude that fathead minnows can distinguish between predators and non-predators and base their reproductive decisions, such as where to nest and when to defend a nest, on their estimates of risk. Chemosensory recognition may be the primary method used to assess predation threat.

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Chapter One

General Introduction

Predation may have a strong effect on population dynamics, and community structure (Connell 1975; Sih *et al.*, 1985). Among fishes, predation may affect growth and behaviour of prey species (Reimchen 1988; Tonn and Paszkowski 1991) and the size and structure of prey populations (Tonn and Paszkowski 1986). The effects of predation on prey may be direct and/or indirect (non-lethal). The mortality of prey is the direct effect. Nonlethal effects arising from the presence of a predator may influence abundance and species diversity (Sih 1987; Wootton 1990), morphology, physiology, life history and behaviour of prey.

Declines in prey populations may be a result of direct effects of predators. However, indirect (non-lethal) effects of predation may affect reproductive success of prey. For example, Fraser and Gilliam (1992) found that killifish (*Rivulus harti*) exposed to a predator produced fewer eggs; this contributed to a decline in their population.

Indirect (non-lethal) effects of predation on reproductive behaviour are not well known. For example, courtship behaviour of guppies (*Poecilia reticulata*) (Endler 1987) and oviposition ("egg-laying") sites of female tree frogs (Resetarits and Wilbur 1989) are affected by the presence of a predator, but there is "little evidence that mature animals facultatively alter reproductive behaviour when the threat of predation is changed" (Fraser and Gilliam 1992). I addressed this issue by examining the reproductive behaviour of the mature male fathead minnow in response to a northern pike. Since fathead minnows exhibit a variety of behavioural patterns, such as parental care of eggs and alarm responses, they are an ideal species on which to study the indirect effects of predation on reproductive behaviour. Knowing that alarm signals exist helps in designing valid experiments that make use of visual and chemical predatory cues (Smith 1992) and in determining how these cues affect fathead behavioural responses.

A dominant piscivore in Alberta and throughout the Holarctic is the northern pike (*Esox lucius*), a solitary, ambush predator often viewed as a keystone species within lakes (Eklöv 1992). Access to appropriate-sized prey is critical for adequate growth in pike (Diana 1987). The efficiency of pike predation can have strong negative impacts on populations of some prey species (Robinson 1989).

The fathead minnow (*Pimephales promelas*) is a common, small-bodied fish in northern lakes that is vulnerable to piscivores (Gillen *et al.*, 1981; Robinson and Tonn 1989). In Alberta, fathead minnows grow for 2 years. In their third summer, they become reproductively active for approximately 2 months after which they die (Unger 1983; Price *et al.*, 1991). During this month of reproductive activity, males guard nests which contain clutches of eggs deposited by several females (Unger 1983). Nests are found in territories beneath floating or submerged objects (McMillan 1972). Males guard their clutches against egg predators (*i.e.* juvenile and female fatheads) by chasing them from the nest site. Males must also clean the eggs. Cleaning and protection of eggs by the male is crucial to egg survival (McMillan and Smith 1974). Fathead minnows may adjust their reproductive behaviour (*e.g.* nest defense) in reaction to their immediate circumstances. Male fatheads, subjected to egg predation from crayfish (*Orconectes* spp.), spent less time rubbing and cleaning their eggs and more time chasing crayfish (Sargent 1988).

Fathead minnows use visual and chemical cues to detect and avoid predators (Smith 1992). Visual recognition of a predator incites a fright response of dashes and skitters by prey minnows, but may not provide enough time for evasion (Mathis and Smith 1992). Fathead minnows possess a second mechanism for detecting and evading predators, a chemical alarm system. When a fathead is injured it releases a pheromone into the water from club-shaped cells ("alarm substance cells") in the epidermis. Nearby minnows detect the alarm pheromone and exhibit a fright response. Breeding male fathead minnows lack alarm substances but retain the fright response (Smith 1973). In this way, alarm signaling may indicate presence of a predator, and may alter predator-prey interactions (Smith 1992).

I conducted a study with both field and laboratory components to answer the following questions:

(1) (a) Are males in the wild sensitive to changes in predation risk? Do extrinsic and nest factors affect male's behavioural responses to risk?

(b) Are patterns of nest locations and densities of fathead minnow nests related to relative densities of northern pike in a boreal lake?

(2) Does predation threat (visual and chemical) in the laboratory alter nest defense in male fathead minnows? Are the behaviours in the laboratory consistent with what occurs in nature?

I hypothesized that reproductive activities of fathead minnows in the wild, especially the use of nests, would be depressed or altered by piscivores. Therefore, nesting would be less likely to occur in areas where pike density is the greatest. In the presence of a predatory pike, I predicted that territorial defense and egg-guarding would vary with breeding status of the male, egg number and egg age. Males should respond to differential predation threat by altering the "riskiness" of territorial behaviour in relation to severity of threat. Hence, I predicted that combined visual and chemical threats should invoke greater change in territorial defense than either visual or chemical threats alone.

Small-bodied fish, like the fathead minnow, are pivotal links in freshwater food webs and are important prey for economically valuable gamefish. However, responses to predation, especially during their critical reproductive season, are poorly known. My study will contribute to a better understanding of decision-making by reproductive prey in the presence of a predator. Moreover, it will provide fundamental knowledge about the non-lethal effects of predators and their role in structuring fresh water assemblages in boreal lakes.

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Chapter Two

Effects of northern pike on reproductive behaviours of male fathead minnows in a boreal lake

Introduction

Animal behaviour is often a complex response to a variety of biotic and environmental factors. Animals make decisions about where to feed, when to feed, what to eat, how vigilant to be, and with whom to mate. Predator avoidance is one of the main factors influencing these decisions (Lima and Dill 1990). Ecologists are now acknowledging the complexity of predators' effects on their prey, especially those *indirect* effects such as predator avoidance that can translate into changes in habitat use, feeding patterns, morphology, and growth (Sih 1980; Dill and Fraser 1984; Helfman 1986; Craig 1994; Godin and Crossman 1994). "However, little is known about how **reproductive behaviour** is influenced by predation risk" (Magnhagen 1990).

During reproduction an animal may be more vulnerable to predation than otherwise because of mate attraction tactics (*e.g.* calling or displaying), egg- bearing, courtship and parental behaviours (Ryan 1985; Svensson 1988; Wing 1988; Gwynne 1989; Magnhagen 1991; Peckarsky *et al.*, 1993). Increased vulnerability to predators during reproduction may lead, obviously, to mortality. The non-lethal (indirect) effects of predators, however, are not so obvious, and may lead to changes in reproductive success of individuals and prey populations as a whole (Reznick and Endler 1982; Dill 1987; Sih, Krupa and Travers 1990; Fraser and Gilliam 1992).

Fathead minnows (*Pimephales promelas*) are a common, small-bodied fish in northern lakes that are vulnerable to piscivores (Gillen *et al.*, 1981; Robinson 1989; Robinson and Tonn 1989). Since fatheads exhibit a variety of behavioural patterns, such as male parental care of eggs and alarm responses, they are an ideal species for studying the indirect effects of predation on reproductive behaviour of prey. The dominant piscivore in boreal lakes and throughout the Holarctic is the northern pike (*Esox lucius*). Piscivory by northern pike is thought to be one of the main factors structuring fish assemblages in lakes; fathead minnows rarely co-occur with pike in central Alberta lakes (Robinson and Tonn 1989).

During the breeding season male fatheads develop dorsal pads, black heads and horny proliferations (tubercles) on their snouts (McMillan 1972). Males defend territories and eggs from other males who are competing for nest sites and from females and juveniles who are egg predators. Males guard eggs until they hatch (7 days to one month depending on water temperature). Alberta fathead minnows guard their eggs in their second year and then die. Presumably site attachment, heightened coloration, and conspicuous defense behaviours, such as chases and egg rubs, make male minnows vulnerable to visual predators like northern pike (Eklöv and Diehl 1994). In this study, I determined the behavioural response of reproductive male fathead minnows, late in the breeding season, to the presence of live northern pike in a small, boreal Alberta lake. Few studies have attempted to quantify behavioural responses in the wild to a live predator; previous studies have used artificial (model) predators that do not present both visual and chemical threats.

I asked three questions regarding sensitivity to predation risk. (1) Are males sensitive to two intensities of predation threat (*i.e.* can males distinguish low threat (control stimulus) versus intense threat (presence of a predator))? (2) What is the behavioural response of males to predation threat? and (3) Do nest characteristics and extrinsic factors, such as water temperature, affect a male's response to predation risk?

I predicted that males could detect differences in predation threat and would respond by decreasing the number of each nest-tending behaviour performed in the presence of a predator. For many organisms, one would predict that late in the breeding season parents would provide low levels of care in order to maximize probability of survival to the next year (Carlisle 1982). However, because most male fathead minnows die after their eggs hatch, I expected them to assume greater risk than they might have earlier in the breeding season, making a tradeoff between being eaten and maintaining their clutch.

I predicted that extrinsic factors such as temperature, nest depth, distance to shore, distance to nearest neighbour, % cover of the nest, and nest type would determine a male's response to predation. A shallow, covered nest under a rock would be the 'safest' because a pike may be less likely to detect a male there; males in safer nests should take more risk than those in "riskier" nests by staying in the nest when a predator was present. I predicted that nest characteristics (age of eggs and number of eggs) would directly influence risk-taking. Males with older eggs (Day 5, 6 or 7) should return to the nest faster (more risk) than those with younger eggs (Day 1, 2, 3). Males with larger numbers of eggs should take more risk than those with small clutches.

Methods

Site

I carried out the study in August 1994 at Armstrong Lake, a clay bottomed, shallow lake (maximum depth 4.5 m) in north central Alberta (54 °24°'N 113° 39°'W). The lake's surface area is 230 hectares (Ducks Unlimited Canada 1994) and is largely undeveloped with limited public access. The area is dominated by agricultural land but stands of aspen (*Populus tremuloides*) and white spruce (*Picea glauca*) surround the lake.

The majority of the lake has an extensive littoral zone with heavy macrophyte cover (Figure 2.1). Emergent vegetation includes cattail (*Typha latifolia*), bulrush (*Scirpus* spp.), and giant bur-reed (*Sparangium eucarpum).* Zones deeper than 1 m are dominated by pondweed (*Potamogeton* spp.) and coontail (*Ceratophyllum demersum*). Inlet areas have heavy growths of yellow pond lily (*Nuphar variegatum*).

Water depth on the east shoreline ranges from 2.0 cm to 50.0 cm. This shoreline is one of the few areas on the perimeter of the lake without extensive emergent macrophyte growth. A patchy distribution of *Potamogeton* spp. is present but this shore is essentially bare because of wave action and the clay substrate. In addition, two lakefront property owners have removed some of the shoreline vegetation in order to tie up small boats.

Armstrong Lake contains northern pike (*Esox lucius*), white suckers (*Castostomus commersoni*), brook sticklebacks (*Culea inconstans*) and fathead minnows (*Pimephales promelas*). There is little recreational fishing on the lake because of poor access roads and the community belief that the northern pike population was eliminated by winter kill.

Fathead minnows nest along the east shore from late May until late August. Waves deposit rocks and driftwood under which fathead minnows nest. Fallen logs and pieces of bark from beaver activity also provide nesting substrate. When the water is calm, fathead minnows and sticklebacks are visible in the littoral zone.

Materials

Nesting male fathead minnows were exposed to either a control treatment or a predator treatment. In each treatment a 'threat' was introduced to the nesting fish. The threat was either a piece of driftwood in a cage (control) or a live pike in a cage (predation treatment). The wire cage was rectangular (57 cm x 8 cm x 10 cm) and was made from a metal frame wrapped in chicken wire. The interior of the cage was partially lined with clear plastic to prevent damage to enclosed pike. Lake water and pike odor flowed through the sides. This design ensured that minnows experienced both a visual and chemical threat when the pike was presented.

Prior to field experiments, I tested the cage in large outdoor fiberglass tanks at the Meanook Biological Research Station. I observed pike in the cage to determine the length of time they would tolerate captivity. I also placed minnows in the same tank as the empty cage for 24 h to determine their response to it and to ensure that the metal frame was not toxic to them. The presence of the cage did not elicit any overt behavioural changes. After 24 h all fish were alive and undamaged.

In May of 1994, I gill-netted three northern pike for this experiment from Armstrong Lake (mean total length = 54 cm ± 2 cm). The piece of driftwood used as the control was approximately the same size as the pike used (53 cm). I used a different pike on each day of the experiment to minimize stress and physical damage to them. Use of pike from Armstrong Lake ensured that they were familiar with fathead minnows and lessened the possibility of the spread of disease.

Experimental design

I was not able to test male minnows over their entire breeding season because high winds reduced visibility of nesters for most of June and July. Thus I was confined to conducting my study on August 4, 8, 9, and 10, 1994 and when there was no wind. The experiment was performed during daylight (10:00-17:00) to ensure visibility of males.

To locate males, I walked the shoreline and canoed deeper waters. Guarding males were visible because of conspicuous behaviours and coloration. Active males were plotted on a map and, of those, I randomly chose five males each day for 4 d. On the first day, I randomly assigned three of the males to the control and two to the predator treatment. I alternated assignment of treatments for the remaining males (*i.e.* on the second day, two males were assigned to the control and three to the predator). Of the 20 males manipulated, only 14 are used in analyses because one had only one egg and others were sickly (bloated and weak) throughout the treatment. The egg number for seven control males was 280 ± 163 while egg number for the predator treatment (n=7) was 150 ± 94.

Each male was observed for 30 minutes. This sampling period was divided into three time intervals: pre-treatment, treatment and post-treatment. I used three intervals to establish baseline measurements and to reduce the effects of individual differences (Martin and Kraemer 1987). The pre-treatment interval was 10 min of baseline behaviours measured immediately before the cage was added. The treatment interval was 10 min of recording while the control or predator was present. Post-treatment behaviours were noted for 10 min after the cage was removed. Males were tested only once to avoid habituation to the stimulus (Magnhagen and Vestergaard 1991).

When the cage was added, males left the nest and exited the territory. I defined **nest** as the substrate to which the eggs were attached and **territory** as the surrounding area (up to 15 cm), including the nest. Nest were under a rock, partially buried twig, or section of fallen log. I measured return time (s), the time for the male to return to his nest after introduction of the cage. Time away from the nest (return time) is a measure of risk-taking and reproductive decision (Lachance and FitzGerald 1992). I assumed that males returning sooner were more vulnerable to predators like northern pike or great blue herons (*Ardea herodias*) and were taking a greater risk (Pressley 1981; Magnhagen and Vestergaard 1991; Lachance and FitzGerald 1992) because by returning sooner the probability of being detected and eaten by a 'sit and wait' predator may have been higher.

I distinguished 2 main types of reproductive behaviour: territory defense and egg-guarding. **Egg-guarding** included behaviours directed towards the eggs such as cleans. I defined **territory defense** as the suite of behaviours not directly oriented towards the eggs, such as chases of conspecifics or circles around the nest and the territory.

For each male, I measured eight reproductive behaviours including both territorial defense and egg-guarding type behaviours. I recorded chases, cleans, circles (wide and tight), forage rate, tail beats, number of encounters with a female, and number of rolls with a female. Of these eight, five behaviours were the most consistent; they were exhibited by all males with similar frequency. The remaining three behaviours occurred infrequently and were often difficult to measure. Thus, although I measured eight behaviours, I used only five in my analyses. These five are defined below.

Behaviours

(1) **Chases** were vigorous lunges at territory intruders or conspecific and heterospecific 'passerby's'. Intruders were other male, female or juvenile fatheads, invertebrates or sticklebacks. Chases were often followed by head-butts (direct contact of male frontal tubercles with the intruder) or tail-beats (swatting at nearby fish or trespasser) (McMillan and Smith 1974). Occasionally males chased females towards the territory and nest instead of away. Chases were considered as part of the territory defense behaviours.

Cleans were nest and egg-oriented behaviours characterized by two distinct motions: rubs or nibbles (McMillan 1972).
(2) Rubs were a conspicuous abrading motion of the dorsal pad on the underside of the nest. The dorsal pad contains mucous and taste cells that may function in determining suitability of nest site and ensuring adhesion and cohesion of eggs (Smith and Murphy 1974).
(3) Nibbles were behaviours where fish assumed a vertical position in the water column underneath the nest during which males placed their mouths, nostrils and tubercles in contact with the ceiling of the nest and individual eggs (McMillan 1972). Cleans were defined as egg- guarding behaviours.

Circles. Males made two types of movements around their nests and territories: tight circles and wide circles.
(4) Tight circles had the same circumference as the nest. Whether a nest was a rock face or underside, an excavation under a fallen log or branch or twig, a tight circle involved a revolution around the nest.
(5) Wide circles were revolutions from inside the nest area to the perimeter of the male's territory. They were larger than tight circles because they extended the full circumference of the male's *territory*, not just the nest. Circles occurred in the presence and absence of eggs and were considered territory defense.

To determine how extrinsic factors influenced return time and reproductive behaviours, I measured physical variables (extrinsic factors) and nest characteristics after the behavioural observations on all males were completed. **Extrinsic factors** were nest type (1=log, 2=twig, 3=rock), nest depth (cm) from the substrate to the water surface, distance to nearest nesting neighbour (cm), distance to shore (cm) from the nest, and cover of each nest determined as the % cover of the nest. % cover was estimated by what percentage of the nest was visible to an observer in a male's territory. I measured water temperature (°C), dissolved oxygen and pH at each nest but found all three parameters to be similar in each nest. The mean temperature was 20°C (s.e. 1.0), dissolved oxygen was 7.9 (s.e. 1.4), and the mean pH was 8.6 (s.e. 0.2).

Age and number of eggs were measured to determine if **nest characteristics** influenced return time, egg- guarding and nest defense behaviours. Age of eggs was determined by egg characters. Egg age varied between 0 to 6 days (control 5.4 ± 1.0; predator 4.3 ± 2.2). New eggs were clear pink. As they aged, they became "eyed" (day 4) and were wigglers (black eyes moving in the egg) by day 5. Most eggs hatched on Day 6 and 7; these eggs were characterized by wiggling fry with gold eyes. I noted what percentage of each clutch had fungus and the number of eggs that were inviable (opaque). In addition, I gave the guarding male a color score (1 - 5) in each of the three time periods to determine if males altered their coloration in advanced stages of egg care and to see if dark males remain conspicuously colored in the presence of a threat. Males generally paled after introduction of the cage but regained some of the nuptial coloration in the post-treatment interval. Color scores were based on McMillan and Smith (1974) and Unger (1983); males that were pale scored 1 and those that were darker with a pronounced dorsal pad scored 2, 3, and 4; higher scores indicated increasing darkness in color. Those with a score of 5 were not only dark with a developed dorsal pad, but had two white or golden bands encircling the body.

Analysis

Multiple regression was used to analyze return time, nest characteristics and extrinsic factors. Data were analyzed with Systat® version 5.2.1 (Macintosh) (Wilkinson 1992). Because there was such uniformity in pH, dissolved oxygen and temperature, I did not include them in analyses of return time.

To test whether reproductive behaviour differed between treatments over sampling intervals, I used a nested split-plot design analyzed with a repeated measures ANCOVA and repeated measures MANCOVA (see Appendix One for assumptions). I did a repeated measures ANCOVA on the rubs and a repeated measures MANCOVA on total activity, where total activity was calculated as the sum of the number of chases, rubs, and circles for each time interval. I used 'total activity' because behaviours other than rubs were infrequent; since all males tested had eggs in their nests, rubs were the primary behaviour. Total activity was a measure of both egg-guarding and territorial defense. All behaviours were analyzed as number of acts in each interval.

In the repeated measures design, treatment was the whole-plot factor (between-subject effect) and sampling interval was the split-plot factor (within-subject effect); thus I tested two basic questions with the ANOVA's: (1) was there a time effect? and (2) was there a treatment effect? Individual males were nested within treatment. Age of eggs was analyzed as the covariate. I examined the data and residual plots to ensure that the assumptions of the linear model were not violated. ANOVA's and regressions were analyzed as mixed models with treatment a as fixed effect and male as a random effect.

Results

When the control or predator was introduced all males left their nests and lost a portion of their breeding coloration. Most males decreased 2 color scores after the cage was added. Males usually left their territories and hid in nearby vegetation. Most males returned within the 10 min that the cage was present (Table 2.1). Two males (one in each treatmentment) came back to their nests only after the cage was removed. One male in the predator treatmentment spawned and received eggs from a female during the time the cage was present. No male inspected the pike or the control.

Do males in each treatment take the same amount of risk?

Return time (time to return to the nest after presentation of the cage) was my measure of risk-taking. I assumed that males returning sooner were taking more risk than those that were slower to return. Return time was variable among males (Table 2.1). Males in the predator treatment took longer to return than control males (Wilcoxon z=-2.82, p = 0.010).

Do extrinsic variables and nest characteristics influence the willingness of a male to take risks under predation threat?

Contrary to my predictions, return time was not dependent on any of the nest characteristics such as number of eggs or age of eggs (F = 0.189). In addition, it was related to only one of the extrinsic factors, distance to nearest neighbour.

Using multiple regression, I analyzed return time with distance to nearest neighbour and number of rubs before the cage was added (pre rubs). There was a significant relationship between return time, distance to nearest neighbour and rubs (F=10.74, p =0.001, $r^2 = 0.86$) (Table 2.2). Intensity of rubs before the treatment was not significant but there was a significant interaction between rubs and distance to nearest nesting neighbour. A male with a close neighbour rubbed more and returned to the nest sooner than males with distant neighbours. Using a multiple regression, I analyzed the number of rubs with percentage cover of the male as well as color score with egg age and number of eggs. Males with high percentage nest cover were less visible and rubbed more frequently than males with less concealed nests (F=6.319,p = 0.027). Male coloration was related to egg - guarding and territorial defense; males with more rubs and chases were darker in color (F =16.17, p = 0.001).

Do reproductive behaviours differ between treatments between sampling intervals?

(1) Rubs

I analyzed the number of rubs for 14 individuals before (pre-treatment) and after (post-treatment) presentation of the cage (Table 2.3). I did not include the treatment period because rubs were non-normally distributed. Rubs from the treatment period were thus analyzed with a Mann-Whitney U non-parametric analysis, testing if the number of rubs per min in the nest was significantly different between treatments. I used rubs per min in the nest to account for the time absent from the nest when a stimulus was present. Mean rubs per min in the nest was 6.6 (s.e. 2.19) for predator-treated males and 6.07 (s.e. 1.00) for control males. Differences between predator and control males in the treatment interval were non-significant (U= 24.0, p =0.949). There were significant differences in number of rubs between treatments in the post-treatment sampling interval (Figure 2.2). There was a slight but non-significant increase in rubs over time (sampling intervals) (F =0.289, p =0.602) for both treatments. Males in the predator treatment had a smaller increase in rubs than did males in the control.

One male was spawning with a female during a predator treatment. He had 250 eggs in his nest after the 30 minutes of observation. His number of rubs were not significantly different from other males, but age of eggs became a significant covariate (F=11.820, p =0.006) because his eggs were less than one day old whereas the others range from 2 to 6 days. Although the age of his clutch is underrepresented in the sample of males, I chose to leave him in the analysis because other courting and spawning males were visible on the shoreline.

(2) Total activity

I analyzed the total activity over pre-treatment, treatment, and post-treatment sampling intervals for all males (Table 2.4). Differences in total activity between treatments in post-treatment intervals were significant (F = 5.305, p =0.042); age of eggs was a significant covariate (F = 9.469, p =0.011). Males in both treatments increased their total activity over sampling intervals but it was not a significant increase for either treatment (within-subjects factor (sampling interval) F = 1.193, p =0.322, with Greenhouse-Geiser adjustment p =0.318). Males in the predator treatment displayed a lower increase in activity upon returning than did control males (Figure 2.3).

Discussion

Egg-guarding male fathead minnows altered their reproductive behaviour when subject to predation threat from a live pike. Egg-guarding and nest defense differed between males in control and predator treatmentments after the threat was presented. I conclude that males can differentiate between disturbances from predators and non-predators. For both treatmentments, there was a slight but non-significant increase in behaviours after the cage was added. Males under threat from the pike had a smaller increase in rubs and total activity than control males. This suggests that males can alter their behaviour (parental investment) in response to an unpredictable predation threat (*sensu* Carlisle 1982) and "base certain reproductive decisions on estimates of the prevailing risk of predation"(Lima and Dill 1990).

Fathead minnow males that guard eggs are conspicuous because of their behaviours and bold color (Isaak 1961; McMillan and Smith 1974). An increase in the occurrence of behaviours increases the probability of detection by a predator, particularly a visual predator like northern pike (Diana *et al.* 1977; Endler 1987). Matity *et al.* (1994) suggested that the conspicuous territorial behaviour of breeding fathead males made them more vulnerable than juvenile or female fatheads to predation by garter snakes (*Thamnophis radix*). Fatheads exposed to the predator treatmentment did not increase their egg-guarding behaviour and territorial defense as much as control males, even after being away from the nest significantly longer. I hypothesize that males with eggs delay their return to the nest to avoid exposure to predators. A test of this hypothesis would be to record behaviours over a longer period of time after presentation of the pike to determine how long males are affected. My results are similar to Sargent's (1988) who showed that fathead males subject to predation risk from crayfish exhibited lower rates of rubbing than those not at risk.

I found that males assumed different amounts of risk based on predation threat. Both sets of males left their nests, but those in the control treatmentment returned faster than males in the predator treatmentment. Males may detect pike both visually and chemically through alarm pheromone from minnows in the pike's diet. Sensitivity to pheromones increases survival of minnows (Mathis and Smith 1993). Individuals may survive, but time away from the nest and lower levels of parental care could lead to egg predation, the usurping of nests (Unger 1983) and a decline in nesting success (Sargent 1988). This non-lethal effect of predation, as well as direct mortality, could help explain why fathead minnows do not commonly co-occur with large predatory fish in many lakes (Central Alberta -Robinson and Tonn 1989; Wisconson -Tonn and Magnuson 1982; Rahel 1984).

Because most males die after their eggs hatch and have few opportunities for multiple clutches, especially late in the breeding season, I expected males to take greater risk for older eggs than for younger eggs. While males in the control treatmentment seemingly assumed greater risk by returning sooner than males presented pike, the response was not related to age or number of eggs. Lachance and FitzGerald (1992) also found no relation between clutch size and parental investment in the three-spine stickleback (*Gasterosteus aculeatus*). Magnhagen and Vestergaard (1991) found only a small relationship between egg age and risk-taking in common gobies (*Pomatoschistus microps*). Sargent (1988,1989), however, showed *positive* correlations between number of rubs and number of eggs and number of rubs and age of eggs in fathead minnows. Small-mouth bass have also been shown to increase aggressive, nest defence behaviours when age of brood increased (Ongarato and Snucins 1993). Age of eggs significantly affected the frequency of egg - guarding behaviours with the inclusion of the male that acquired eggs during the predator treatmentment. Rubs and total activity *decreased* as eggs got *older*. This contradiction to earlier work may stem from the age of eggs present in the sample of males. A larger sample size of males with younger eggs could more convincingly confirm this pattern.

Extrinsic factors may influence a male's behavioural decisions. Males returned faster when they were closer to other nesting males and when their initial parental investment (rubs prior to presentation of the cage) was high. Sullivan and Atchison (1978) suggested that most fathead minnow anti-predator behaviours were based on social interactions among minnows rather than on individual behaviour. Unger (1983) found that males nesting alone (solitary males) lost more weight and remained paler in color compared to those guarding in a competitive setting. In Armstrong Lake, males nested close to one another (range 12 - 700 cm). This may be a function of a limited number of nest sites but it may also serve as "safety in numbers" to minimize predation. If the latter is true, males in high density nesting areas may return sooner, regardless of egg number or age, because the probability of being eaten is reduced while the probability of egg predation and nest usurpation is high. Fathead minnows may choose to breed based upon where they assess local predation risk to themselves may be lowest (Lima and Dill 1990).

There may be an ecological tradeoff between guarding in a competitive setting and solitary defense. Near neighbours may relieve predation pressure on an individual (*sensu* the selfish-herd theory; see Krause 1993) but because of competition for females and nest sites, neighbours may cause males to increase agonistic and reproductive behaviours; consequently, guarders increase in reproductive coloration (Unger 1983; personal observation) and may become more easily detected by visual predators like northern pike, garter snakes and great blue herons.

Whoriskey and FitzGerald (1985) and Lachance and FitzGerald (1992) reported that depth and distance to shore were significant predictors of return time in three-spined sticklebacks threatened by an artificial great blue heron. Presumably, a deep nest far from shore would be less risky for sticklebacks subjected to heron shore attacks. I predicted that it would be the opposite situation for fatheads threatened by northern pike; a shallow nest near shore would be safer from a predator coming from deeper water For fathead minnows in Armstrong Lake, nest type, distance to shore and depth of nest were not significant in predicting return time. Perhaps this lack of significance was because of the habitat structure of the lake where the littoral zone (nesting area) varies little in water depth. Hence, males nested at the same depth (28-32 cm) no matter the distance to the shore. Thus, contrary to my predictions, depth or proximity to shore may not be an advantage in avoiding predators in this lake.

In three-spined stickleback, males with concealed nests (high degree of cover) had greater reproductive success and lower variability of hatching success (Sargent and Gebler 1980). However, for fathead minnows, cover (visibility of male) did not significantly influence return time but it did affect the frequency of rubs and chases as well as male coloration. This observation suggests that males choose nests with cover, near neighbours, and adjacent vegetative refuge, in order to minimize predation risk. If this is the case in fathead minnows, proximity to vegetative refuge and nesting neighbours may be the extrinsic factors influencing return time and possibly reproductive success.

In conclusion, I have shown that fathead minnow males in the wild can detect predators and respond to predation threat by altering their reproductive activities. The ability to detect an unpredictable predation threat and exhibit a behavioural response that may minimize consumption risk, especially during the breeding season, may help explain how fathead minnows can co-exist with northern pike in this lake. In central Alberta, however, there are few lakes in which fathead minnows exist with large predatory fish. Piscivory by northern pike is suggested to be one of the dominant processes contributing to this almost mutually exclusive pattern of co-occurrence (Robinson and Tonn 1989), but perhaps behavioural avoidance tactics using visual and chemical predator recognition systems (Mathis, Chivers, and Smith 1993) compensate for lack of morphological defense (*e.g.* spines) that often protects small fish from predators (Robinson and Tonn 1989) and allow for co-existence in certain lakes. Piscivory may result in direct mortality of fathead minnows or in a disruption of normal reproductive patterns and success.

Future studies should determine if variation in responses to predation affects reproductive success and if the pattern of fathead response is the same over the entire breeding season and for males of various ages and sizes. Moreover, new studies should examine if the pattern of behavioural response is similar in other rare lakes that contain cyprinids and northern pike. Also of interest is determining what the pattern of response to predation is when fathead are faced with a different predatory strategy (*e.g.* yellow perch (*Perca flavescens*) (see Gywnne 1989).

	PREDATOR	CONTROL
Mean Return time (s)	149.57 *	61.86
Standard Deviation	219.31	62.65
Range	1-600	2-159

Table 2.1 Mean, standard deviation and range of return times (time for male to return to the nest after introduction of a stimulus) for predator and control treated males in Armstrong Lake.
* Males in the control treatment returned faster than males in the predator treatment (Wilcoxon z=2.82, p=0.01).

Independent variable	**Std Reg Coef**	*p*
pre rub	0.191	0.549
Distance near neighbour	1.419	0.003
pre rub*Neighbour	-1.258	0.017

Table 2.2 Multiple regression of return time with distance to nearest neighbour and pre treatment rubs ($F = 10.74$, $p=0.001$, $r2= .86$).

	PRE	POST
PREDATOR	30.20	46.92
CONTROL	47.51	75.94
S.E.	6.20	11.52

Table 2.3 Adjusted least squares means and standard errors for number of rubs in each time interval.
* There was a significant difference in number of rubs between treatments in the post interval (F=8.769, p=0.013).

	PRE	TREAT	POST
PREDATOR	46.40	50.93	59.41*
CONTROL	74.17	82.65	98.16
S.E.	6.76	16.24	12.77

Table 2.4 Adjusted least square means and standard errors for total activity in each time interval.

* There was a significant difference in total activity between treatments in the post interval (F=5.305, p=0.042). Differences in activities over time within treatments were not significant (F=1.193, p=0.322).

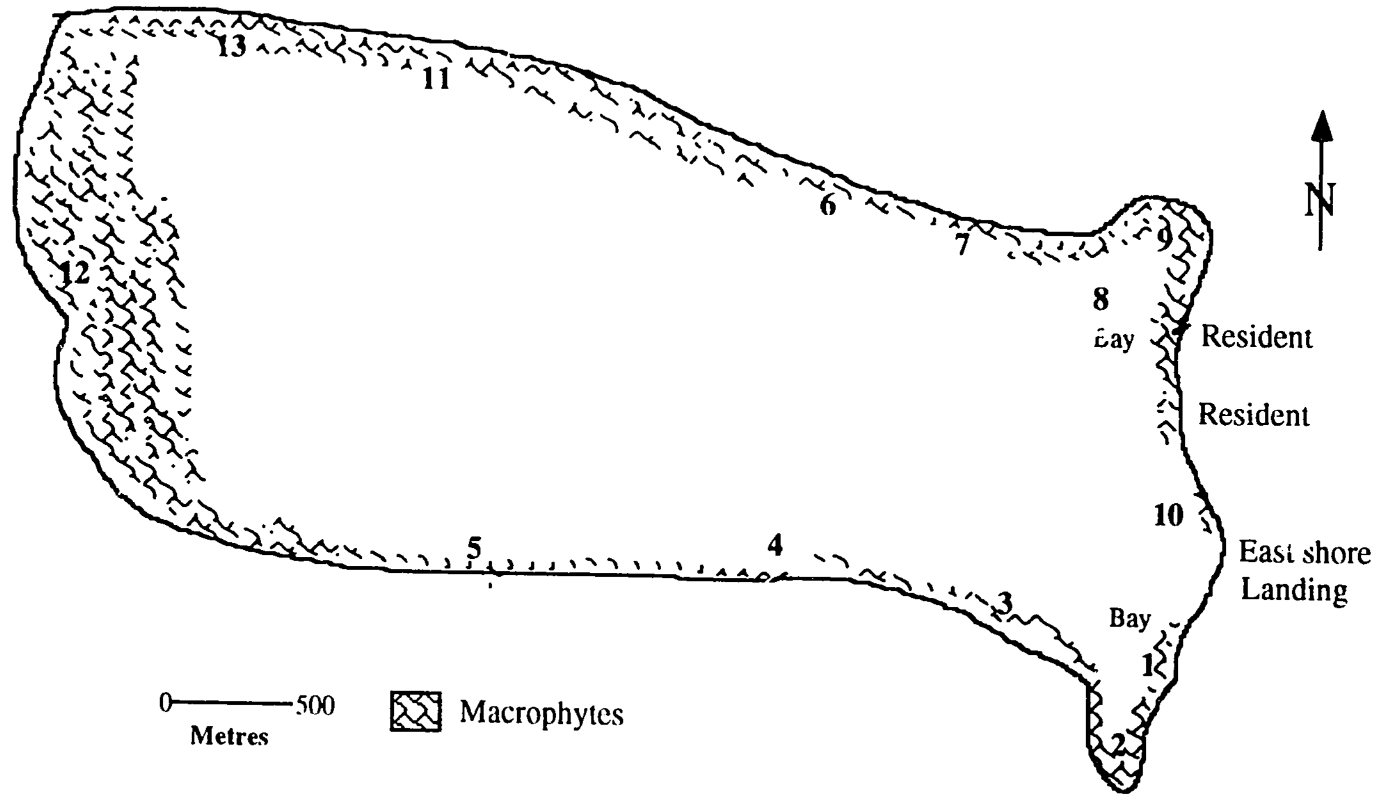

Figure 2.1 Armstrong Lake (230 ha): macrophyte cover and sampling locations. Note fewer macrophytes at Locations 4, 6 and 10. Numbers indicate locations for sampling of northern pike (Chapter Three). See Table 3.1 for decriptions of sampling locations.

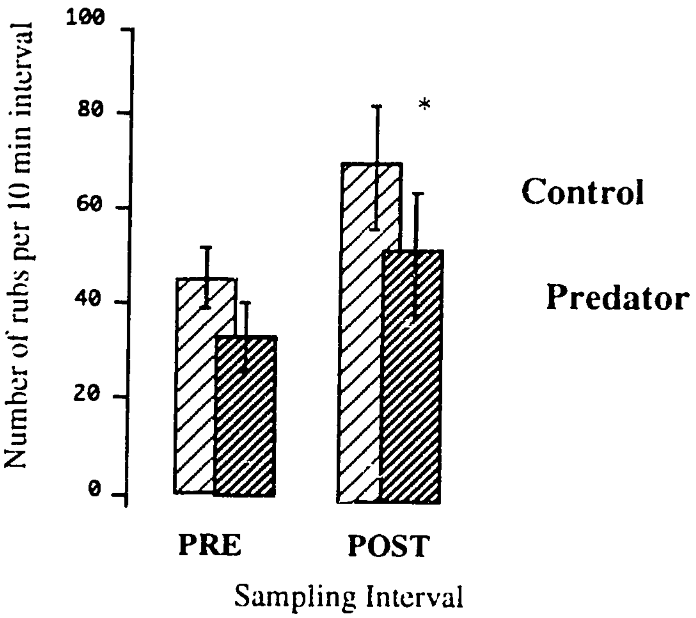

Figure 2.2 Number of rubs per 10 min sampling interval. Bars indicate one standard error. Treatment sampling interval was excluded and analyzed separately. * Number of rubs between treatments in the post intervals were significantly different (F=8.769, p =0.013).

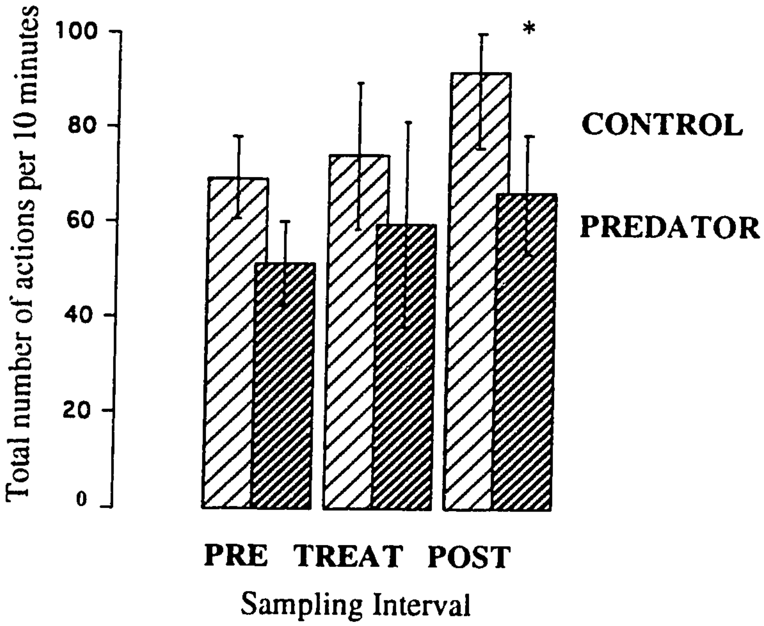

Figure 2.3 Total activity per 10 minute sampling interval. Bars indicate one standard error. * Predator-treated males had significantly fewer total activities than control males (F= 9.469, *p* =0.011).

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Chapter Three

Use of nests by fathead minnows in relation to density of northern pike in a boreal lake

Introduction

Do prey shift their location in response to predators? "An individual prey animal should be able to reduce its susceptibility to predators by adjusting the place where it engages in various important activities, such as breeding and feeding" (Helfman 1986). Some studies have reported that prey avoid areas where predation risk is high, "either because predators are especially abundant there or because protective cover such as vegetation is scarce" (Dill 1987). The trade-off in these situations may be that the prey sacrifices food or mating opportunities for reduced vulnerability to predation. Werner and Hall (1988) reported that bluegill sunfish (*Lepomis macrochirus*) exhibited ontogenetic habitat shifts in response to largemouth bass (*Micropterus salmoides*) predation. Juvenile bluegills switched to habitats where risk of predation was lower but abundance and quality of food were also decreased.

Predator-mediated habitat shifts made while foraging have been documented (Werner *et al.*, 1983 a,b), but, there is a paucity of data on how predators influence choice of breeding territory, nest use and fecundity of prey (but see suggestions in McKaye 1984). Presumably, if prey are vulnerable during reproduction, they should choose nests and breeding territories that both maximize reproductive success and minimize vulnerability to predators. Some studies have documented changes in reproductive behaviours in the presence of predators (Reznick and Endler 1982; Sih 1988; Magurran and Seghers 1990; Peckarsky *et al.*, 1993) but data are lacking here as well (Magnhagen 1991).

Fathead minnows (*Pimephales promelas*) are a common, small-bodied boreal fish that are vulnerable to piscivores (Gillen *et al.*, 1981; Moody *et al.*, 1983; Robinson 1989; Robinson and Tonn 1989). Fatheads are an ideal species for studying the effect of predation on locations of nests because nests are easily found on shorelines of boreal lakes. A dominant piscivore in boreal lakes and throughout the Holarctic is the northern pike (*Esox lucius*). Pike occupy shallow, vegetated areas and are solitary, ambush predators. Northern pike preferentially eat fathead minnows over most other prey species (Mauck and Coble 1971; Moody *et al.*, 1983; Wahl and Stein 1988; Robinson 1989).

From late May to August, fathead minnow males move offshore (Price *et al.*, 1991), set up territories in the littoral zone and defend eggs until hatching. During this period, males develop dark nuptial coloration and tubercles on their snout (McMillan and Smith 1974). Site attachment, increased coloration, and conspicuous territorial behaviours may make nest-guarding males vulnerable to northern pike. Further, territorial males are usually aggregated. It is plausible that ambush, littoral predators like northern pike could cue in on local aggregations and exploit them. Pike are efficient predators around prey refuges in the littoral zone (Eklöv and Diehl 1994). Moreover, Ivlev (1961) concluded that the intensity of northern pike feeding depended not only on the amount of food available, but on the patchiness of distribution; food concentrated in patches was consumed more intensively than food that was scattered evenly.

Predators usually alter species composition through complete exclusion of predation-intolerant species (Robinson and Tonn 1989). Fathead minnows do not commonly co-occur with pike (Harvey 1981; Tonn and Magnuson 1982; Rahel 1984; Robinson and Tonn 1989); however, Armstrong Lake (54"24"'N113" 39"'W) contains both species. Although there is evidence of winter kill of the pike population in this lake (Robinson and Tonn 1989), minnows and pike have undoubtedly co-existed intermittently in this lake since 1950 (Ducks Unlimited 1994). This lake, therefore, provides a unusual opportunity to examine nesting patterns of a vulnerable prey species in relation to predator densities.

In this study, I determined if patterns of nest use by prey fish in a boreal lake were related to predator densities. In 1993, I observed that minnows used only a small proportion of the potential nests available to them. I hypothesized that threat of predation by pike was an important factor determining nest locations for male fathead minnows. Therefore, the objectives of my study were (1) to determine if there were areas in the lake with high or low relative densities of pike, and (2) to determine if the number of clutches on artificial and natural nests of fatheads was related to density of northern pike. I also sought to estimate the rate at which males encountered northern pike in May, June and July. By quantifying encounter rate, I estimated the degree of threat an individual nesting male experienced. Male fathead minnows can detect predators and respond to predation threat by altering their reproductive behaviours (Chapter 2). Hence, minnows may "base certain reproductive decisions on estimates of the prevailing risk of predation" (Lima and Dill 1990). Thus, I predicted that fathead minnow males would be less likely to use nests (both artificial and natural) in areas where pike density was the greatest, *i.e.* if nesting male minnows were vulnerable to predation, they should choose to nest in locations where pike density was relatively low ("safer" locations).

Methods

Density of northern pike in Armstrong Lake

Local residents reported that northern pike had winter killed in Armstrong Lake. Thus I sampled the lake with gill nets to determine if northern pike remained in the lake. Potentially low numbers of pike might have suggested that threat to minnows in this lake was minimal and thus I wanted to estimate the density of northern pike relative to other lakes with pike. To determine the relative density of northern pike in Armstrong Lake (refer to Chapter 2 for lake description) and to determine regions of "high-pike density" and "low-pike density", I used a mark, release and recapture technique (Blower *et al.*, 1981). I captured fish by gill netting from the first day the lake had open water (April 27, 1994) to the first day of ice cover (October 21, 1994).

I used a random stratified sampling design to minimize variability between samples (Schaeffer *et al.*, 1986) while estimating relative densities. Stratified sampling is a common technique used when estimating space use and abundance of northern pike (DeLury 1951; Turner 1984; Hilborn and Walters 1992). After surveying the lake, I chose 13 locations for sampling. Locations were chosen based on their accessibility and having representative features such as substrate type and vegetation (Table 3.1). These locations maximized information on both pike and minnow habitat use within the lake relative to structures, such as vegetation and fallen trees. I measured pH, depth, dissolved oxygen, and temperature in each location every month to determine that locations were physically and chemically similar.

Locations for sampling were numbered clockwise around the lake starting from the east shore boat launch (Figure 2.1). All locations had emergent and submergent littoral vegetation except locations 4 and 10. Locations 2 and 9 were bays on the south and north ends, respectively, of the lake. I sampled the most northerly or southerly areas in these bays ("interior" of each bay) and then sampled the exterior of these bays (locations 1 and 8) that led to the main body of the lake. Locations 3, 7, 11, and 13 were characterized by the presence of beaver lodges that served as nests for minnows. Locations 4 and 6 had fallen spruce trees in the littoral zone that were used as nests for minnows; Location 4 had a sand substrate with patchy vegetation. Location 5 had a large fallen dock in the littoral vegetation that was used as a minnow nest site. Location 10 (the east shoreline) had sand and rock substrate with little emergent vegetation but significant submergent vegetation. Location 12, the west shore, was dominated by emergent and submergent vegetation. In late June when vegetation density was high, locations 5, 11 and 12 became inaccessible for sampling.

Of the 13 locations, I randomly chose 4 per sampling period. I placed one gill net (50 m length, experimental mesh sizes) in each of the four locations. Northern pike are thought to be crepuscular (Ivanova 1969; Christiansen 1976) so I set nets twice daily at dawn and dusk for 3 hours per set; thus, eight locations were sampled per day. A short set was desirable because it minimized mortality of northern pike; minimal loss of the population during marking is essential for mark, release, and recapture (Krebs 1989). I gill-netted 3 d weekly in late April and May because I expected high pike mobility immediately after their spring spawning (Ivanova 1969; Diana *et al.*, 1977); this offered increased opportunity for marking fish. For June, July, August, September and October, I gill-netted 3 d a week every 2 wks. Sampling was incomplete in August (only 7 gill-nets set) because I did not want to capture newly hatched chicks of red-necked grebes (*Podiceps grisengena*) on the lake.

Sampling was random and all locations were sampled once before any were sampled again. All captured fish were marked with a caudal fin clip, had their total length (cm) recorded and were released.

In addition, I sampled the east shore (location 10) with one gill net twice a week in late May, June and July. Minnows nest on the east shore and thus use of the additional sampling in this location allowed me to estimate average daily encounter rate with pike for these minnows.

Mark recapture analysis

Mark-recapture data were analyzed with the Schumacher-Eschmeyer method and Schumacher-Eschmeyer confidence intervals were estimated (Seber 1982; Krebs 1989). The assumptions of this method are constant population size without recruitment and losses, random sampling, and that all individuals have the same chance of capture. The relationship between the number of marked fish and the proportion of marked fish in each sample was approximately linear, indicating that assumptions likely were met.

Encounter rates

Encounter rates were estimated from the catches in Location 10 using catch per hour multiplied by length of daylight (corrected for pike activity patterns (Ivanova 1969; Christiansen 1976) to estimate average number of pike encountered by a nesting minnow per day. I assumed that pike are mobile at dawn and dusk and so to avoid overestimation of encounter rates, I calculated amount of time active as a proportion of the total daylight length (8 of 15 h in May, 10 of 17 h in June, and 11 of 17 h in July). I used the length of daylight for Edmonton, Alberta (lat. 53.6° long. 113.5°). I then multiplied each per day calculation by 10 to estimate the number of pike a male minnow might encounter during an average 10 d nest period. I did not estimate the encounter rate for August because I did not want to disturb nesting male minnows that were to be used in another experiment (Chapter 2). All means are presented with their associated standard errors.

Estimation of high and low density pike locations

For May and early June, I used catch per unit hour by location (Figure 3.1) as an estimate of density patterns (n=24 nets). I used these results to define areas of high and low densities. I defined locations with consistent high catch per unit hour (CPUH) of pike over the sampling period as high-density-pike locations and low catch per unit hour as low-density-pike locations. Locations 2, 3, 4, and 6 showed consistently higher CPUH (mean 1.5 fish per hour) whereas locations 9, 10, and 13 had lower catches (mean 0.6 fish per hour). I could not use locations 1 and 5 as they were often inaccessible. I pooled capture data for June, July and August and calculated the CPUH.

Artificial and Natural Nest Use in each location

Minnows nested under logs, rocks, twigs, and bark. Each of the seven locations (defined above) had suitable nesting substrate for fathead minnows, except the bay (Location 2), where minnows could nest only under the leaves of yellow lilies (*Nuphar variegatum*). There was not always an equal number of nests available in each of the high and low-density pike locations. There were more potential nests at Location 4 and Location 10 than at other locations. Thus, I used artificial nests to offer similar nesting opportunities. In the four high-density-pike locations and three low-density pike-locations, I placed artificial nest boards on the water surface (mean depth to the bottom of lake from the board was 23 ± 3 cm). Boards were 13 x 25 cm and were anchored to rocks with nylon cord. Nine boards (three sets of three boards tied together) were placed in each of the seven locations in early June. Preliminary investigations in 1993 indicated that male fathead minnows consistently used these boards as nests. I defined use of a board as the presence of eggs on the underside of the board.

I monitored the boards twice a month in June, July and August. The length of the nest period (time for eggs to hatch) in June, July and August was typically 10 d. Thus, when monitoring nests twice a month, I did not resample eggs.

In addition to artificial nests, I surveyed the seven locations twice a month for use of natural nests. I looked for nests under the abundant leaves of yellow water lilies (McMillan and Smith 1974; B. Gingras, pers. comm.), but none were located. I located natural nests either by walking the shorelines and overturning potential sites, or by canoeing to deeper locations and examining nests for the presence of eggs. Presence of conspicuous males with breeding coloration was also used to indicate possible presence of a nest. I could not document the total number of natural nests in any location because persistent high winds continually altered the availability of nesting substrate (*i.e.*, waves continually deposited and removed debris, rocks and fallen logs that minnows used as nests).

To determine if minnows were present at all locations, I set minnow traps once a month in the early summer in locations where the artificial boards were placed. I set one to three unbaited minnow traps in each of the seven locations. I set traps in early June and again in early July 1995. When traps could not be set, I sampled locations with a gill net (experimental mesh size).

I analyzed the use of artificial and natural nests between high and low-density locations for all months combined with Friedman's nonparametric test (Zar 1984) using Systat® 5.2.1 (Wilkinson 1992). Data were pooled between locations by density. I inspected boards twice a month in all 7 locations for three months; thus my sample size was 18 for low-density locations and 24 for high-density locations.

Results

Mark-recapture analysis

Gill nets were set a total of 35 times in the sampling period (April to October); there were 4 nets per set (n=140 nets) and thus each location was sampled approximately 10 times throughout the season. I captured 122 northern pike and recaptured seven of those. There was a bi-modal size distribution of northern pike (Figure 3.2). I captured 135 white suckers (*Catostomus commersoni*), all between 37-45 cm in total length.

I estimated the density of northern pike to be 421 (95% confidence intervals: 265, 1077). This is equivalent to 1.7 pike per hectare with a range of 1.1 to 4.1 pike per hectare based on the upper and lower confidence intervals. These density estimates are low compared to some other north temperate lakes (Turner 1984). Roi Lake in central Alberta contained 22.5 pike per hectare (Turner 1984) and a Wisconsin Lake was estimated at 2.2 - 10.1 pike per hectare (Kempinger and Carline 1978).

Encounter rates

I used encounter rate to estimate of the frequency at which a nesting minnow may encounter a northern pike. Estimated mean encounter rates with northern pike for minnows on the east shore differed between summer months. In May, I only caught 1.0 ± 1.0 pike per day. This number rose to 2.8 ± 1.3 pike per day in June. In July, when northern pike post-spawning mobility was low (Diana *et al.*, 1977), the encounter rate was 0.3 ± 0.3 pike per hour. Thus, I estimated that a male at Location 10, nesting for an average of 10 d, could encounter 3 to 28 pike, depending on when it nested.

Artificial and Natural Nest Use

As predicted, minnows used artificial nest boards more frequently in areas with low-pike densities than in areas with high-pike densities (Figure 3.3). In the three low-density locations, mean board use was 1.7 ± 3.0. In high-density locations, mean use was 0.7 ± 1.6 . Variability was high because there were very few nests at any of the locations in June and early July due to persistent high winds creating wave action that precluded nest defense. Nest boards in locations 2 and 9 were not used.

This pattern was similar for natural nests. Significantly more natural nests with eggs were found in locations with low-pike densities than in locations with high-pike densities (Figure 3.4). Low-density locations had on average 5.7 ± 9.7 nests with eggs, whereas high-density locations had 1.4 ± 2.1 . Again variability was high because there were so few nests in June and early July. I found no nests with eggs in either locations 2 or 9.

Minnows were present at all locations in early June and in early July (Table 3.2). The proportion of each sex caught was variable; more males were trapped in June while more females were trapped in early July.

Discussion

I conclude that there was differential use of habitat by northern pike in this lake during summer. There was consistent pike activity in the bays (Locations 2 and 8), near beaver lodges (Location 3) and fallen spruce (Locations 4 and 6); there was submergent and emergent vegetation and fallen trees in each of these areas. Pike were found in less vegetated areas (Location 10) but less frequently than in areas where habitat complexity was greater. Chapman and Mackay (1984) reported that pike select from the habitats available to them, "relying most heavily upon the shallow vegetated areas of the lake". "Hot spots" and "cold spots" for pike activity may be partially responsible for the observed pattern of distribution of fathead minnow nests.

Estimates of encounter rates of nest-guarding males with northern pike varied throughout the season. In May and June, when post-spawning mobility for pike was high, and when minnows first defend territories, there was high variability in the number of pike caught near nesting minnows; males could have encountered 0.3 to 2.8 pike per day and thus 3 to 28 pike per 10 d nesting period. This data, though variable, suggests that nesting minnows experience pervasive predation threat. While there were other fish (female and juvenile fatheads and brook sticklebacks (*Culaea inconstans*)) present on the shoreline to which pike may have been attracted, nesting male minnows and competing males without nests were the most common (unpubl. data). Granted, not all pike found on the shoreline were necessarily a direct (lethal) predation threat, but even presence of a predator near a nest affects parental behaviours of minnows in their nests (Chapter 2) and potentially reproductive success of males.

It was important to document in-lake encounter rates in order to estimate the degree of predation threat. Field estimates of encounter rates can be also used in laboratory experiments (Chapter 4) to realistically simulate the frequency of predator activity (Helfman 1986).

The population of northern pike in Armstrong Lake was estimated to be 421 (95% CI 265, 1077). The low population estimate could suggest that the predation threat to minnows was minimal. My low estimate could be due to few recaptures throughout the season. Low recaptures usually indicate high population numbers but in this instance it could reflect inadequacy in sampling. In addition, local residents reported northern pike winter kills that may have reduced the population. I suggest that though pike densities were low, the threat to minnows was great because fathead minnows may have been eaten preferentially by pike (Robinson 1989).

The lake contains fathead minnows, brook stickleback and white suckers, all of which are potential prey for northern pike. However, pike preferentially eat fathea minnows (Robinson 1989; 8 of 10 northern pike stomachs that I examined as a result of gill-netting mortality contained fathead minnows only). Hart and Connellan (1984) showed that pike grew fastest when eating small minnows. Sticklebacks possess spines that pike often avoid (Wahl and Stein 1988; Robinson 1989). Adult white suckers may have been too large a prey item (mean 46 cm) for gape-limited predators like northern pike (Hart and Hamrin 1988). Further, because suckers often require flowing water, *e.g.*, streams for reproduction (Scott and Crossman 1973), and because I did not capture any small (< 37 cm) suckers, I suggest that suckers may not use this lake for reproduction and thus juvenile suckers may not be available to pike as food. Presumably, the lake could maintain a large population of small-sized pike but because the pike caught were large relative to other populations (Turner 1984), the lake may only support a small population of large individuals. It is plausible that the large pike (60^+ cm) in the lake (Figure 3.2) were subsisting almost exclusively on fathead minnows; thus, relative to other northern pike lakes with different prey assemblages, this lake may be able to sustain only a small population of pike.

As predicted, minnows used artificial and natural nests more frequently in locations with low densities of pike than in locations with high densities of pike. This suggests that male minnows make decisions about where to nest based on their estimates of prevailing risk (*sensu* Lima and Dill 1990). I cannot conclude that predation pressure is the only factor influencing the observed pattern of artificial and natural nest use. Three alternative hypotheses could explain why males nested less often in "risky" areas. First, nesting patterns may be driven by the distribution of male and female minnows and not distribution of pike, although the two distributions were likely related. Because there were always male and female minnows present in all of the locations (Table 3.2), I suggest that absence of nests in the high-pike-density locations likely cannot be explained by absence of reproductive males and females in these areas.

Food availability is a second alternative hypothesis. However, because nesting male fatheads forage little during reproduction (McMillan and Smith 1974; pers. obs.) food may not be a factor determining nest site. However, females may choose males on the basis of food near their nests. My study did not determine if females are affected by predation. In convict cichlids (*Cichlasoma nigrofasciatum*), females laid significantly more eggs in secure areas than in risky areas (Wisenden 1992). Further work should examine responses to predation by both sexes.

The third hypothesis is that there is a trade-off between maximal visibility to females for mating and minimal visibility to predators. Males may choose nests on the basis of this tradeoff, making visibility to females the dominant factor influencing choice of nest site. If a trade-off exists, predation might still play a role, albeit a reduced role.

One last consideration is that nest use may simply be a function of nest availability. Availability of nests, however, was complicated by the relationship between habitat used by minnows and habitat used by pike. Female and juvenile minnows used vegetated and unvegetated areas as well as areas with fallen trees. Breeding male minnows predominantly used less vegetated areas as well as areas with fallen trees and beaver lodges because nests were available there. Northern pike were primarily found in areas that were structurally complex, *i.e.*, areas with emergent and submergent vegetation and fallen trees and beaver lodges. It may be that male minnows simply nest in locations where nests were available, rather than deciding to nest where there were few pike. It is difficult to differentiate the effects of differential habitat use by pike and minnows and the true role of predation in determining the pattern of nest use for fathead minnows. The use of artificial boards, though, ensured that nests were available in all locations. There were many unused nests, both artificial and natural, in the high-density-pike areas whereas the low density areas consistently had few nest vacancies.

My results are consistent with those of Fraser and Gilliam (1992) who found that space use by guppies (*Poecilia reticualta*) and killifish (*Rivulus hartii*) in predator environments was suppressed. Moreover, intimidation by a predator suppressed egg production of killifish by 50%. Presence of a predator created "spatial patchiness (more eggs laid in safer pools) and temporal patchiness (pulses of eggs) in egg production" (Fraser and Gilliam 1992). I noted pulses of fathead minnow eggs during the summer months. However, these "pulses" may have been more strongly influenced by environmental factors, particularly the strong winds throughout May, June and most of July that made nest-guarding difficult (see Reznick and Yang 1993 as an example of the influence of environmental factors).

My data suggest that male fatheads may choose to nest in areas where they assess predation risk to be lowest. In addition, local predation "hot spots" may be sites where alarm pheromone is common, and minnows may choose to avoid these areas based on chemical cues (Chapter Four; Mathis and Smith 1992). Fathead minnows are a vulnerable prey species and thus tolerance to predation must be aided by behavioural adaptations such as avoidance of areas where predation may be high.

Spatial segregation of minnows to shallow areas with little vegetation (behavioural avoidance) may be an adaptation that allows pike and fathead minnows to co-occur.

Location	Description	Vegetation
1	South bay exterior	emergent and submergent
2	South bay interior	emergent and submergent
3	Beaver lodge	submergent
4	Fallen spruce tree	sparse submergent
5	Fallen dock	emergent and submergent
6	Fallen spruce tree	submergent
7	Beaver lodge	emergent and submergent
8	North bay exterior	emergent and submergent
9	North bay interior	emergent and submergent
10	East shore	sparse submergent
11	Beaver lodge	emergent and submergent
12	West shore	emergent and submergent
13	Beaver lodge	emergent and submergent

Table 3.1 Locations for gill nets in Armstrong Lake. Figure 2.1 illustrates locations. Locations were chosen for accessibility, vegetation and habitat structure. Minnows nested in areas with structures such as beaver lodges, a dock and fallen trees. Locations were sampled with random stratified sampling. Locations 5, 11 and 12 became inaccessible because of vegetation growth.

Date	**Pike Density**	**Location**	**# of traps**	**gill nets**	***Males***	***Females***
June 9 1995	high	2	2	1	36	11
	high	3	3	0	199	99
	high	4	2	0	173	86
	high	6	1	0	36	9
	low	9	0	1	31	6
	low	10	1	0	21	72
	low	13	0	1	22	11
July 6 1995	high	2	1	1	41	22
	high	3	3	0	42	122
	high	4	1	1	56	81
	high	6	0	1	9	41
	low	9	1	1	22	59
	low	10	1	0	3	61
	low	13	0	1	11	32

Table 3.2 Number of minnows trapped or netted in four high-density-pike and three low-density pike locations for one trapping date in June and in July 1995. High and low pike-density locations were defined in Figure 3.1.

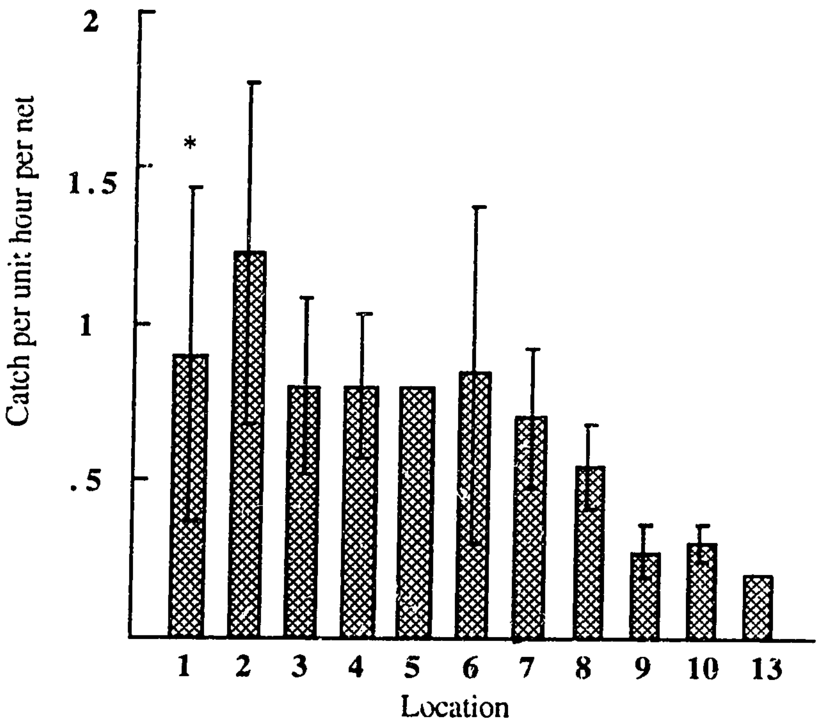

Figure 3.1 Catch per unit hour (CPUH) of northern pike for May and June 1994 in Armstrong Lake, Alberta. Error bars indicate one standard error. Locations 2, 3 4, and 6 were "high-density-pike" locations. Locations 9, 10 and 13 were "low-density-pike locations".* Locations 1 and 5 were not chosen as high-density- pike locations; I could not place artificial boards there because of extensive vegetation growth.

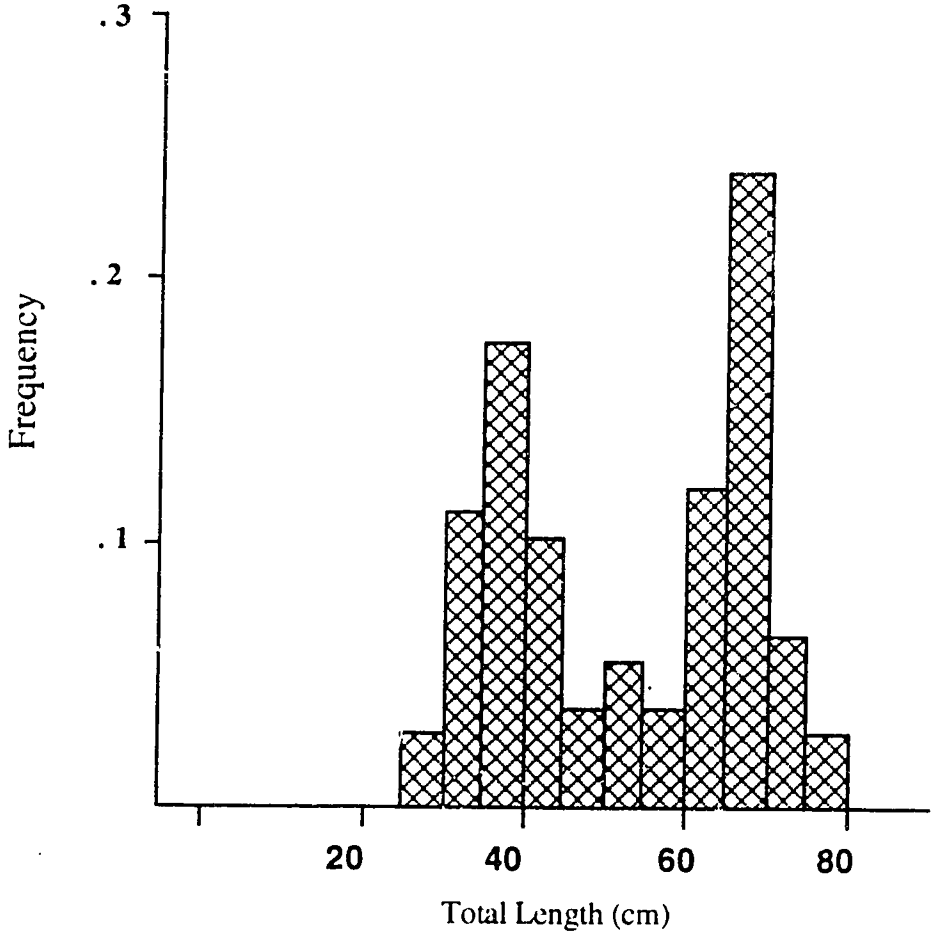

Figure 3.2 Frequency distribution of total length (cm) of northern pike in Armstrong Lake (n=122).

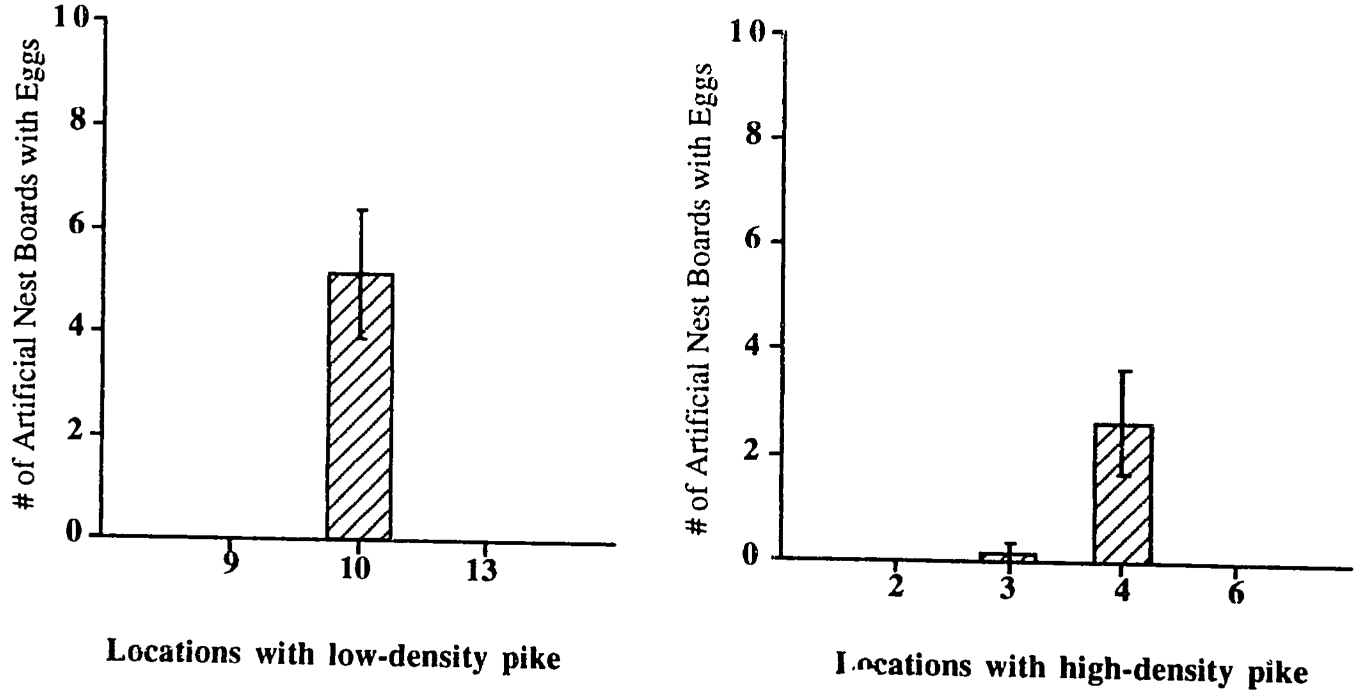

Figure 3.3 Use of artificial nest boards by fathead minnows in Armstrong Lake, Alberta (June, July and August 1994). Bars represent one standard error. Total number of artificial boards available per location was 9. Boards in low density locations had significantly more clutches than boards in high density locations (Friedman=34.4, $p < 0.001$). No eggs were found on nestboards in locations 2, 6 and 9.

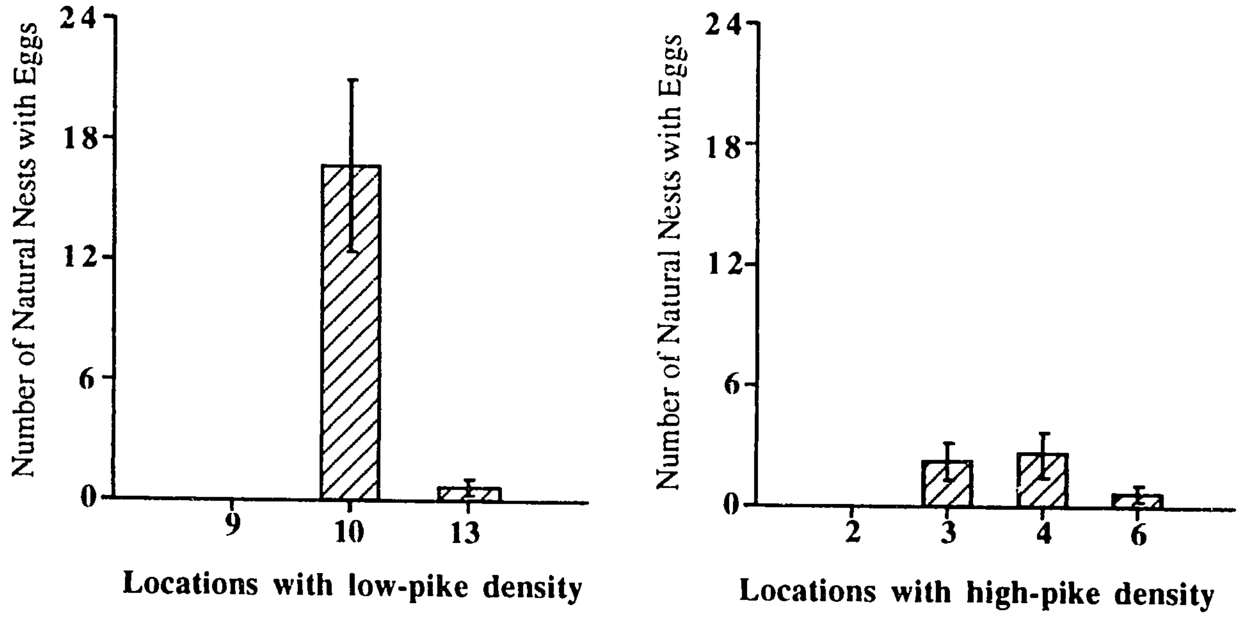

Figure 3.4 Number of natural nests used by fathead minnows in Armstrong Lake (June, July and August 1994). Bars represent one standard error. There were significantly more clutches in locations with low densities of pike (Friedman=16.1, $p < 0.001$). Although there were more potential nest sites at Location 10 and 4 than at the other locations, there were still many more nest vacancies at Locations 3, 4, and 6 than at 10. I did not find nests with eggs in Locations 2 or 9.

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Chapter Four

Effects of visual and chemical exposure to northern pike on territorial behaviours of fathead minnows

Introduction

Predation is now acknowledged as one of the main factors influencing prey behaviour (review in Lima and Dill 1990). Predator avoidance can translate into changes in habitat use, feeding, morphology and growth of prey (Sih 1980; Dill and Fraser 1984; Helfman 1986; Craig 1994; Godin and Crossman 1994). Most investigations of predation risk address escape responses, patch use, and vigilance (Martel and Dill 1993). Predatory impacts on **reproduction** are not well known (Sih 1987; Lima and Dill 1990; Magnhagen 1990). Whereas some studies show that guppy courtship behaviour (Endler 1987) and female frog (*Hyla chrysoscelis*) oviposition ("egg-laying") sites (Resetarits and Wilbur 1989) are altered in the presence of a predator, there is little evidence that "mature animals facultatively alter reproductive behaviour when the threat of predation is changed" (Fraser and Gilliam 1992). The "magnitude of prey response to predation is [likely] proportional to predation risk" (Sih 1987), but what are the effects of conflicting demands, such as mating opportunity versus visibility to predators, on anti-predator responses?

The fathead minnow (*Pimephales promelas*) is a common, small-bodied fish of boreal lakes that is vulnerable to predation (Robinson 1989; Robinson and Tonn 1989). Because fatheads exhibit a variety of behavioural patterns, such as courtship (Cole and Smith 1987), male parental care of eggs, and alarm responses (McMillan and Smith 1974), they are an ideal species for studying the effects of varying predator regimes on reproductive behaviour. Further, northern pike (*Esox lucius*), a dominant piscivore in boreal lakes preferentially eat fathead minnows over most other prey species (Mauck and Coble 1971; Moody *et al.*, 1983; Wahl and Stein 1988; Robinson 1989).

In Alberta, fathead minnows grow for 2 years, are reproductively active for approximately 2 months, then die (Unger 1983; Price *et al.*, 1991). Prior to reproduction, males develop dark nuptial coloration and tubercles on their snouts (McMillan and Smith 1974). Male courtship consists of approach and leading of females to appropriate nest sites (Cole and Smith 1987). Nesting is in territories beneath floating or submerged objects (McMillan 1972). Males clean and defend nests with clutches of eggs deposited by several females (Unger 1983) by rubbing the eggs and chasing egg predators and nest competitors. Site attachment, increased coloration, and conspicuous territorial behaviours may make reproductive males vulnerable to ambush, littoral predators such as northern pike (Diana *et al.*, 1977; Eklöv and Diehl 1994). Matity *et al.* (1994) showed that breeding male fatheads were more vulnerable to predation from garter snakes (*Thamnophis radix*) than juveniles, non-breeding males and females.

Fathead minnows use vision and chemical cues to detect and avoid predators (Smith 1992; Chivers and Smith 1993). Visual recognition of a predator induces a fright response of dashes and skitters by prey, but may not provide enough time for evasion (Mathis and Smith 1993 a). Fathead minnows possess a second mechanism for detecting and evading predators, a chemical alarm signal system. They have club-shaped epidermal cells ("alarm substance cells") that release a pheromone into the water when fish are injured. Nearby minnows detect this and perform a fright reaction. Breeding male fathead minnows lack alarm substances but retain the fright response to the pheromone (Smith 1973). Alarm signaling as an indication of predatory threat, may alter predator-prey interactions (Smith 1992); for example, in a field experiment, Mathis and Smith (1992) reported that minnows avoided traps where alarm pheromone was present. Moreover, exposure to conspecific alarm pheromone improved the survival of minnows during encounters with northern pike (Mathis and Smith 1993 a)

I conducted a laboratory study to determine if fathead minnows males altered their territorial behaviours associated with nest-guarding in response to combinations of visual and chemical cues from predators. I used the presence of a pike as a visual cue and fathead minnow alarm pheromone as a chemical cue. I examined territorial defense of males prior to egg-guarding. During this period, males spend the greatest time away from the nest itself (see Appendix Three) and exhibit conspicuous behaviours such as approaching and leading females (Cole and Smith 1987) that take them up to 1 m away from their nests (unpub. data). Presumably those activities make them more vulnerable to "sit and wait" predators like northern pike than during egg-guarding when males spend most of their time in the protective cover of the nest. In addition, I compared reproductive behaviours of fathead minnows in the laboratory and in a boreal lake to determine if behaviours in the laboratory were comparable to those of minnows breeding under natural conditions.

I predicted that temporary increases in risk (24 h of exposure to cues from predators) would result in less rigorous defense (Carlisle 1982; Clark and Ydenberg 1990). I predicted that males would exhibit a "graded" response and alter their behaviours when the threat of predation was changed; males would decrease territorial behaviours when either visual or chemical cues were presented, but would dramatically decrease behaviours in treatments where visual and chemical threats were combined.

Methods and Materials

Experimental fish

Three northern pike were gill netted from Amisk Lake (54° 35 ''N 112° 37''W) in May of 1993 and three from Armstrong Lake (54° 24° 'N 113° 39''W) in May of 1994. Total length of each pike was recorded. Pike were held outdoors in 1500 l holding tanks at Meanook Biological Research Station (MBRS), Alberta. Each pike was fed five fathead minnows twice weekly. Pike that were used in experiments were transported indoors to a 300 l glass-fronted, holding tank to acclimate them to laboratory conditions. They were not fed for 5 d before a trial to ensure that all minnow exudates passed from the pike prior to experimental use (Chivers and Smith 1993) and to ensure that pike were hungry during treatments. Pike were rinsed with clean water prior to introduction in an experimental tank to remove any remaining minnow residues from the pike's skin (Chivers and Smith 1993).

I collected reproductive male and female fathead minnows from late May to early August in 1993 and 1994. To ensure that experimental minnows were "predator experienced" (Mathis and Smith 1993 b), minnows were collected from Armstrong Lake where fathead minnows coexist with northern pike (Robinson and Tonn 1989). Fish were collected with unbaited minnow traps and were transported to MBRS and placed in 1500 l outdoor, fiberglass holding tanks. Fathead minnows were fed commercial flaked food (Tetramin) once daily.

Experimental Protocol

Laboratory experiments were conducted at MBRS from May to October in 1993 and 1994. Experiments were performed in four, 450 l glass-fronted fiberglass tanks. Each tank was divided, resulting in two 90 x 45 x 60 cm chambers ; halves of the tank were separated by a perforated metal screen. The left chamber of the tank contained 3 artificial minnow nests and 2 plastic plants (11 cm in height). Each artificial nest was constructed of wood (13 cm^2 x 1.5 cm) and was anchored 11 cm above a 10 cm rock with nylon twine. Nests were placed a minimum of 18 cm apart, at right angles to one another, to ensure that male territories would overlap little (Anon. 1985) and that all males were visible.

Six randomly chosen, reproductive fathead minnows (four males, two females) were placed in the left half of each tank. I assumed that minnows were in reproductive condition when males exhibited a pronounced dorsal pad and when females appeared gravid with a pronounced ovipositor (McMillan and Smith 1974). I chose six fish so there would be competition between males for nest sites and for females. Competitive males appear more robust and frequently engage in more conspicuous behaviours (Unger 1983; pers. obs.). Minnows were tested only once to avoid learned responses (Chivers and Smith 1994) and habituation to stimuli (Martin and Kraemer 1987; Martin and Bateson 1993). Minnows were maintained on a 18 : 6 light-dark photoperiod at 21° C and were fed Tetramin once daily. A 8 x 5 x 10 cm charcoal filter was placed in the right chamber of the tank so males could not use it as a nest site. Water was changed between treatments.

I allowed minnows to acclimate to the tanks and males to establish territories around the artificial nests for 4 d before stimuli were introduced. I defined territory as the area underneath and immediately surrounding the nest. Prior to stimuli, there were no nests with eggs. After the acclimation period, a male with consistent territorial fidelity was chosen as the focal male.

I measured seven territorial behaviours of the focal male. Territorial behaviours included chase, rub, tight circle, wide circle (see Chapter Two for definitions), forage, tail beat, and head butt. Forage was any bite of flaked food either on the surface or bottom of the tank. Tail beat was a sweep of the focal male's tail towards a territorial intruder (McMillan 1972). Head butts occurred when two males engaged in a "mutual snout-butting contest", resulting in direct contact of tubercles (McMillan 1972). I also recorded color score (1 - 5) (see Chapter Two) in each of the intervals to determine if males' color altered when threatened. Behavioural observations were recorded by an observer or video equipment. Video equipment allowed observers to view behaviours in an adjacent room without disturbing minnows.

There were four treatments : control, visual, chemical, and visual and chemical. For clarity, these treatments are hereafter referred to as control, Treatment V, Treatment C, Treatment V/C. Presence of a pike acted as a visual predation threat and fathead minnow alarm pheromone was used as a chemical cue of predation threat. In the treatments involving alarm pheromone, minnows that "donated" alarm pheromone were non-reproductive juveniles or females. No males were used because testosterone eliminates alarm pheromone in reproductive males (Smith 1973). Treatments were randomly assigned to each tank. In each treatment, the predator stimulus was added to the right chamber of the tank and remained present for 24 h. 24 h was likely a realistic period of predation threat given that I estimated that males in the wild may encounter 0.3 to 2.8 pike in 24 h (Chapter Three).

Control treatments involved the introduction of a 45 cm brick to the right chamber of a tank. The size of the brick was similar to the size of the northern pike used in Treatments V and V/C.

In the *visual treatment (Treatment V)*, one randomly selected northern pike (56 ± 3 cm total length) was fed two brook sticklebacks (*Culea inconstans*) (5.6 ± 0.5 cm). Brook sticklebacks possess a chemical alarm signal, but fathead minnows do not exhibit a fright response to skin extract from brook sticklebacks (Chivers and Smith 1994). It was necessary for pike to feed during this treatment for the methodology to be consistent with Treatment V/C where pike were fed fathead minnows. While pike "odour" was a chemical signal minnows likely detected, there was no conspecific chemical cue in this treatment.

In the *chemical treatment (Treatment C)*, I added fathead minnow alarm pheromone and a 45 cm brick. Pheromone was obtained by scraping the lateral sides of two non-reproductive donor fathead minnows with a dull blade and rinsing the damaged skin in the experimental tank (*sensu* Brönmark and Pettersson 1993). Donor fish were then fed to pike in the outdoor holding tanks.

In the *visual and chemical treatment (Treatment V/C)*, a randomly selected pike (56 ± 3 cm) was fed two fathead minnows. Before minnows were fed to the pike, their lateral sides were scraped. This ensured that alarm pheromone was available immediately upon introduction of the pike; therefore, there was no time delay between addition of the pike (visual cue) and the pheromone (chemical cue).

Focal males were observed for nine 30 min periods throughout the experiment. These periods were divided into 3 pre-stimulus, 3 stimulus-1 (< 12 h after stimulus introduction), and 3 stimulus-2 (> 12 h after the stimulus) samples. The final pre-stimulus sample was recorded immediately preceding addition of the stimulus. Immediately after introduction of the stimulus, focal males were observed for one 30 min interval, and then for two 30 min intervals within 12 h of the stimulus. During the interval immediately after introduction of a stimulus, observers recorded the response of every fish in the tank in order to document the effect of simulated threat on both sexes. I sampled stimulus-2 behaviours in three random intervals 12 h after a stimulus was introduced.

Experimental Analysis of Treatments

I used a multivariate repeated measures analysis of variance (MANOVAR) using Systat® 5.2.1 (Wilkinson 1992) to test whether behaviours differed between treatments over sampling intervals (Potvin *et al.*, 1990). Systat® used an alternate sum of squares to test hypotheses appropriate to the unbalanced design (Wilkinson 1992; Shaw and Mitchell-Olds 1993). Although the data were doubly multivariate (greater than one response variable per individual per treatment), I analyzed each behaviour separately in order to meet the assumptions of MANOVAR (von Ende 1993; see Appendix One for assumptions).

Data from each 30 min observation period were averaged within the three samples to mitigate the effects of individual variation (Martin and Kraemer 1987; Martin and Bateson 1993). This resulted in one pre-stimulus interval, one stimulus-1 (<12 h after stimulus) interval, and one stimulus-2 (>12 h after stimulus) interval per replicate. Means and standard errors for each sampling interval are presented in Table 4.1. Percentage change in number of behaviours performed in each sampling interval was calculated from this data; only significant changes are reported. Percentage change in numbers of each behaviour performed served only to describe the pattern of response of behaviours but because percentages can be misleading, particuluarly with low sample sizes, caution was exercised when interpreting them. Moreover, interpretation was restricted to significant contrasts (see below).

I used a 4 treatment x 3 sampling interval design with seven replicates per treatment. Fungal infections and failure of the screen divider, however, reduced these replicates (control n=4, visual n=2, chemical n=3, visual and chemical n=7). Nine focal males were impaired by fungal infections prior to addition of any stimulus and could not be used in any further trials. Three focal males were eaten by pike when the screen barrier failed upon introduction of the predator. Because there was only pre-stimulus data from each of these males, their behaviours were not useful for any analyses. Moreover, preparation of each trial took 10 d per tank and 12 d per tank if the barrier required repairs; if a trial was unsuccessful it could not be repeated.

Data were square root + 0.5 transformed (Martin and Bateson 1993) to meet assumptions of normality and homogeneity of variance. Tail beat, head butt, and forage were not used in analyses because they occurred infrequently and were difficult to observe. Cleans, while easily observed, violated the sphericity assumption of MANOVAR (Greenhouse-Geisser epsilon = 0.7850) because there were so few recorded and thus were not used in analyses. Chase, tight and wide circle behaviours were analyzed.

In the repeated measures design, treatment was the between-subject effect and sampling interval was the within-subject effect (von Ende 1993). Planned orthogonal multiple comparisons were performed after a significant MANOVAR (Maxwell and Delaney 1990). With four treatments, there were 6 possible contrasts of interest. Simultaneous analysis of 6 contrasts per behaviour, however, may have resulted in spurious significance (Hand and Taylor 1987; Maxwell and Delaney 1990; von Ende 1993). Thus I used a Bonferroni correction to control error rate (Maxwell and Delaney 1990). The adjusted level of significance was ∝ /number of contrasts = 0.01. While this may be overly conservative, especially for behavioural experiments (Maxwell and Delaney 1990), it controls the experimentwise error rate (probability that one or more contrasts will be falsely declared significant) at 0.05 (Maxwell and Delaney 1990). Discussion of contrasts was limited to those significant at ∝ = 0.01.

Behavioural comparisons between laboratory and lake

I compared reproductive behaviours of fathead minnows in the laboratory at Meanook Biological Station and in Armstrong Lake, Alberta to determine if behaviours in the laboratory were comparable to those of minnows breeding under natural conditions. In June and July of 1993 and July of 1994, I recorded reproductive behaviours of 40 nest-guarding male fathead minnows (20 laboratory males and 20 "lake" males).

Laboratory males were randomly chosen from outdoor holding tanks and were allowed a 4 d acclimation period to indoor tanks before observation (see above for tank description). Males in Armstrong Lake were located by shoreline observation. Of the males present on the shore, I randomly chose 3 males per day for 5 d. These five days were "hapahzardly" chosen (Martin and Bateson 1993) in June and July of 1993 when weather conditions allowed field observation. I measured males with and without eggs in their nests. In July of 1994, I observed five additional males to equalize the sample size between the laboratory and the lake. Each laboratory and "lake" male was observed only once.

In a 30 min observation period per male, I measured seven reproductive behaviours including chase, clean (rub), tight circle, wide circle, forage, head butt, tail beat (defined above). Head butts and tail beats were often impossible to record in the field and thus were not analyzed. Males foraged in both the laboratory and the lake while they were nest-guarding, but this occurred too infrequently to analyze.

Numbers of chases, cleans, tight circles and wide circles were compared separately between locations (laboratory and lake) with a Mann-Whitney U non-parametric analysis (Sokal and Rohlf 1981).

Results

In the 16 trials, all focal males responded to simulated predation threat with reduced activity, increased use of refuge (vegetation) and loss of breeding coloration. When a stimulus was added, focal males abandoned territorial defense and often hid behind the nests and artificial vegetation. The remaining five fish in each treatment hid with the focal male. Before addition of a stimulus, fish did not enter the vegetation. Control fish returned to their territories within 1 ± 5 min and chased intruders (fish that were "hiding" behind the nest) from the nest. Both males in Treatment V were motionless for 2 ± 3 min and then returned to their nests. Non-focal fish in Treatment V inspected the screen divider that separated the minnows from the pike. They initiated aggressive behaviours towards one another approximately 2 cm from the divider. This inspection alternated with non-focal males returning to their nests and back to the screen. In the stimulus-2 intervals, fish did not inspect the screen. Before the stimulus, control and Treatment V males were scored on average for breeding color as 3 ± 1 (n=9), indicating that they were dark with a pronounced dorsal pad (see Chapter Two). Color score remained at 3 ± 1 during both stimulus-1 and stimulus-2 intervals.

In Treatment C (chemical) and V/C (visual and chemical), all ten focal fish and all non-focal fish performed a fright reaction and then remained "relatively" inactive for up to 24 h. In the stimulus-1 intervals (<12 h after stimulus) when pike were present, all fish inspected the pike near the tank divider and often initiated aggressive behaviours towards one another near the divider; males butted the divider with their tubercles. This aggressive behaviour alternated with periods of inactivity ("hanging in the water column") near the divider. Within 17 ± 11 min after the introduction of a stimulus, nine of ten focal males in these two treatments repeated an inspection of the screen (where the pike's head was present), then swam towards their nests and back to the screen; they did not resume pre-stimulus activity levels. In the three Treatment C and seven Treatment V/C trials, all non-focal males completely abandoned their territories for the entire sampling period of 24 h and continued to swarm near the screen divider and initiate aggressive behaviours towards one another near the divider. They did not compete with the focal male for any of the nests. Males in the chemical treatment consistently scored 3 for breeding coloration in the pre-stimulus intervals and then 2, following introduction of a stimulus. Males in the visual and chemical treatments scored 4 ± 2 before addition of a pike, and scored 1± 2 after addition of the pike. One male returned to his pre-stimulus coloration within 12 hours after the pike was present.

Female response to predation threat was similar to the males. Generally, females sought cover immediately upon introduction of the stimulus. In control and Treatment V, females seemed to return to pre-stimulus levels of activity, while in Treatment C and V/C, females spent more time in vegetation and hiding under nests than in the pre-stimulus intervals. Interestingly, in two replicates of Treatment C and five of seven replicates of Treatment V/C, females deposited eggs in the focal males' nests. One clutch was found in a non-focal male's nest. Clutch size varied from 2 to 45 (n=7). I could not determine if eggs were fertilized.

Although pike tended to be inactive for most of the 24 h they were exposed to the minnows, their immediate response to introduction in tanks was to lunge and prod the divider and consume the prey fish provided. Approximately 1 h after introduction, pike became relatively inactive. When minnows approached the divider, pike lunged and attempted to attack the minnows.

Does differential predation threat alter territorial defense in males?

Chase behaviour

Within-subject effect (sampling intervals)

As predicted, there was an overall decrease in the number of chases performed between the sampling intervals (F = 11.579, $p < 0.001$, Greenhouse-Geisser $p < 0.001$). Specifically, the number of chases after introduction of a stimulus was significantly less than pre-stimulus chases for all treatments except the control (F = 6.860, $p = 0.004$). Males in Treatment V decreased the number of chases 68 % after introduction of the stimulus. Males in both Treatments C and V/C decreased the number of chases by 54 % (see Table 4.1 for data from which this was calculated). There were no differences in the number of chases between stimulus-1 and stimulus-2 sampling intervals (F = 1.137, $p = 0.385$). There was no interaction between treatment and time (sampling intervals) (Pillai's trace = 0.611, F = 1.760, $p = 0.150$). Thus, males in all treatments were similar in their response to stimuli over time, although control males did not decrease the number of chases over sampling intervals as the other males did.

Between-subject effect (treatment)

The number of chases performed by focal males was significantly different between treatments (F = 7.863, p = 0.004; Figure 4.1) for stimulus-1 and stimulus-2 intervals but not pre-stimulus. In the pre-stimulus interval, there were no differences in the number of chases between the control and treatments (F = 3.087, p = 0.104). In the stimulus-1 interval, Treatment V, C and V/C males chased less than control males (see Table 4.1 for significance values; see Table 4.2 for raw behavioural data). Twelve h after the stimulus, Treatment C and V/C males chased less than control males (Table 4.2).

Tight circles

Within-subjects effect (sampling interval)

As predicted, there was an overall decrease in the number of tight circles performed over the sampling intervals (F = 5.340, p < 0.012, Greenhouse-Geisser p = 0.012). Specifically, the number of tight circles after introduction of a stimulus was significantly less than pre-stimulus tight circles (F = 4.706, p = 0.016) except for the control. In the visual treatments, males decreased their tight circles by 32 %, males in the chemical treatment decreased by 87% and males in the visual/chemical treatments decreased their tight circles by 57% (But note, while the decrease is significant, there were no significant differences between the treatments (see between subjects effect)). There were no differences in the number of tight circles between stimulus-1 and stimulus-2 intervals (F = 1.265 , p = 0.337). The interaction between treatment and time (sampling intervals) was not significant (Pillai's trace = 0.627, F = 1.828, p = 0.136).

Between-subjects effect (treatment)

The only differences in the number of tight circles between treatments (F = 2.179, p = 0.143), was in the stimulus-1 sampling interval (within 12h of the stimulus), where males in Treatments V, C and V/C had significantly fewer tight circles than control males (F = 9.970, p = 0.008; Figure 4.2).

Wide Circles

Within-subject effect (sampling interval)

As predicted, an overall decrease in the number of wide circles was observed between the sampling intervals (F = 15.796, p < 0.001, Greenhouse-Geisser p <

0.001). Specifically, there were significantly fewer wide circles after introduction of a stimulus than in pre-stimulus intervals for Treatments V, C and V/C; control males did not decrease the number of wide circles. Treatment V males reduced their wide circles by 81%, Treatment C males by 79% and Treatment V/C males by 62%. The number of wide circles did not differ significantly between stimulus-1 and stimulus-2 sampling intervals (F = 1.199, p = 0.361). The interaction between treatment and time (sampling intervals) was not significant (Pillai's trace = 0.375, F = 0.924, p = 0.495), indicating that control and treatments were equivalent in their response to stimuli over sampling intervals.

Between-subject effect (treatment)

Overall, the number of wide circles performed by focal males was significantly different between treatments (F = 6.220, p = 0.009; Figure 4.3). In the pre-stimulus interval, however, there were no differences in the number of wide circles between controls and treatments (F =1.758 , p = 0.210). In the stimulus-1 interval, males in Treatment C circled less than control males (Table 4.2). Although males in Treatments V and V/C decreased their wide circles from pre stimulus, this did not differ from the decrease in number of wide circles performed by control males (F = 3.276, p = 0.095). Twelve h after the stimulus, control males circled more than Treatment C males (see Table 4.2 for significance values).

Did males in the laboratory and Armstrong Lake behave similarly?

Differences in the number of behaviours performed per 30 min of observation between twenty males in the laboratory and twenty males in Armstrong Lake were significant for cleans (rubs) (U= 75, p = 0.001) and wide circles (U=336.5, $p<0.001$). Neither the number of chases (U= 253.0, p = 0.151) nor the number of tight circles (U= 180, p = 0.588) was different between locations. Appendix Two lists the behavioural data and the means and standard errors of each of the four behaviours.

Discussion

Male fathead minnows altered their nesting behaviours when subject to increases in predation threat. Males did not change behaviours when presented control stimuli and returned to pre-stimulus levels of activity within 12 h when subject to the visual treatment (pike fed stickleback). Chemical, and visual and chemical cues combined elicited a dramatic decrease in three behaviours for up to 24 hours after stimuli were introduced. The design of the experiment allowed me to determine that minnows were responding to chemical cues, in the form of the alarm pheromone, and not simply to "pike odor" (Mathis and Smith 1993 b); chemical cues appeared to signal the greatest threat. The observed declines in chases and circles are not surprising given that males had a high expectation for future reproductive success and would thus attempt to decrease predation threat (Sih 1987). Minnows in the laboratory were not performing exaggerated or unrealistic behaviours as the numbers of each behaviour performed were similar to those performed by minnows nesting under natural conditions. The numbers of rubs and wide circles were higher in the "lake" males but this was not unexpected as males in the field had eggs that required cleaning (rubbing) and laboratory minnnows did not. Males in the lab likely circled their territories more than those in the field to attract females (McMillan 1972).

Minnows must make a trade-off between mating opportunity and nest defense and the visibility to predators. Presumably males subjected to predation threat decreased behaviours such as wide circles that may have been "risky". Vigorous chases and wide circles around the tank were very conspicuous and might easily be detected by a visual predator. While males did decrease the number of tight circles after introduction of a stimulus, the number of tight circles did not differ between treatments over time. Perhaps because this was a nest-oriented behaviour that, while obvious to the observer, did not take males away from the nest (a "safe" structure near vegetation) as did wide circles and chases. Tight circles are likely obvious to competing males and may signal to females that the male is defending his territory, though less vigorously. The inability to analyze other behaviours such as head butts and tail beats does not imply that these behaviours are less important in the behavioural repertoire of the fathead minnow, nor does it indicate that "ecologically interesting" behavioural alterations do not occur in the presence of predators in regard to these behaviours. It simply reflects the inability to consistently record behaviour accurately enough for statistical analysis.

That minnows did not decrease behaviours in response to control treatments suggests that, consistent with my field experiments (Chapter Two), males can distinguish between disturbances from predators and non-predators (the brick). Perhaps males do not respond as dramatically to visual cues alone because by inspecting the pike near the tank divider they determined that it was not a threat. Undoubtedly, a larger sample size of Treatment V could more convincingly document this response. In Treatments C and V/C, inspection was paired with chemosensory recognition of the pike through alarm pheromone and thus the pike may have been perceived as a threat. Many prey species "can evaluate predation risk at a gross level " (tell predators from non-predators) (Sih 1987) and even detect actively foraging predators from non-foraging predators (Sih 1987; Eklöv 1992). It is apparent that fathead minnows can adjust nest defense behaviours such as chases in response to risk. Endler (1987) and Magurran and Seghers (1990) documented similar patterns in guppies (*Poecilia reticulata*) where males, in the presence of a predator, altered their courtship behaviour.

I did not expect the high incidence of predator inspection in the three treatments. In ten of twelve trials (treatments only), minnows inspected the screen divider. I observed no predator inspections in my field study of egg-guarding fathead minnows responding to northern pike (Chapter Two) but inspection by fatheads were previously reported by Savino and Stein (1989). Laboratory fish may be somewhat habituated to their "safe" surroundings and thus fathead minnows may have inspected the pike to confirm its identity and to determine the severity of threat. Likely, they determined that the pike, while "normally" dangerous, was incapable of "reaching" them because of the divider. Under natural conditions, such inspection may be uneccesary because minnows learn that chemical cues from northern pike indicate "danger" (see Mathis and Smith 1993 b) and that delays in response to this cue are likely costly. In laboratory experiments, other fish such as the three-spined stickleback (*Gasterosteus aculeatus*), displayed continual "boldness" towards predators even when they were nest-guarding (Huntingford 1976). In addition, the European minnow (*Phoxinus phoxinus*) commenced inspection behaviour when confronted with a pike (Magurran and Pitcher 1987); it is thought that in populations of European minnows that naturally occur with pike, anti-predator behaviours such as predator inspection are inherited (Magurran 1990) .

Another unexpected finding in this study was the egg-laying by female minnows in Treatment C and V/C. Presumably females deposited eggs only in nests of focal males because focal males were those with established territories and conspicuous breeding coloration, making those males attractive to females. I could not determine if eggs were fertilized. However, because the eggs were deposited in a nest, and apparently not "dumped" by the female, it is possible that they were fertilized. This result differs from Wisenden (1992), who documented that female convict cichlids (*Cichlasoma nigrofasciatum*) laid significantly fewer eggs in risky caves than secure caves.

Two alternative hypotheses may explain why females deposited eggs under threat of predation. First, females may weigh the benefits of mate choice. If risk is perceived to be high (*i.e.*, detection of the alarm pheromone), females may decide to disregard any potential benefits she gains from selecting a high quality mate, and spawn with a low quality male (Wisenden, pers. comm.). This is consistent with the theoretical prediction that increased pre-mating risk should decrease choosiness of mates (Crowley *et al.*, 1991). A second hypothesis, not necessarily unrelated to the first, is that under predation threat, females may decide that future reproduction is unlikely; females simply decide to invest in current reproduction (Wisenden *in litt.*).

The anti-predator response (reduced activity, use of vegetative cover, and fright reaction) of fatheads in this study is consistent with other reports of non-breeding fathead minnow behaviours under threat of predation (Sullivan and Atchison 1978; Moody *et al.*, 1983; Mathis and Smith 1993 b). Territorial males reduced behaviours such as wide circles and chases in Treatments C and V/C. Chasing nest competitors and females and circling wide "on patrol" were behaviours that often forced males away from refuges (nest and vegetation). Males may have reduced these behaviours to lessen the probability of encounter with a predator. The (qualitative) decrease in breeding coloration in males in addition to these behaviours suggests that coloration makes males differentially vulnerable to visual predators. This supports the suggestion of Matity *et al.* (1994) that males were more vulnerable to garter snake predation than were females or juveniles because of their coloration and conspicuous territorial behaviours.

I had expected that the combined visual and chemical threats would elicit the most dramatic decline in behaviours. However, contrary to my predictions, males responded similarly in all behaviours to Treatments C and V/C. It may be that because the fatheads used in this study were familiar with northern pike, they were conditioned to respond to alarm pheromone as a severe threat (*sensu* Mathis and Smith 1993 b; Chivers and Smith 1994) more so than visual cues. However, it is likely that the strength of the chemical cue was high because I used alarm pheromone in a contained environment, whereas, under natural conditions, water flow must presumably reduce the strength of "raw" alarm pheromone. Nonetheless, this suggests that chemosensory predator recognition may be the primary method used to assess predation threat. Moreover, anti-predatory response to pheromone could be an adaptation in response to particular modes of predation (see for example, Jedrzejewski *et al.*, 1993). Cryptic ambush predators, like northern pike, could potentially always have an advantage if minnows relied solely on vision to detect them (*i.e.*, pike use crypsis and ambush predatory attacks and thus it is often impossible to have the advantage of an "advance" warning of an attack). Because fathead minnows often live in systems of high turbidity and/or high algal density (Scott and Crossman 1973), where visual information is often unreliable and difficult to obtain, use of an additional mechanism for detecting and evading predators could improve the probability of survival (see Mathis and Smith 1993 a). Obviously chemosensory recognition of predators and a fright reaction in response to this recognition is advantageous for a non-armored, non-cryptic species such as the fathead minnow, living in systems where visual information is infrequent, for it could allow receivers of alarm signals to avoid areas where predation risk is high or reduce conspicuous activities.

I conclude that male minnows can assess and respond to differential predation threat, weighing nest defense against predation risk. The decrease in behaviours for up to 24 hours in some fish could affect male fitness. The decision to stop vigorously defending a nest could result in a male losing his nest to competitors, allowing more robust males to acquire nests. The decision to stop conspicuously courting, approaching and leading females to the nest could force males to spawn quickly with females when the threat of predation is lower, potentially resulting in a small clutch. Finally, if predation risk is high late in the breeding season, males who are not vigorously defending territories may have to delay reproduction to the following year. The ecological consequences of predation threat for females minnows may be alterations in mate choice, forcing them to make "hasty" decisions about who to mate with and whether or not to delay reproduction. Moreover, because females are not site attached, they may simply leave areas that they perceive to be "high risk" and seek males elsewhere.

Treatment	*Interval*	*Chase*	*Clean*	*Tight Circle*	*Wide Circle*
Control	pre-stimulus	**91.75**	**6.25**	**51.75**	**73.50**
(n=4)		16.33	0.75	6.50	17.35
	stimulus-1	**103.25**	**7.75**	**68.75**	**44.00**
		23.77	1.55	24.05	13.57
	stimulus-2	**87.75**	**4.50**	**37.25**	**37.50**
		27.41	2.40	7.92	3.48
Visual	pre-stimulus	**98.50**	**8.95**	**42.15**	**74.15**
(n=2)		6.50	7.75	4.15	2.15
	stimulus-1	**32.00**	**4.25**	**28.50**	**13.75**
		4.00	4.25	16.50	5.25
	stimulus-2	**51.50**	**6.25**	**54.50**	**47.40**
		3.50	4.75	39.50	9.10
Chemical	pre-stimulus	**36.67**	**4.00**	**59.00**	**33.00**
(n=3)		6.53	4.00	32.14	7.94
	stimulus-1	**17.00**	**0.33**	**7.67**	**6.67**
		8.54	0.33	6.23	6.67
	stimulus-2	**13.33**	**0.33**	**13.33**	**9.00**
		13.33	0.33	8.10	9.00
Vis/Chem	pre-stimulus	**53.74**	**32.71**	**55.86**	**50.86**
(n=7)		11.15	14.59	9.96	7.85
	stimulus-1	**24.71**	**9.43**	**23.57**	**19.14**
		5.83	5.44	6.69	9.22
	stimulus-2	**19.29**	**7.86**	**18.00**	**14.86**
		5.68	3.09	5.09	3.71

Table 4.1 Non-transformed means (with standard errors) of four behaviours in the four treatments in three sampling intervals. Mean average decrease (see text) for chase, tight and wide circles in each time interval was calculated from these means.

Behaviour	Sample Interval	Contrast	F	*p*	significance
Chase	pre-stimulus	control vs C	7.09	0.021	
	stimulus-1	control vs V	6.90	0.022	
	stimulus-1	control vs C	19.37	0.001	*
	stimulus-1	control vs V/C	19.06	0.001	*
	stimulus-1	V vs C	9.77	0.009	*
	stimulus-2	control vs C	12.12	0.005	*
	stimulus-2	control vs V/C	16.64	0.007	*
Tight Circle	stimulus-1	control vs all	9.97	0.008	*
Wide Circle	pre-stimulus	control vs C	4.81	0.049	
	stimulus-1	control vs C	7.82	0.016	*
	stimulus-2	control vs C	9.93	0.008	*
	stimulus-2	control vs V/C	5.61	0.035	
	stimulus-2	V vs V/C	5.80	0.033	

Table 4.2 Multiple contrasts of chase/circle behaviours between treatments. Only contrasts between treatments over the three sample intervals (pre,stimulus-1,and stimulus-2) that were significant at the .05 level are presented. This significance level was adjusted (Bonferroni) for the number of contrasts per sampling interval (contrasts=6). Contrasts were then only accepted as significant if alpha was ≤.01. Treatment contrasts are labelled V=visual treatment, C=chemical treatment, and V/C=visual and chemical treatment.

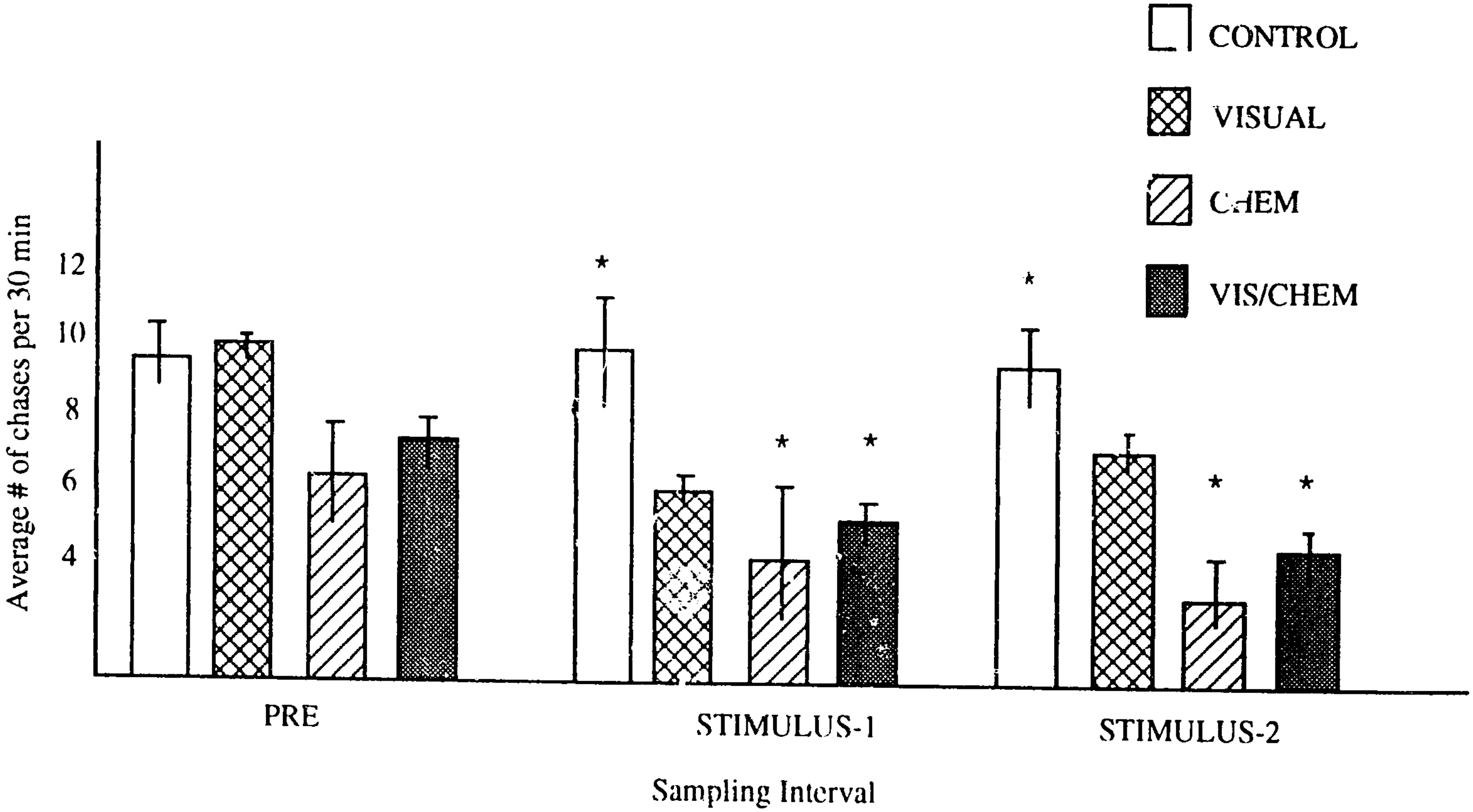

Figure 4.1 Average number of chases (sqr. root + 0.5 transformed) per 30 min per treatment in each sampling interval (n=4 control, n=2 visual, n=3 chemical, n=7 visual and chemical). Bars represent one standard error. Pre behaviours were averaged from three 30 min recordings 12 h before the stimulus was added. Stimulus-1 behaviours were averaged from three 30 min recordings taken immediately after introduction and within 12 h of the stimulus. Stimulus-2 behaviours were averaged from three 30 min recordings 12 h after the stimulus. The number of chases decreased from pre to stimulus-1 and stimulus-2 sampling intervals (F = 11.579, p<0.001). * In the stimulus-1 and stimulus-2 sampling intervals, chemical and visual/chemical treated males had fewer chases than control males (Table 4.2).

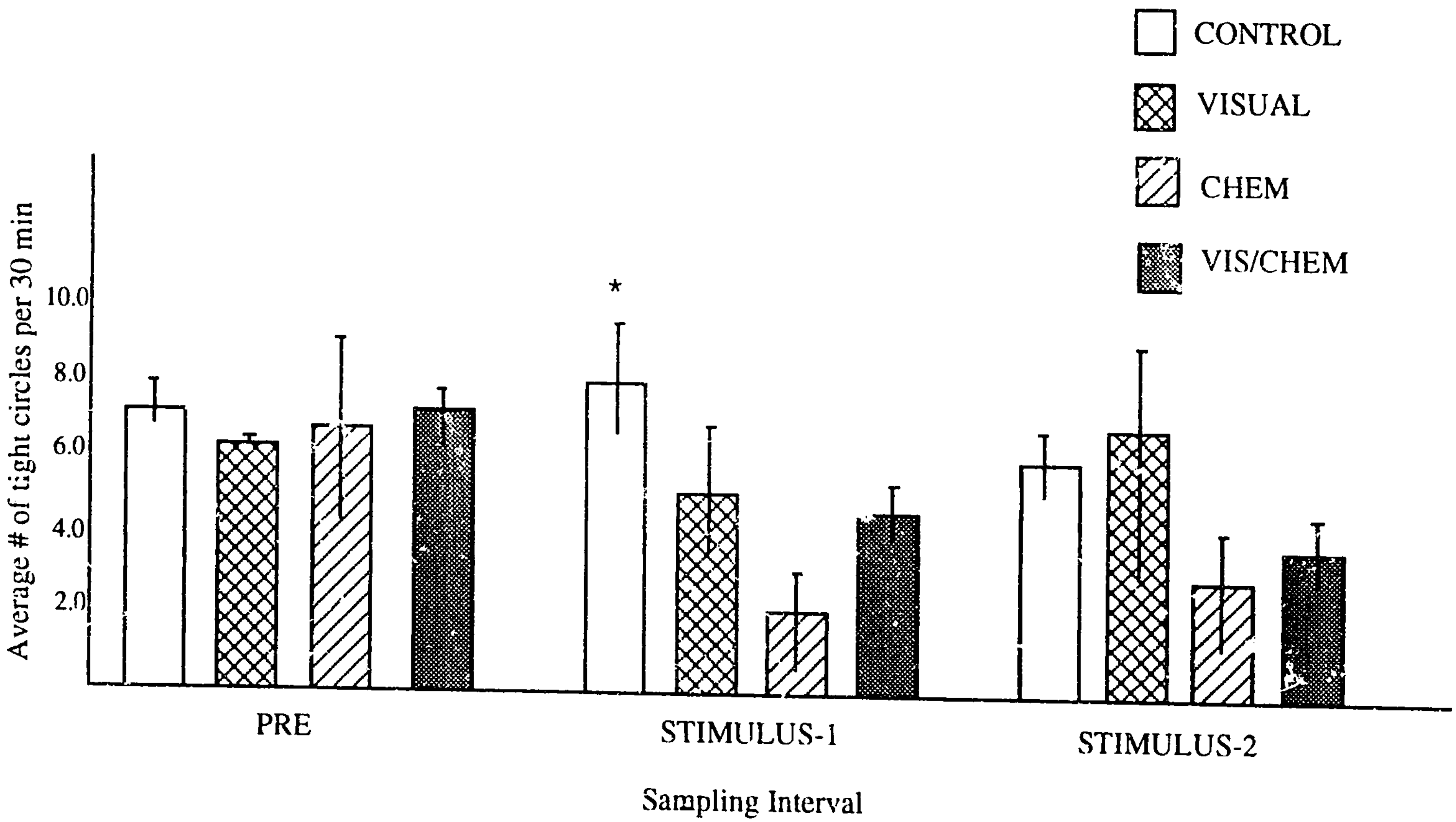

Figure 4.2 Average number of tight circles (sqr. root + 0.5 transformed) per 30 min per treatment in each sampling interval (n=4 control, n=2 visual, n=3 chemical, n=7 visual and chemical). Bars represent one standard error. Pre behaviours were averaged from three 30 min recordings 12 h before treatment. Stimulus-1 behaviours were averaged from three 30 min recordings taken immediately after introduction and within 12 h of the treatment. Stimulus-2 behaviours were averaged from three 30 min recordings 12h after the treatment. There was a significant reduction in the number of tight circles from pre-stimulus to stimulus-1 sampling intervals (F=4.706, *p* =0.016). * Control males had significantly more tight circles in the stimulus-1 sampling interval than males in the other treatments (F=9.970, p=0.008).

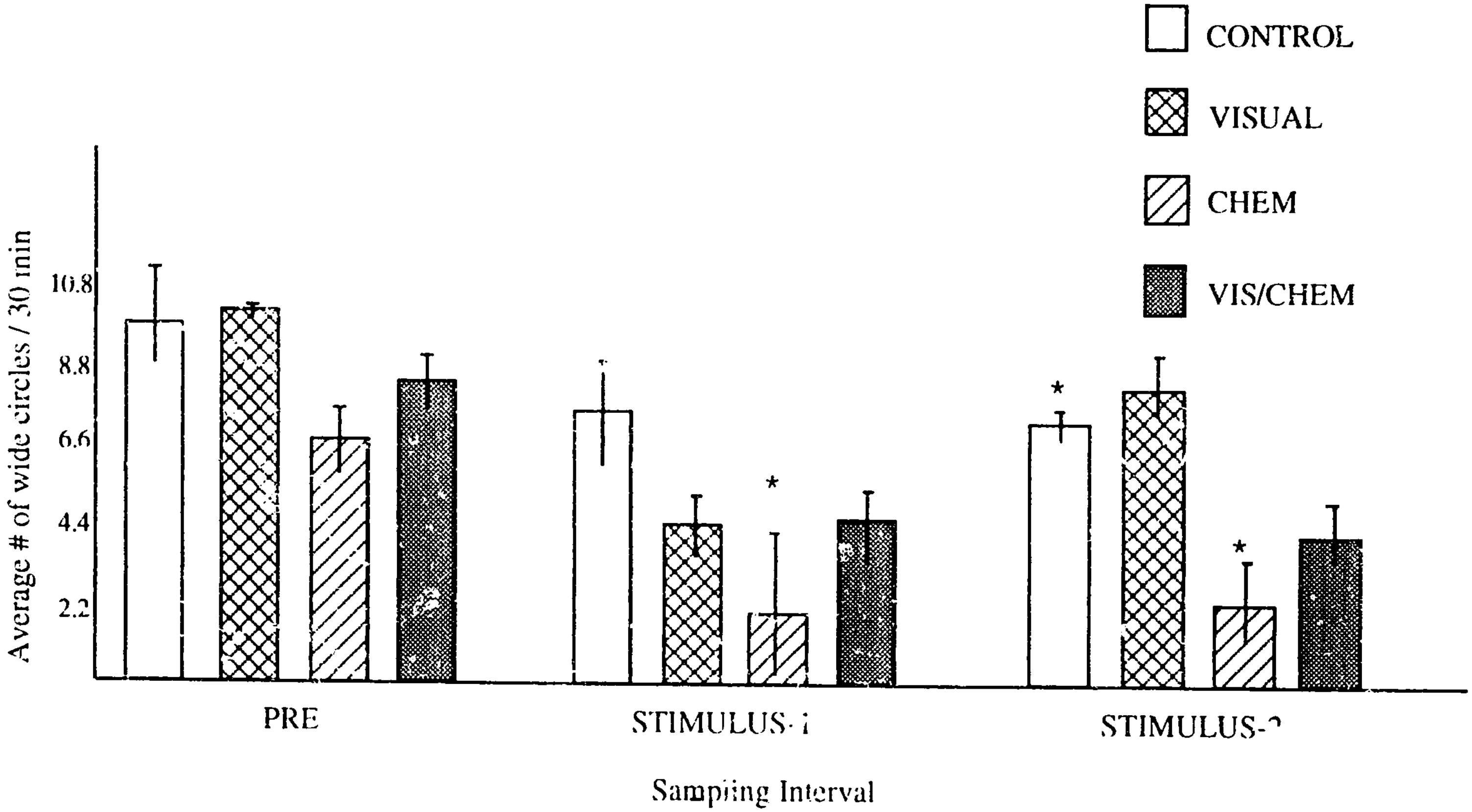

Figure 4.5 Average number of wide circles (sqr. root + 0.5 transformed) per 30 min per treatment in each sampling interval (n=4 control, n=2 visual. n=3 chemical, n=7 visual and chemical). Bars represent one standard error. Pre behaviours were averaged from three 30 min recordings 12 h before the stimulus was added. Stimulus-1 behaviours were averaged from three 30 min recordings taken immediately after introduction and within 12 h of the stimulus. Stimulus-2 behaviours were averaged from three 30 min recordings 12 after the stimulus. There was a significant reduction in the number of wide circles over sampling intervals (F=6.860, p =0.004). * During the stimulus-1 interval, only the chemical treatment was different than the control. In the stimulus-2 interval, the control was significantly greater than the chemical treatment, the visual treatment was not different from the chemical or visual and chemical treatments. Refer to Table 4.2 for significance values.

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Chapter Five

General Discussion

This study documented the behavioural response of breeding male fathead minnows to predation threat. Specifically, my objectives were to determine (1) if males in the wild were sensitive to changes in predation risk and if extrinsic and nest factors affected male's behavioural responses to risk? (Chapter Two), (2) if patterns of nest locations and densities of fathead minnow nests with clutches related to densities of northern pike in a boreal lake? (Chapter Three), and (3) if differential predation threat (visual and chemical) in the laboratory altered nest defense in male fathead minnows and if the behaviours were consistent with what occurs in nature (Chapter Four).

Nesting male fatheads in the wild and in the laboratory (Chapters Two and Four) took different amounts of risk based on predation threat, indicating that they could distinguish between disturbances from predators and non-predators. Males in predator treatments consistently took less risk than control males. Egg-guarding males in the wild took longer to return to their nests when a pike was present than when a control stimulus was present. Nest-guarding males in the laboratory significantly reduced their chasing and circling behaviours (by more than 50%) for up to 24 h when threatened with stimuli from northern pike and often abandoned their nests altogether. Decreases in conspicuous behaviours such as wide circles and chases may minimize the probability of detection by a predator. Egg-guarding males, however, did return to their nests, obviously indicating that they had made an ultimate and perhaps maximal investment in reproduction and must take increased risk over males without eggs to protect their investment. Nevertheless, for males with and without eggs, the choice to leave the nest and/or decrease behaviours for sustained periods of time may be interpreted as a choice to put less effort into reproduction in the presence of a predator and this may have effects on male success.

The consequences for males with eggs that choose to depart from the nest when the threat of a predation is high may include egg-predation by nest intruders such as juvenile and female fatheads and nest usurpation by rival males without territories that may be willing to take more risk in the presence of a predator. The consequences for males without eggs that decrease behaviours such as chasing intruders and circling their territory and whose breeding coloration appears to fade when a predator is present may be nest usurpation by more robust males or declines in mating opportunity. Likely, females gather some information about potential mates based on quickly and easily assessable characters (see Sullivan 1994) such as coloration and "vigorousness" of behaviours. Males with less "vigor" may be assessed by females as "low quality". In addition, if females determine that an area is "high risk" they may seek males elsewhere. A natural threat of 0.3 to 2.8 pike per day (Chapter Three) near nesting male minnows suggests that males and females may frequently be faced with these decisions.

In Chapter Three, the observed pattern of artificial and natural nest use suggested that males make decisions about where to nest based on their estimates of prevailing risk (Lima and Dill 1990). Minnows used artificial and natural nests more frequently in locations with low densities of pike than in locations with high densities of pike. I conclude that predation was one of the factors influencing the observed pattern of nest use. Undoubtedly, nest site availability was also important. In one location with high densities of pike, few natural nests appeared to be available, although it was difficult for an observer to assess what would be used as a nest because of the ephemeral nature of the habitat (*i.e.* wave action deposited, removed and re-deposited material potentially used as nesting substrates). Nonetheless, in other locations where there were many pike, there appeared to be numerous available unused natural nest sites in the form of anchored branches and rocks. In addition, there were many artificial nest boards available but unused in these locations. My data suggest that males may choose to nest in areas where they assess predation risk to be lowest.

These findings support the laboratory and field studies of introduced predation threat, indicating that male fathead minnows can assess and respond to predation risk, weighing nest defense, courtship and egg-guarding against exposure to predators. Males respond to conflicting demands such as visibility to predators and egg-defense with reduced activity that is proportional to predation risk (Sih 1987). Male fatheads can "alter their reproductive behaviour when the threat of predation is changed" (Fraser and Gilliam 1992). Fathead minnows are a vulnerable prey species (Moody *et al.,* 1983; Robinson 1989) and thus tolerance to predation must be aided by behavioural adaptations such as avoidance of areas where predation may be high and facultative declines in "risky" nest-defense behaviours and by use of chemical and visual cues to recognize and respond to predators.

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Appendix One

Testing statistical assumptions of Repeated Measures MANOVA

To test the homgeneity of slopes assumption for ANCOVA's (Sokal and Rohlf, 1981), I used a repeated-measures multivariate analysis on the treatment and covariate factors. There was no significant interaction between treatment (control / predator) and the covariate (age of eggs) (n = 14; F = 0.832, *p* = 0.449).

MANOVA/ MANCOVA is a statistical procedure that generally has less power than traditional methods such as ANOVA (Gurevitch and Chester 1986) but it is often a more informative method of analysis with multivariate data (Gurevitch and Chester 1986). Potvin *et al.* (1990) have shown that the number of dependent variables (k) that can be analyzed in a MANOVA is dependent on both sample size (N) and the levels of treatment groups (M). They use MANOVA only when N - M > k. More conservative statisticians recommend that N - M > k + 9 before using the analysis (Maxwell and Delaney 1990). In my study, N - M was greater than k + 9 (i.e. 14 - 2 > 2+ 9).

Repeated-measures (RM) analyses make a number of assumptions about the variance and covariance matrices involved in calculating F values (Morrison 1990). RM's require that these matrices be *circular*, *i.e.* the difference between any two levels of the within-subject factor equals the same constant (Winer *et al.*, 1990). One way of assessing circularity is by the matrix characteristic *sphericity* (von Ende 1993). Sphericity is calculated by using orthogonal transformations of the variance-covariance matrix (Winer *et al.*, 1990). Sphericity is an especially important assumption to check when time is the split-plot or within-subject factor. If the condition of sphericity is not met, F statistics for the within-subject factors are inflated (von Ende 1993).

To test whether variance and covariance matrices met the required assumption of sphericity, I examined two estimators, the Greenhouse-Geisser and Huynh-Fedlt epsilons. Both epsilons range from 0 to 1; the smaller a value the greater the departure from sphericity (von Ende 1993). For my data, the Greenhouse-Geisser epsilon was 0.859 and the Huynh-Fedlt epsilon was 1.000. I concluded that these matrices met the assumptions of sphericity and could be used to test behaviours for differences between treatments over sampling intervals.

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Appendix Two

Location	Chase	Clean	Tight circle	Wide circle
Laboratory	0.0	317.0	45.0	0.0
Laboratory	21.0	170.0	47.0	59.0
Laboratory	0.0	124.0	31.0	27.0
Laboratory	0.0	144.0	20.0	54.0
Laboratory	40.0	251.0	22.0	91.0
Laboratory	11.0	115.0	81.0	81.0
Laboratory	111.0	66.0	0.0	33.0
Laboratory	82.0	59.0	0.0	33.0
Laboratory	120.0	98.0	0.0	23.0
Laboratory	74.0	43.0	15.0	3.0
Laboratory	31.0	0.0	17.0	67.0
Laboratory	49.0	61.0	55.0	68.0
Laboratory	33.0	[illegible]	33.0	50.0
Laboratory	115.0	[illegible]	21.0	47.0
Laboratory	87.0	0.0	24.0	39.0
Laboratory	42.0	12.0	50.0	63.0
Laboratory	16.0	30.0	48.0	32.0
Laboratory	4.0	105.0	13.0	62.0
Laboratory	1.0	59.0	49.0	83.0
Laboratory	6.0	113.0	38.0	46.0
Mean	**42.2**	**91.5**	**30.5**	**48.1**
Std. Error	**9.3**	**18.5**	**4.7**	**5.5**
Armstr. Lake	24.0	126.0	27.0	60.0
Armstr. Lake	6.0	159.0	72.0	99.0
Armstr. Lake	0.0	279.0	69.0	12.0
Armstr. Lake	3.0	129.0	39.0	0.0
Armstr. Lake	0.0	123.0	90.0	15.0
Armstr. Lake	12.0	122.0	36.0	30.0
Armstr. Lake	39.0	171.0	39.0	21.0
Armstr. Lake	27.0	216.0	15.0	9.0
Armstr. Lake	6.0	189.0	18.0	12.0
Armstr. Lake	10.0	57.0	123.0	12.0
Armstr. Lake	12.0	18.0	6.0	12.0
Armstr. Lake	30.0	273.0	27.0	12.0
Armstr. Lake	15.0	252.0	12.0	0.0
Armstr. Lake	3.0	198.0	18.0	3.0
Armstr. Lake	3.0	150.0	9.0	0.0
Armstr. Lake*	9.0	219.0	66.0	3.0
Armstr. Lake*	57.0	261.0	39.0	0.0
Armstr. Lake*	48.0	171.0	66.0	6.0
Armstr. Lake*	51.0	249.0	15.0	3.0
Armstr. Lake*	27.0	165.0	6.0	6.0
Mean	**19.1**	**176.4**	**39.6**	**15.9**
Std. Error	**4.0**	**15.7**	**7.1**	**5.4**

Appendix 2. Number of behaviours per 30 min for 40 male minnows. Twenty males were observed in the MBRS laboratory; twenty males were observed in Armstrong Lake, Alberta. Most observations were made during June and July, 1993. * Data collected in July 1994. Cleans and wide circles were significantly different between locations (see Chapter 4).

Appendix Three

I measured time away from the nest in reproductive male fathead minnows with eggs and without eggs. In June, July and August of 1993, I measured the time spent out of the nest for 29 laboratory males (n=11 males with eggs; n=18 males without eggs).

Experimental protocol

Males were observed in 30 l glass tanks. Three sides of the tank were covered in black plastic, allowing one side for observation. Each tank contained a suspended artificial nest (see Chapter 4 for details) and one 11 cm plastic plant. Two reproductive males (56 ± 8 cm, [illegible]8) were placed in a tank with one gravid female (52 ± 9 cm; n=25 to ensure competitive behaviour (Unger 1983) and allow eggs to be laid in the nest. Water was filtered with a 8 x 10 cm charcoal filter. Minnows were maintained on a 18 : 6 light-dark photoperiod at 21° C and were fed Tetramin once daily.

The male observed utilizing the artificial nest was chosen as the focal male. Two observers, one with a stopwatch, recorded total amount of time spent away from the nest in 30 min. There was not a random allocation of "with eggs" and "without eggs" to males. If a male acquired eggs then he was used as a "with eggs" male.

Time away from the nest between males with eggs and without egg was analyzed with a Wilcoxon signed ranks test (Sokal and Rohlf 1981).

Results

Males with eggs spent more time in their nests than did males without eggs (Wilcoxon $z = -2.490$, $p = 0.013$). Males with eggs spent on average 8.53 min (s.e. 0.65) away from the nest in a 30 min recording period, whereas males without eggs spent 17.50 min (s.e. 1.49) away from their nests. When males were away from the nest they were feeding, chasing the female and male, or circling the tank.

Discussion

Minnows with eggs, not surprisingly, spend more time in the nest to clean their eggs, avoid egg- predation and nest-usurpation. Males without eggs need to attract females and defend a newly selected territory from competitors, involving time away from the nest itself.

Reproductive male fathead minnows may be vulnerable to "sit and wait" predators because of site attachment, conspicuous behaviours, and increased coloration. This vulnerability may come at any moment throughout the breeding season but may be increased prior to the period when males have eggs to defend. Prior to defending eggs, males select their nest and territory and defend it from competing males and intruding females and juveniles (McMillan 1972). In this "pre-egg" period, males spend more time away from the nest itself than males with eggs and more time chasing intruders and presumably advertising to females. I suggest that males defending territories (and not yet eggs) are at greater risk from predators than males with eggs because they spend more time out of the nest. Time away from the nest potentially makes males more vulnerable to visual predators that likely cue on mobility of prey, in particular the chasing and circling performed by male fatheads.

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