University of Alberta

Population-level responses of fathead minnow (*Pimephales promelas*) to alarm substances and predator odour

by

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

Master of Science
in
Ecology

Department of Biological Sciences

Spring 2010
Edmonton, Alberta

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Abstract

Alarm substances, released by injured prey, and odours from predators, such as northern pike, are chemical cues associated with increased predation risk in aquatic ecosystems. In laboratory studies, individual prey can respond to the presence of such cues by reducing conspicuous behaviours, such as foraging and by seeking shelter. These responses may reduce growth and reproduction, which could have effects at the population-level. The objective of my study was to determine if alarm substances or pike odour have population-level effects on fathead minnow. In the cattle trough experiment, alarm substances and pike odour had no effect on breeding behaviour and recruitment of young; however, spawning occurred earlier with exposure to alarm substances relative to water controls. In a larger-scale pond experiment, alarm substances had no effect on reproduction or recruitment. Despite individual-level effects in the laboratory, exposure to alarm substances and pike odour had no impact at the population scale.

Acknowledgements

In addition to individual persistence, research projects require a great deal of help and support from others. Field work would never have been completed without my undergraduate assistants. I thank John Perdue for the monumental effort of helping get this project off the ground, ridding the ponds of dividers, vegetation and fish (twice), and tolerating the exciting chore of egg counting. To Homun Yee, computer genius assistant, for revolutionizing how things were done from my first summer. Finally, I thank Jay Rasmussen for continuing to be cheerful no matter how frustrating and disgusting working with fish got to be.

The staff and researchers at the Meanook Biological Research Station must be thanked for providing just about everything; including food, shelter, lab and field space, a truck, extra hands, and of course afternoon and evening entertainment. Thanks and best of luck to the energetic and spirited WISEST students Tara Howatt and Lisa Lorenz who came out to help with my project, but ended up fishing, swimming and roaming among all the projects at the field station.

Thanks to the P-T lab, an ever evolving bunch of crazy people with seemingly endless amounts of random knowledge and laughter. Their lack of inhibition in criticizing has greatly improved my work. Kudos and thanks to the poor souls, Arthur, Helen, Candra and Teslin, who co-habit my office space and have graciously tolerated the hourly interruptions for advice or a second opinion.

I am grateful for the people I have met in this program for they are my support group away from home and many have become good friends. Without the endless stream of non-academic socializing, life would have been very lonely. I owe a great deal to my parents, who despite not knowing exactly what it is I study, have been my quiet, solid supporters (driving me half-way across the country twice!) and defenders (Are you done yet?) of my unconventional decisions.

Funds for this study were provided by the Alberta Conservation Association, Alberta Sport Recreation Parks & Wildlife Foundation, the Canadian Circumpolar Institute and by the Natural Sciences & Engineering Research Council of Canada via a Discovery Grant to Bill Tonn. I also acknowledge the funding and support provided by the University of Alberta, Department of Biological Sciences. I thank my supervisory committee, Drs. Bill Tonn, Nadir Erbilgin and Norm Stacey for their encouragement, advice and varying perspectives. In particular, I thank my supervisor, Dr. Bill Tonn for financial and logistical support, and most importantly for focusing and directing me through this experience. I have never had so many important conversations conducted entirely in whispers.

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Introduction

Predator-prey interactions are integral processes in governing animal behaviour and structuring biological communities. Predators can have profound effects on prey populations directly through capture and consumption, and indirectly by altering prey behaviour (Sih 1994, Lima 1998). Many ecological studies of predator-prey interactions have focussed on lethal effects, with less attention to non-lethal effects. Non-lethal effects on prey behaviour are increasingly being recognized as important, particularly in aquatic systems, where upwards of 50% of the total effect of predators on prey can be attributed to non-lethal effects (Luttbeg and Kerby 2005, Preisser et al. 2005, Peckarsky et al. 2008). Non-lethal effects can impact the entire prey population, not just the attacked individual, and usually occur chronically throughout the lifetime of the prey (Peacor and Werner 2001).

To survive and reproduce, prey species have evolved a variety of methods to defend themselves against predation, including changes to morphology, physiology, life history and behaviour (Fuiman and Magurran 1994, Kats and Dill 1998). Among the latter, many organisms have developed communication systems to provide advanced warning of predators. Alarm signals can elicit behavioural responses from others towards to potential threats, and can incorporate visual displays, audible distress calls and/or chemical cues (Kats and Dill 1998, Alcock 2001).

Chemical signals are particularly important in aquatic environments, where chemicals are easily transmitted and vision is often limiting (Brönmark and Hansson 2000, Wisenden 2000). Two basic sources of chemical signals in predator-prey interactions are those released from the predator (kairomones) and those released by the prey (disturbances cues or alarm substances) (Brönmark and Hansson 2000). In many prey fishes, alarm substances are stored in the epidermis and are released only upon injury (Pfeiffer 1963).

Laboratory-based studies consistently show strong behavioural responses from a variety of prey fishes to predator odours and alarm substances. Common responses include fleeing from the area, decreased movement, increased sheltering (hiding and schooling), changes to activity schedules and increased vigilance (Smith 1992, Chivers and Smith 1998). Although behavioural responses vary widely among prey fish species, these responses are generally the tactics that specific prey species uses to evade their natural predators (Pfeiffer 1977, Chivers and Smith 1998). Although modifying behaviours can reduce the risk of predation and thereby increase short-term survival, they require time and energy that could otherwise be devoted to foraging or reproduction.

Studies on prey fish cognition have shown that chemical signals can be used to determine the level of predation-risk in the environment, and that individuals may adjust their behaviour appropriately to minimize predation risk (Brown et al. 2006, Ferrari and Chivers 2006, Ferrari et al. 2006a). For example, convict cichlids (*Archocentrus nigrofasciatus)* exposed to subthreshold concentrations of alarm substances will adopt a 'heads-up' foraging posture. At higher concentrations; however, the same fish will significantly reduce foraging (Foam et al. 2005). Perch (*Perca fluviatilis)* will switch to feeding on smaller, less profitable prey when exposed to visual and chemical cues from northern pike (*Esox lucius*) (Mikheev et al. 2006). Reduced foraging can persist for several days after a single exposure to predator cues and consequently can have negative effects on growth, body condition and survival (Ficke 1987, Jachner 1997, Jachner and Janecki 1999). For example, convict cichlids showed reduced growth after 41 consecutive daily exposures to alarm substances (Pollock et al. 2005).

Although the presence of chemical cues can limit energy intake, additional energy is required for active defensive mechanisms against predators. Increased schooling behaviour, induced morphological defences and altered habitat use incorporating safer but less profitable areas are energetically expensive (Pettersson and Bronmark 1999, Herczeg et al. 2009). Japanese minnows (*Pseudorasbora parva)* exposed to higher predation risk reduce activity levels and move to shallow riffles; however, swimming in these higher velocities riffles increased their energy expenditure (Sunardi et al. 2007). Rapid growth and good body condition is particularly important in regions that experience long and harsh winters, such as northern Alberta (Danylchuck and Tonn 2006, Divino and Tonn 2007). Fathead minnows (*Pimephales promelas*) from lakes prone to over-winter oxygen depletion have shorter lives, but mature earlier and allocate proportionally more energy to growth and reproduction than those from benign environments (Danylchuk and Tonn 2006). Adding high predation risk to such harsh environments may prevent individuals from acquiring the energy resources needed to survive and reproduce.

Reproduction can be risky and energetically costly, and therefore may be compromised in a high-risk environment. Establishing territories, finding mates, and producing and caring for young all require substantial amounts of energy. In addition, successful breeding is often associated with risk-taking behaviours (Lima and Dill 1990, Nelson and Paetz 1992, Sih 1994). Conspicuous males that are brightly coloured and perform elaborate displays tend to acquire more mating opportunities, but incur higher predation rates (Sih 1994). Under conditions of high predation risk, males will reduce courtship behaviour and employ alternative safer mating tactics (Sih 1994, Candolin 1997). Cleaning and actively guarding nests from predators are also risky behaviours that greatly increase reproductive success (Divino and Tonn 2008), but may be reduced under predation threat (Sih 1994, Steinhart et al. 2008). Similarly, females modify their behaviours in risky environments by becoming less choosy, employing mate copying more often or choosing to lay eggs in sheltered nesting sites (Forsgren 1992, Wisenden 1993, Briggs et al. 1996, Godin and Briggs 1996).

In addition to short-term behavioural changes, in the laboratory reproductive strategies can also change in response to chemical information. For example, guppies (*Poecilia reticulata*) increased their reproductive output by shortening gestation time, decreasing brood intervals and increasing brood sizes when exposed to chemical cues from a predator (Evans et al. 2007, Dzikowski et al. 2004). Convict cichlids bred earlier when exposed to alarm substances, although the total number of eggs produced was unchanged (Pollock et al. 2005). Early reproduction is advantageous if there is a high likelihood of being eaten in the near future. Young produced earlier in the breeding season, in turn, have more time to grow, potentially increasing recruitment rates (Divino and Tonn 2007). However, Evans et al. (2007) found that accelerated development under high predation risk produced young with locomotor impairments.

Although reduced growth and reproduction, and producing young of poor quality can be detrimental to a population, the vast majority of research on predatory chemical cues has been conducted in the laboratory. As a result, almost all the information on the behavioural responses of fishes is from short-term studies on individuals, typically on the scale of minutes. These laboratory studies consistently show strong responses to alarm substances and predator odours; however, these may be misleading as behaviour and reproductive effort are dependent on social context (Danylchuk and Tonn 2001, Wisenden et al. 2003, Pollock et al. 2006a). The few studies conducted in natural waters often focus on avoidance of alarm substances and predator odours, rather than how a population responds as a whole to predatory chemicals (Magurran et al. 1996, McPherson et al. 2004, Friesen and Chivers 2006). In addition, results from these few studies on alarm substances in natural waters are ambiguous and mixed (Wisenden 2004). To better understand the consequences of alarm substances and predator odours in natural waters, studies must be conducted at the population scale.

The objective of my study is to determine if chemical signals associated with increased predation risk will cause modifications to reproduction or recruitment at the population-level. Fathead minnow was used as a model to assess the effects of alarm substances and pike odour (water tainted by northern pike kairomones) on reproductive effort, recruitment and growth. Two population-level experiments were conducted at Meanook Biological Research Station (MBRS; 54°37'N, 113 °35'W), where a successful protocol quantifying reproduction and recruitment of young into populations of fathead minnow has previously been established in experimental ponds (Danylchuk and Tonn 2001, Grant and Tonn 2002). By employing this protocol, this study will be the first to explicitly examine the consequences of chemically mediated, risk-reducing behaviours of prey fish at the population level.

Study system

Northern pike and fathead minnow range over much of North America and are commonly found in the small lakes of boreal Alberta (Becker 1983, Page and Burr 1991, Nelson and Paetz 1992). Despite their high frequency of occurrence and overlapping distributions, rarely do they co-occur within the same lake (Robinson and Tonn 1989, Paszkowski and Tonn 2000). Winterkill, a result of oxygen depletion during prolonged winters, is a strong factor in limiting the persistence of fish populations in northern Alberta. Low oxygen levels are preferentially detrimental to larger fish; as a consequence, northern pike is typically restricted to larger and deeper lakes (Tonn et al. 2003, Hurst 2007). Fathead minnow is less susceptible to winterkill and can persist in lakes unsuitable for northern pike. Although consumption likely has a major role in reducing fathead minnow populations where northern pike is present, it is unclear why total exclusion occurs in the vast majority of lakes.

Fathead minnow belong to the family Cyprinidae and to the larger Super Order Ostariophysi (Page and Burr 1991, Helfman et al. 1997). Alarm substances were first discovered and are now attributed synonymously to the Ostariophysi group (Helfman et al. 1997). Fathead minnow possess club cells, whose proposed function is to store alarm substances, located in the epidermis (Pfeiffer 1963). Although alarm substances were isolated and identified as hypoxanthine-3(N)-oxide in the 1970s, its identity is now under debate (Pfeiffer et al. 1985, Brown et al. 2000, Brown et al. 2003, Carreau-Green et al. 2008). In laboratory-based behavioural studies, fathead minnows respond to alarm substances by increased schooling and sheltering, decreased movement and fleeing from the area (Lawrence and Smith 1989, Chivers and Smith 1998). Similar responses are elicited when exposed to northern pike odours (Kusch et al. 2004).

The spawning season in northern Alberta typical begins in June when mean temperatures reach ~15°C and extends through to August (Andrews and Flickinger 1979, Nelson and Paetz 1992). Males normally establish breeding territories under submerged or floating objects such as logs, stones or lily pads, but will readily accept artificial surfaces (Benoit and Carlson 1977, Nelson and Paetz 1992). Fathead minnow are promiscuous, so nests can contain eggs from several females and be at different developmental stages. Conspicuous courting behaviours include rapid approaches, visual displays and leading females to their territory (Cole and Smith 1987). Males continue to provide parental care to the eggs until hatching by cleaning, aerating and actively guarding the nests from predators (Wynne-Edwards 1932, Jones and Paszkowski 1997a).

Courting, guarding and parental care are conspicuous, and therefore potentially risky, behaviours in the presence of predators. Reproductive success in fathead minnow is largely dependent on male parental care; nests with less paternal care are shorter in duration, have fewer eggs, and higher rates of failure (Sargent 1988, Divino and Tonn 2008). This system of risky reproductive behaviours and severe consequences of not performing them, make fathead minnow a good species to study the impacts of predation cues. Alarm substances and pike odour are both chemical cues that increase the perceived risk of predation for fathead minnow. Laboratory experiments by Jones and Paszkowski (1997a) found that exposure to alarm substances and pike odour caused male fathead minnows to change territorial behaviour and abandon their nests. Therefore, I predicted that behavioural responses to alarm substances or pike odour would negatively impact reproduction by reducing the number of nests initiated, eggs acquired and hatching success of the nests.

Fathead minnow are fractional spawners and a single female is capable of producing upwards of 10,000 eggs (Gale and Buynak 1982). Because of this reproductive strategy, females may have some flexibility in changing the time intervals between batches and the size of each batch. Increased brood size and decreased retention of embryos has been reported for guppies in response to predation risk cues (Dzikowski et al. 2004, Evans et al. 2007). Convict cichlids also began breeding earlier when exposed to alarm substances; however, fewer breeding attempts were made and overall production was unchanged (Pollock et al. 2005). I predicted that reproduction in the fathead minnow would occur earlier in the season and production would be negatively affected by alarm substances or pike odour as gravid females are more susceptible to predation and reduction in foraging is expected in response to predation cues (Lima and Dill 1990, Magnhagen 1991, Chivers and Smith 1998).

In environments with high predation risk, prey species decrease their foraging efficiency while increasing their energy expenditure. Therefore, I predicted that growth rate, body condition and survival of stocked fish would decrease with exposure to alarm substances and pike odour. Since young-of-the-year (YOY) show no response to alarm substances until 48-56 days post-hatching, the greatest influence from alarm substances and pike odour is likely through the adults via changes to reproductive behaviours (Carreau-Green et al. 2008). Since I predicted reduced overall reproductive effort in the presence of alarm substances or pike odour, I further predicted that there will be a negative impact on the quantity of young-of-the-year recruited into the population. The quality of YOY was predicted to increase as there would be fewer adult fathead minnows foraging and fewer YOY to compete with for food.

Methods

Experimental design

This study was composed of two experimental components, conducted in cattle troughs and ponds, respectively, at the Meanook Biological Research Station (MBRS; 54°37’N, 113 °35’W), located near Athabasca, Alberta. The pond experiment was conducted to determine whether alarm substances have a population-level effect on fathead minnow, using four constructed ponds in 2007 and six in 2008. The ponds are similar in morphometry and water quality (Table 1). Depth at the centre of the ponds was held at 1.5 m in 2007 and 1 m in 2008.

To facilitate reproductive monitoring, existing potential nesting substrate (woody debris and aquatic vegetation) was removed and replaced with floating artificial nesting surfaces prior to stocking fathead minnow populations. These nesting surfaces consisted of a fence board (55 cm x 14 cm x 2 cm) covered in black tarpaulin and anchored at the ends using bricks with approximately 0.5 m of twine. Twenty nesting boards were placed along the perimeter of the ponds. Data loggers (HOBO) recorded water temperature at a depth of 25 cm every 2 hours from June to August in 2007 and 2008. Water samples were collected on June 4, 2008 at a depth of 30 cm from the centre of each pond. These were processed at MBRS for conductivity (Accumet) and pH (Accumet), and then by the Biogeochemical

Analytical Laboratory at the University of Alberta for total nitrogen (TN; µg/L), total phosphorus (TP; µg/L) and chlorophyll-a (Chl-a; µg/L). Dissolved oxygen measurements were taken at a depth of 30 cm in the centre of each pond on July 27, 2008.

In 2008, a cattle trough experiment was added to determine if alarm substances and pike odour have differing effects on behaviour, reproduction and recruitment of fathead minnow. The bottom of 15 plastic cattle troughs (height = 0.75 m, diameter = 2.55 m) were covered with 5 cm of silty sand. Troughs were filled with 2.65 m^3 of pond water and 3 nesting boards (described above) were added to each.

Fathead minnows were collected using Gee minnow traps from Rochester Lake (ca. 35 km SE of MBRS) and transported live to MBRS. This is a small, productive lake that contains fathead minnow and brook stickleback, but not northern pike. Fathead minnows were temporarily housed in large (~1,500 L) outdoor tanks and maintained on fish flakes. Fish were sorted into 3 life-history categories: mature females, mature males and juveniles (defined as fish lacking distinguishable secondary sexual characteristics; Danylchuk and Tonn 2001). Fish were then anaesthetized with clove oil (30 mg/L) and marked subcutaneously with acrylic paint for later identification when monitoring reproduction and growth. Fish were batch marked based on size and life-history category. In 2007, females and males were divided into three 3-mm size classes and juveniles into two 3-mm size classes by total length. In 2008, females, males and juveniles were divided into three 3-mm size classes. Pond populations were stocked on June 8 in 2007 and June 13 in 2008 (Julian day 159 and 165 respectively) with equal proportions of females, males and juveniles at a total density of approximately 1 fish/m^3. Only mature female and male fathead minnows were used in the cattle trough experiment. Five females and five males were individually marked and stocked on June 23, 2008 (Julian day 175) resulting in a density of 4 fish/m^3. Both densities are within the natural range observed in boreal Alberta lakes (Danylchuk and Tonn 2003).

Treatments

To signal an increase in predation risk, alarm substances and pike odour were added as chemical cues. Alarm substances were derived by homogenizing fathead minnow skin extract, following the methods of Ferrari and Chivers (2006). Only skin from female and juvenile fish was used, as males do not produce alarm substances during the breeding season (Smith 1976). Donor fathead minnow were euthanized by a blow to the head and/or decapitation. Skin filets were removed from both sides and homogenized using a tissue grinder to release alarm substances. For the pond experiments, individual doses were comprised of 6 cm^2 and 11 cm^2 of skin extract in 2007 and 2008, respectively. Doses were frozen into approximately 90 mL aliquots at -5°C until needed.

Ponds were assigned to alarm substances or control treatment (Table 1). Alarm substances were added every fourth day in 2007 at an average concentration of 1 cm^2: 20,000 L and every second day in 2008 at 1 cm^2: 10,000 L. Each dose of alarm substances was thawed and mixed into approximately 15 L of pond water. An equal volume of pond water was added to control ponds. In 2007, treatments were added evenly from a boat travelling along a transect through the middle of the pond. Treatments were divided into 20 portions and added between nesting boards in 2008.

Alarm substances concentrations were based on values found in the literature, preliminary trials and the use of logistically practical numbers of donor fish (Figure 1). Unfortunately, there is no standard concentration of alarm substances. Concentrations used in the literature range from 1 cm^2: 25 L to 1 cm^2: 1,776,000 L, with concentrations up to 1 cm^2: 296,000 L being effective in eliciting anti-predatory behaviour in fathead minnow (Ferrari et al. 2005, Ferrari and Chivers 2006, Ferrari et al. 2006b; Figure 1). Although the concentrations in my study are not among the highest, they were nearly 15 and 30 times more concentrated, for 2007 and 2008 respectively, than the consistently low value reported of 1 cm^2: 296,000 L (Figure 1). Concentrations in my study were also well above the concentrations, 1 cm^2: 58,824 L (Lawrence and Smith 1989) and 1 cm^2: 888,000 L (Ferrari et al. 2005), at which the strength of the anti-predatory behaviours began to decrease from complete cessation of activity. In addition, during preliminary trials using minnows from Rochester Lake, freezing and sheltering behaviours were observed at concentrations as low as 1 cm^2: 40,000 L (Jung unpublished data).

Cattle troughs were assigned to alarm substances, pike odour or control. Pike odour was produced following the protocol in Ferrari et al. (2006a; Table 2). Northern pike were collected by angling from Narrow Lake, 10 km west of MBRS, and transported live to MBRS. This lake contains yellow perch and northern pike, but not fathead minnow (Mitchell and Prepas 1990). Northern pike were housed individually in large (~1,500 L) outdoor tanks and fed live fathead minnow. Prior to collection of pike odour, pike were starved for 4 days to allow dietary alarm substances to pass from their system (Jones and Paskowski 1997a). Two pike were than each placed individually into 60 L of aerated but not filtered water for 24 hours (Ferrari et al. 2006a).

Cattle troughs were treated every second day. Pike odour was added at a concentration of 1 L: 600 L, based on concentrations found in the literature (Kusch et al. 2004, Ferrari et al. 2006a; Table 2). Alarm substances were added at a concentration of 1 cm^2: 10,000 L and water was added to the controls. The volumes containing the treatments were equalized to 4.5 L and frozen. Freezing water tainted with predatory odours and fathead minnow skin extracts is common in such studies and has been shown to elicit anti-predatory behaviour (e.g. Smith 1992, Mathis and Smith 1993a, Ferrari et al.

2006a). Treatments were added by suspending the frozen blocks above the cattle troughs and allowing them to melt. The ice blocks would take approximately 6 to 16 hours to melt depending on the weather conditions. A black bucket was placed on top of the frozen blocks to reduce photo degradation.

Data collection

Nesting boards were monitored manually for the presence of nests every second day in the 2007 pond experiment. In 2008, daily photographs of nesting boards for the pond and cattle trough experiment were taken and later analyzed using ArcGIS. The location and date of appearance of eggs were recorded for each nest to calculate the number of nests established and the average start date of the nests in the cattle troughs and ponds. Eggs were counted on days the nest was present to determine the size of each nest and the total productivity. The number of times new eggs appeared at a nest was counted as the number of egg batches acquired per nest. Nest duration is defined as the number of days a nest was present. To determine the fate (hatched or predated) of eggs, developmental stage was recorded at each observation following Vandenbos et al. (2006). Fathead minnow eggs progress through three distinguishable developmental stages: clear, black eye and gold eye. Eggs disappearing prior to reaching the third stage were recorded as predated and those disappearing after reaching the gold eyed stage were recorded as successfully hatched (Vandenbos et al. 2006). From this information, percentage of eggs successfully hatching in each cattle trough or pond was calculated. Evidence of fungal infections was also noted during nest monitoring and is presented as percentage of nests showing evidence of infection in the cattle trough or pond. Total failure is defined as a nest with no eggs surviving to hatching (Divino and Tonn 2008). Spawning season was calculated as the number of days between the laying of the first and last egg for each pond or cattle trough, peak date is the day with the highest number of eggs present and end date is the last day new eggs were laid.

While monitoring nests in the cattle trough and pond experiment, characteristics of nest-guarding males were recorded by adapting protocols from Divino and Tonn (2008). These characteristics included whether the male was present and what level of guarding he was exhibiting. The percentage present was calculated from the number of times a male was present at a nest divided by the total number of observations made over the course of his nest. I defined guarding levels as 0 (male not present at nest), 1 (male present but fled on approach), 2 (passively guarding: male was nearby but reluctant to approach observer), and 3 (actively guarding: male head butted, nipped, or otherwise attempted to repel the observer from the nesting site). Maximum parental care is the highest recorded level of guarding observed for each male.

Experiments were terminated when no nests were present for 10 consecutive days. Fish were removed from the ponds and cattle troughs to determine recruitment, growth and survival. Gee minnow traps were set in the ponds for 3 consecutive days to remove the majority of adults. The ponds were then drained to approximately 30 cm in depth. Remaining adults and YOY were removed by repeated seine netting. Seining continued until 3 consecutive passes captured less than 5 fish. The cattle troughs were drained to approximately 10 cm depth and fish were removed by dip netting. Dip netting continued until no fish were observed by 2 observers. Fish were euthanized with clove oil and preserved by freezing for later processing. Adult fish were counted, weighed and measured (TL) prior to stocking and again at the end of the summer to determine changes to growth, body condition ($K = \{\text{mass (g)} / \text{length (cm)}^3\}*100$) and survival. In addition, gonadosomatic index ($GSI = (\text{gonad mass (g)}/\text{total body mass (g)})*100$) was measured in the 2008 pond experiment on a subset of females in June, July, and August. All YOY from the ponds and cattle troughs were counted to determine first-summer recruitment. Mass and total length were measured for all YOY in the cattle trough and a sub-sample (~500) from the ponds to determine mean length and biomass.

Analyses

Data from the pond and cattle trough experiments were analyzed separately for effects of alarm substances and pike odour on several reproductive and recruitment parameters. Due to differences to experimental procedure and alarm substances concentrations, pond data were also analyzed separately for 2007 and 2008. Comparisons among treatments at the pond or cattle trough level for spawning season, start, peak and end dates, number of nests, total production of eggs, percentage of nests showing signs of infection, percentage of failed nests, percentage of eggs hatched, stocked fish growth, body condition and survival, and YOY length, biomass and abundance, were analyzed with Welch's t-tests and 1-way ANOVAs for the pond and cattle trough experiments respectively. Square root, log or arcsin-square root transformations were applied to non-normally distributed data prior to analysis. Female GSI and egg accumulation over time were analyzed at the pond or cattle trough level using repeated measures ANOVA. Parameters that are affected by localized differences at the nest-level, average start date, egg batches acquired, average nest size, duration of the nest, observed male presence at the nest and maximum parental care, were analyzed using nested ANOVAs, nests nested within pond or cattle trough, with a Satterthwaite approximation for unequal subgroups. Significance level was set at $p \leq 0.05$. Statistical analyses were performed using S-plus 8.0.4.

Table 1. Experimental treatments and morphometry of experimental ponds at Meanook Biological Research Station (MBRS). Alarm substances (AS) or water (control) was added throughout the summer season. Concentration and frequency of alarm substances differed between 2007 and 2008 (see Methods). Temperature data were collected every 2 hours and averaged over most of 2007 and all of the 2008 breeding season. Dissolved oxygen (DO) was measured at 30 cm depth in ponds on July 27, 2008. Water samples were collected June 4, 2008, from centre of ponds at 30 cm depth. Conductivity and pH were measured at MBRS. TN, TP and Chl-a were analyzed at the Biogeochemical Analytical Laboratory at the University of Alberta.

	Pond 1	**Pond 2**	**Pond 3**	**Pond 4**	**Pond 5**	**Pond 6**
Pond assignments						
2007	-----	Control	AS	Control	AS	-----
2008	AS	AS	Control	Control	Control	AS
Size (depth = 1m; 2008)						
Surface area (m^2)	179	177	202	154	135	175
Volume (m^3)	109.9	122.8	140.8	83.8	86.6	118.0
Temperature (°C)						
2007 (Jun 14-Jul 29)	n/a	22.5	n/a	22.8	n/a	22.2
2008 (Jun 15-Jul 29)	n/a	21.8	21.8	20.7	20.2	20.0
2008 water chemistry						
TN (µg/L)	485	448	383	396	490	465
TP (µg/L)	13	14	6	18	18	16
Chl-a (µg/L)	<0.2	<0.2	<0.2	0.47	0.63	0.94
pH	10.23	10.32	10.39	10.37	10.12	9.98
conductivity (µS/cm^2)	201.2	227.7	245.9	215.3	219.9	201.4
DO (µg/L)	12.38	10.36	10.05	13.05	12.72	10.01

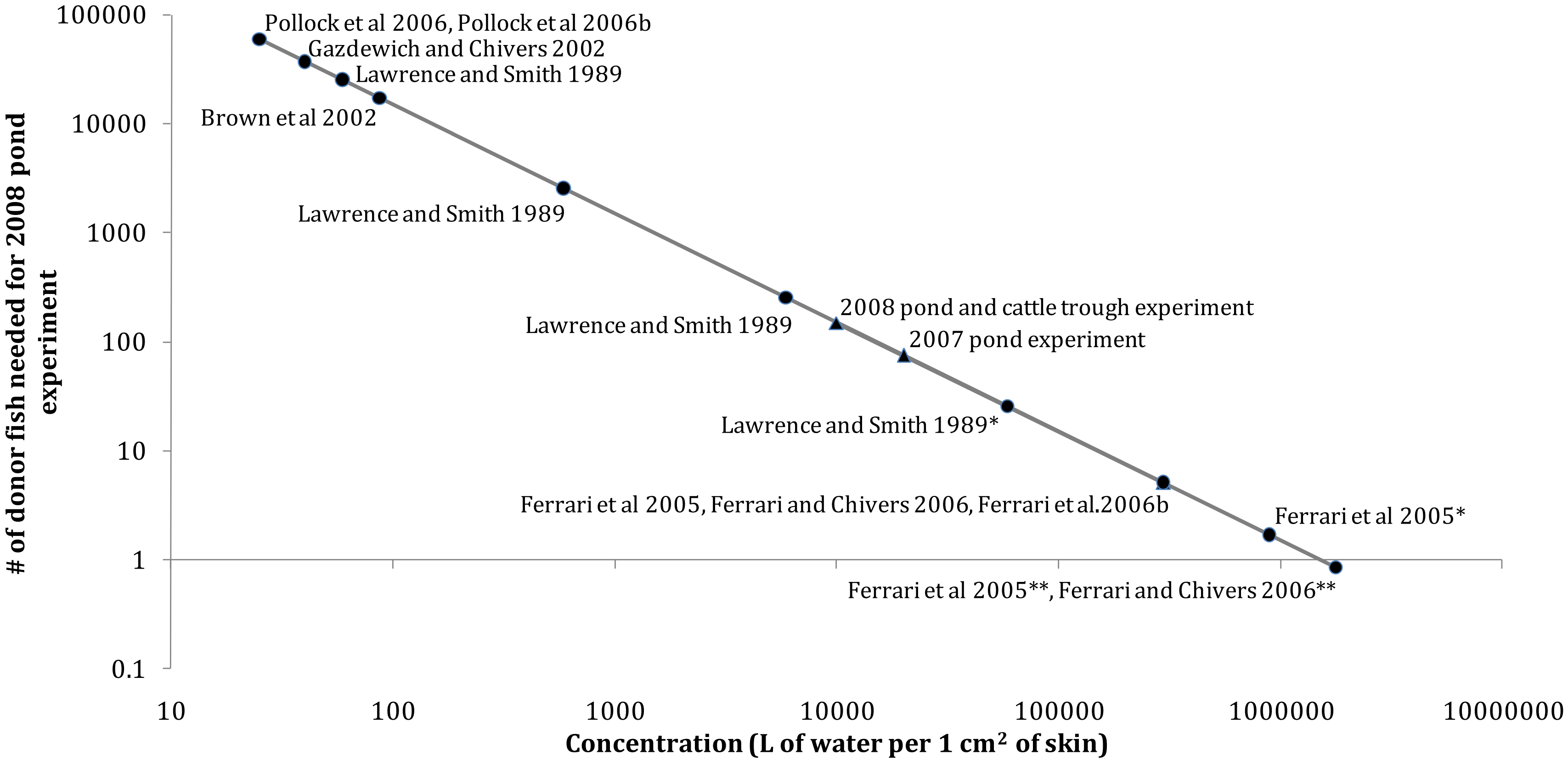

Figure 1. Summary of alarm substances concentrations used in literature for experiments on anti-predatory responses in fathead minnow and the corresponding number of donor fish that would be needed for the 2008 pond experiment (3 ponds treated 25 times). Alarm substances were introduced at a concentration of 1 cm^2: 20,000 L every 4th day in 2007 pond experiment, and 1 cm^2: 10,000 L every 2nd day in 2008 pond and cattle trough experiments.
* anti-predatory and exploratory responses, ** no response

Table 2. Comparison of protocols for pike odour collection and concentrations used to elicit anti-predatory behavioural responses in fathead minnow from 4 studies and the cattle trough experiment of this study. The number of pike is the number of individuals placed together in the collecting water. Pike were recorded as total length (TL), standard length (SL) or fork length (FL). The resultant concentration is the volume of aquarium water (L) that 1 L of the collected water would treat.

Study	# of pike	Size of pike (cm)	Volume (L) of collecting water	Collecting time (hr)	Resultant concentration (L water/ 1L collected water)
Chivers and Smith 1993	3	TL = 19.2 ± 3.55	1,200	72	8,100
Kusch et al 2004	2	SL = 22.8 and 25.0	100	24	308 to 1,233
	2	SL = 63 and 66	100	24	308 to 1,233 *
Ferrari et al 2006a	12	TL = 16.5 ± 1.5	444	24	617
	2	TL = 16.5 ± 1.5	74	24	617
Ferrari et al 2006b	1	TL = 20 and 22	60	24	617 and 1,850
Cattle trough experiment	1	FL = 46 and 48	60	24	600

* fathead minnow only responded to 308 to 617

Results

Female reproductive investment

Eggs began appearing on the nesting boards 1 to 4 days after fathead minnow populations were stocked into the ponds in both 2007 and 2008. Spawning commenced 5 to 9 days after stocking in the cattle trough experiment, with the exception of a single cattle trough treated with pike odour that did not begin until 29 days after stocking. No eggs were produced in 3 cattle troughs and have been excluded from the analysis except for stock fish growth and survival parameters. The timing of egg production was not different between the alarm substances and control treatments in the pond experiment for either 2007 and 2008 (repeated measures ANOVAs: 2007 $F_{1,2} = 0.05$, $p = 0.84$; 2008 $F_{1,4} = 0.10$, $p = 0.76$; Figures 2a,b). In contrast, eggs were laid earlier in the alarm substances treatment relative to the pike odour treatment and water control , in the cattle trough experiment (repeated measures ANOVA (treatment x time): $F_{39,78} = 1.55$, $p = 0.004$; Figure 2c).

There was no difference in the duration of the spawning season between treatments in the 2007 and 2008 pond experiments (2007, $p = 0.25$; 2008, $p = 0.69$; Table 3). Similarly, peak date (2007, $p = 0.50$; 2008, $p = 0.80$) and end date (2007, $p = 0.12$; 2008, $p = 0.75$; Table 3) were unaffected by the addition of alarm substances in the pond experiments. In contrast, exposure to alarm substances in the cattle trough experiment produced a significantly shorter spawning season than the pike odour treatment, but neither alarm substances nor pike odour differed from the water control (p = 0.05; Table 4). Alarm substances and pike odour had no effect on the peak date (p = 0.30) or end date (p = 0.09), although the latter was 11 and 15 days earlier in the alarm substances treatment than the water control and pike odour treatment, respectively (Table 4). Overall, the spawning season in the cattle trough experiment was shorter than the pond experiments (Tables 3,4).

Despite changes to the temporal distribution of egg production in the cattle trough experiment, overall production of eggs per female was similar among treatments (p = 0.62; Table 4). Similarly, there was no difference in egg production between alarm substances and control treatment in the pond experiments (2007, p = 0.73; 2008, p = 1.0; Table 3). In addition, females exposed to alarm substances had comparable gonadosomatic indices as those in the water control during the 2008 pond experiment (repeated measures ANOVA: $F_{2,3} = 8.86$, p = 0.06; Figure 3). There was no difference in the change in body condition or survivorship of females between treatments in the 2007 pond experiment (condition, p = 0.23; survival, p = 0.56; Table 3) or among the three treatments in the cattle trough experiment (condition, p = 0.78; survival, p = 0.85; Table 4). In the 2008 pond experiment, there was no difference in the pooled change in condition and survival for male, female and juvenile between the alarm substances and water control treatments (condition, p = 0.58; survival, p = 0.48; Table 3).

Male reproductive investment

Exposure to alarm substances and pike odour had no effect on the courtship performance of males. Starting date of nests ranged widely, from 1 to 38 days post-stocking in the 2007 pond experiment, 2 to 39 days in the 2008 pond experiment and 5 to 40 days in the cattle trough experiment. On average, start date did not differ among treatments (2007, $p = 0.68$; 2008, $p = 0.39$; cattle troughs, $p = 0.19$; Tables 5,6). Number of nests per male produced in ponds exposed to alarm substances did not differ from water controls in either 2007 or 2008 (2007, $p = 0.96$; 2008, $p = 0.76$; Table 5). Similarly, number of nests produced did not differ among the alarm substances, pike odour and control treatments in the cattle trough experiment ($p = 0.57$; Table 6). Numbers of egg batches that males acquired did not differ among treatments, averaging 2.3, 3.8 and 3.5 in the 2007, 2008 pond experiments and the cattle trough experiment respectively (2007, $p = 0.81$; 2008, $p = 0.15$; cattle troughs, $p = 0.24$; Tables 5,6). In addition, there was no difference in the size of egg batches between alarm substances and control treatments in the pond experiments (nested ANOVAs: 2007 $F_{1,2} = 1.54$, $p = 0.34$; 2008 $F_{1,4} = 1.54$, $p = 0.28$). Similarly, no difference in batch size was detected among alarm substances, pike odour and control treatments in the cattle trough experiment (nested ANOVA: $F_{2,9} = 0.07$, $p = 0.93$). As a consequence, exposure to alarm substances or pike odour had no effect on the total nest sizes in the pond experiments nor in the cattle trough experiment (2007, $p = 0.78$; 2008, $p = 0.74$; cattle troughs $F_{2,7} = 0.92$, $p = 0.45$; Tables 5,6).

Males appeared to invest large but equal amounts of energy towards reproduction regardless of the presence or absence of alarm substances or pike odour. The average duration of a nest was 10 days and did not differ between alarm substances and control treatments in the pond experiments or among alarm substances, pike odour and control treatments in the cattle trough experiment (2007, $p = 0.47$; 2008, $p = 0.31$; cattle troughs, $p = 0.11$; Tables 5,6). Exposure to alarm substances in the pond experiments did not affect the presence of a guarding male during nest monitoring (2007, $p = 0.49$; 2008, $p = 0.50$), although observed presence was 92% higher in 2007 than in 2008 (Table 5). Males exposed to alarm substances in the pond experiments showed the same levels of guarding behaviour as those in the water control (nested ANOVAs: 2007 $F_{1,1.99} = 1.28$, $p = 0.46$, 2008 $F_{1,3.91} = 0.19$, $p = 0.69$; Figure 4a,b). Male guarders were less aggressive when approached in 2008 than 2007 (Table 5). Similarly, exposure to alarm substances or pike odour did not change male guarding behaviour in the cattle trough experiment (nested ANOVAs: observed presence $F_{2,8.45} = 0.29$, $p = 0.75$; parental care $F_{2,8.45} = 0.36$, $p = 0.71$; Table 6, Figure 4c). Exposure to alarm substances in the 2007 pond experiment and alarm substances or pike odour in the cattle trough experiment did not affect male body condition

(2007, p = 0.28; cattle troughs, p = 0.48) or the number of males surviving to the end of the breeding season (2007, p = 0.44; cattle troughs, p = 0.18; Table 5,6). Over the breeding season, male body condition was reduced by 15% in the 2007 pond and 28% in the cattle trough experiment. There was a 66% male mortality rate in the 2007 pond experiment and a 9% mortality rate in the cattle trough experiment.

Reproductive and recruitment success

Exposure to alarm substances or pike odour had little or no effect on the success of nests. In 2007, but not 2008, there was a significantly higher number of infected nests exposed to alarm substances than the water control (2007, p = 0.03; 2008, p = 0.74; Table 7). Detection of fungal or mould infection was 79% lower in 2007 than 2008. Conversely, the percentage of nests failing to produce live young was 67% lower in 2008 than 2007. Alarm substances had no effect on the percentage of nest failures (2007, p = 0.87; 2008, p = 0.59) or the hatching success (2007, p = 0.28; 2008, p = 0.84; Table 7). In the cattle trough experiment, one nest exposed to pike odour showed signs of disease and two nests exposed to alarm substances failed to hatch (Table 8). There was no difference in the percentage of infected or failed nests among treatments (% infected, p = 0.11; % failed, p = 0.51; Table 8). Similarly, the percentage of eggs that successfully hatched was not affected by exposure to alarm substances or pike odour (p = 0.51; Table 8). Hatching rates in the cattle troughs were comparable to those observed in the pond experiments.

The quantity and quality of the young-of-the-year (YOY) surviving to the end of the summer was not affected by exposure to alarm substances or pike odour. There was no difference in the percentage of YOY surviving to the end of the summer between the treatments in the 2007 and 2008 pond experiments or among alarm substances, pike odour and control treatments in the cattle trough experiment (2007, p = 0.97; 2008, p = 0.96; cattle troughs, p = 0.38; Tables 7,8). Survival rate in the cattle trough experiment was 9%, comparable to survival rates in the pond experiments of 6% in the 2007 and 3% in 2008 (Table 7). Exposure to alarm substances or pike odour had no affect on the total recruitment of YOY at the end of the summer (2007, p = 0.63; 2008, p = 0.79; cattle troughs, p = 0.86; Tables 7,8). Average YOY length was also similar among treatments for both of the pond experiments (2007, p = 0.98; 2008, p = 0.26; Table 7), and the cattle trough experiment (p = 0.54; Table 8). Overall, there were no differences in the total biomass of young produced among treatments in the 2007 and 2008 pond experiments (2007, p = 0.75; 2008, p = 0.77; Table 7), or in the cattle trough experiment (p = 0.30; Table 8).

Table 3. Female reproductive investment for fathead minnow populations stocked in experimental ponds exposed to water as a control or alarm substances as a chemical signal of increased predation risk. Spawning season is defined as the duration of time new eggs were being laid. Peak nesting date is the day with the greatest number of new eggs laid and end date is the last day new eggs appeared. Change in body condition is calculated as the difference between average body condition at the end of the summer minus body condition at stocking. Data are averaged across ponds and given as mean ± SE, followed by Welch's t-tests comparing control to alarm substances.

	2007			2008		
	Control n=2	**Alarm substances ($1cm^2$:20,000L) n=2**	**t-tests**	**Control n=3**	**Alarm substances ($1cm^2$:10,000L) n=3**	**t-tests**
Spawning season (days)	26.5 ± 0.5	39.5 ± 5.5	$t_{1.02} = -2.35$	28.0 ± 6.6	32.3 ± 8.0	$t_{3.85} = -0.43$
peak nesting date (days post-stocking)	22 ± 2	23 ± 1	$t_{1.74} = -0.83$	12 ± 2	12 ± 2	$t_{4.00} = -0.27$
end date (days post-stocking)	31 ± 3	45 ± 5	$t_{2.00} = -2.83$	31 ± 6	35 ± 7	$t_{3.80} = -0.34$
Change in body condition (g/cm^3)	-0.27 ± 0.01	-0.14 ± 0.05	$t_{1.03} = -2.61$	0.37 ± 0.20§	0.55 ± 0.16§	$t_{3.72} = -0.59$
% survival	99 ± 1	95 ± 5	$t_{1.07} = 0.81$	39 ± 8§	52 ± 13§	$t_{3.34} = -0.80$
Total production (eggs/female)	1,993 ± 304	2,159 ± 294	$t_{2.00} = 0.39$	2,613 ± 179	2,616 ± 427	$t_{2.68} = -0.01$

§ Due to processing error in 2008, male, female and juvenile measurements for change in body condition and % survival data have been pooled.

Table 4. Female reproductive investment for fathead minnow stocked in cattle troughs exposed to chemical signals of increased predation risk via alarm substances or pike odour, with water as a control. Spawning season is defined as the duration of time new eggs were being laid. Peak nesting date is the day with the greatest number of new eggs and end date is the last day new eggs appeared. Change in body condition is calculated as the difference between average body condition at the end of the summer minus body condition at stocking. Data are averaged across ponds and given as mean ± SE followed by 1-way ANOVA comparisons.

	Control n=4	**Alarm substances ($1cm^2$:10,000L) n=4**	**Pike odour n=4**	**1-way ANOVAs**
Spawning season (days)	13.5 ± 3.0	7.3 ± 1.3	14.5 ± 0.5	$F_{2,9} = 4.32$*
peak nesting date (days post-stocking)	14 ± 4	9 ± 1	20 ± 7	$F_{2,9} = 1.37$
end date (days post-stocking)	24 ± 5	13 ± 1	28 ± 6	$F_{2,9} = 3.25$
Change in body condition (g/cm^3)	-0.49 ± 0.03	-0.53 ± 0.04	-0.05 ± 0.07	$F_{2,12} = 0.26$
% survival	92 ± 8	96 ± 4	96 ± 4	$F_{2,12} = 0.17$
Total production (eggs/female)	571 ± 182	405 ± 174	441 ± 148	$F_{2,9} = 0.50$

* alarm substances treatment is statistically different from pike odour treatment, $p < 0.05$

Table 5. Measures of male reproductive effort from fathead minnow populations stocked in experimental ponds exposed to alarm substances (AS) or water control. The concentration of alarm substances was 1cm^2:20,000L in 2007 and 1cm^2:10,000L in 2008. Batch is defined as a new group of eggs added to a nest. The % of observations present is the proportion of daily nest observations that a male was present. Maximum parental care is the highest recorded level of guarding (0 absent, 1 fled, 2 passive and 3 active). Change in body condition is the difference between average body condition at the start and end of the experiment. Data are averaged across ponds and given as mean ± SE, followed by the statistical tests for 2007 and 2008 data respectively.

	2007		2008		
	Control n=2	**AS n=2**	**Control n=3**	**AS n=3**	**Statistical test: 2007; 2008**
Courting					
# of nests/male	0.93 ± 0.08	0.93 ± 0.02	0.69 ± 0.01	0.73 ± 0.12	t-test: $t_{1.11} = 0.05$; $t_{2.03} = -0.35$
average starting date (days post-stocking)	12 ± 1	15 ± 1	9 ± 0.5	12 ± 1	nested ANOVA: $F_{1,2.00} = 0.31$; $F_{1,3.95} = 0.98$
# of egg batches/nest	2.4 ± 0.1	2.2 ± 0.1	4.2 ± 0.3	3.5 ± 0.3	nested ANOVA: $F_{1,2.00} = 0.09$; $F_{1,3.83} = 3.82$
average nest size (# of eggs)	2,138 ± 166	2,317 ± 171	2,907 ± 260	1,793 ± 220	nested ANOVA: $F_{1,1.99} = 0.13$; $F_{1,3.93} = 0.13$
Defence					
% of observations present	59.2 ± 3.0	56.1 ± 2.9	26.0 ± 2.5	33.7 ± 2.9	nested ANOVA: $F_{1,1.99} = 1.06$; $F_{1,3.93} = 0.57$
max parental care	2.0 ± 0.1	2.0 ± 0.1	1.7 ± 0.1	1.5 ± 0.1	nested ANOVA: $F_{1,1.99} = 1.28$; $F_{1,3.91} = 0.19$
Investment					
nest duration (days)	10.5 ± 0.6	9.7 ± 0.6	11.0 ± 0.6	9.5 ± 0.7	nested ANOVA: $F_{1,1.99} = 1.19$; $F_{1,3.89} = 1.48$
change in body condition	-0.24 ± 0.01	-0.14 ± 0.05	0.37 ± 0.20§	0.55 ± 0.16§	t-test: $t_{1.00} = -2.12$; $t_{3.72} = -0.59$
% survival	39 ± 9	29 ± 5	39 ± 8§	52 ± 13§	t-test: $t_{1.47} = 1.37$; $t_{3.34} = -0.80$

§ Due to processing errors in 2008, male, female and juvenile measurements for change in body condition and % survival data have been pooled.

Table 6. Measures of male reproductive effort from fathead minnow stocked into cattle troughs exposed to chemical signals of increased predation risk via alarm substances and pike odour, with water as a control. Batch is defined as a new group of eggs added to a nest. The % of observations present is the proportion of daily nest observations that a male was present. Maximum parental care is the highest recorded level of guarding (0 absent, 1 fled, 2 passive and 3 active). Change in body condition is the difference between average body condition at the start and end of the experiment. Values are averaged across cattle troughs within treatment and given as mean ± SE, followed by the comparative statistical tests.

	Control n=4	**Alarm substances ($1cm^2$:10,000L) n=4**	**Pike odour n=4**	**Statistical test**
Courting				
# of nests/male	0.32 ± 0.08	0.32 ± 0.08	0.40 ± 0.05	1-way ANOVA: $F_{2,9} = 0.60$
average starting date (days post-stocking)	14 ± 3	6 ± 1	18 ± 5	nested ANOVA: $F_{2,8.38} = 2.09$
# of egg batches/nest	4.9 ± 0.9	2.6 ± 0.5	3.1 ± 0.7	nested ANOVA: $F_{2,7.76} = 1.74$
average nest size (# of eggs)	1,783 ± 380	1,276 ± 306	1,116 ± 170	nested ANVOA: $F_{2,7} = 0.92$
Defence				
% of observations male present	49.0 ± 10.2	35.6 ± 12.3	41.5 ± 6.9	nested ANOVA: $F_{2,8.45} = 0.29$
max parental care	1.8 ± 0.3	1.3 ± 0.4	1.5 ± 0.3	nested ANOVA: $F_{2,8.45} = 0.36$
Investment				
nest duration (days)	10.4 ± 1.5	6.6 ± 1.2	11.2 ± 1.3	nested ANOVA: $F_{2,7.48} = 3.14$
change in body condition	-0.29 ± 0.03	-0.36 ± 0.06	-0.37 ± 0.04	1-way ANOVA: $F_{2,12} = 0.79$
% survival	84 ± 4	96 ± 4	92 ± 5	1-way ANOVA: $F_{2,12} = 2.00$

Table 7. Reproductive success and characteristics of the young-of-the-year (YOY) produced by fathead minnow stocked into experimental ponds and exposed to a chemical signal of increased in predation risk via the addition of alarm substances as a chemical cue. The percentage hatched is the proportion of eggs determined to be hatched for each pond. Percentage infected and percentage failed is defined as the proportion of nests showing signs of infection and proportion of nests producing zero hatchlings, respectively. Total length is the average of all YOY individuals in each pond. Biomass is the total mass of YOY in the ponds. Survival is the number of live YOY at the experiment as a percentage of the number of eggs produced in each pond. Data are averaged across ponds in each treatment and given as mean ± SE, followed by Welch's t-tests comparing control to alarm substances for each year.

	2007			2008		
	Control n=2	**Alarm substances ($1cm^2$:20,000L) n=2**	**t-tests**	**Control n=3**	**Alarm substances ($1cm^2$:10,000L) n=3**	**t-tests**
Nest success						
% hatched	2.4 ± 0.6	13.8 ± 1.2	$t_{1.26} = 1.84$*	35.8 ± 19.5	44.3 ± 14.5	$t_{3.17} = -0.22$
% infected	58.8 ± 6.4	46.3 ± 2.3	$t_{1.51} = -8.83$	77.1 ± 2.6	77.7 ± 1.5	$t_{3.69} = -0.35$
% failed	31.8 ± 17.5	35.6 ± 7.3	$t_{1.34} = -0.20$	8.8 ± 2.5	12.0 ± 5.3	$t_{2.84} = -0.61$
YOY characteristics						
Total length (mm)	20.3 ± 1.0	20.4 ± 3.8	$t_{1.13} = -0.02$	19.7 ± 1.5	22.1 ± 0.4	$t_{2.29} = -1.48$
Biomass (g/pond)	741 ± 123	912 ±417	$t_{1.17} = -0.40$	296 ± 151	242 ± 73	$t_{2.88} = 0.32$
Survival (%)	5.9 ± 1.1	6.0 ± 1.7	$t_{1.73} = -0.04$	3.6 ± 0.8	3.5 ± 1.2	$t_{3.45} = 0.05$
Total recruitment (#)	7,759 ± 111	8,550 ± 1,211	$t_{1.02} = -0.65$	2,306 ± 362	2,133 ± 479	$t_{3.72} = 0.29$

* denotes statistically significant difference, $p < 0.05$

Table 8. Reproductive success and characteristics of the young-of-the-year (YOY) produced by fathead minnows stocked in cattle troughs and exposed to a chemical signal of increased predation risk via alarm substances or pike odour. The percentage hatched is the proportion of eggs determined to be hatched for each pond. Percentage infected and percentage failed is defined as the proportion of nests showing signs of infection and proportion of nests producing zero hatchlings, respectively. Total length is the average of all YOY individuals in each pond. Biomass is the total mass of YOY in the ponds. Survival is the number of live YOY at the experiment as a percentage of the number of eggs produced in each pond. Data are averaged across cattle troughs in each treatment and given as mean ± SE, followed by comparative 1-way ANOVAs.

	Control n=4	**Alarm substances ($1cm^2$:10,000L) n=4**	**Pike odour n=4**	**1-way ANOVAs**
Nesting success				
% infected	0	0	12.5 ± 12.5	$F_{2,9} = 1.00$
% hatched	62.9 ± 13.1	66.5 ± 21.2	85.8 ± 3.3	$F_{2,9} = 0.72$
% total failure	0	20.8 ± 12.5	0	$F_{2,9} = 2.78$
YOY characteristics				
Total length (mm)	16.2 ± 0.8	18.0 ± 4.1	15.3 ± 1.4	$F_{2,9} = 0.66$
Biomass (g/trough)	6.7 ± 2.8	10.7 ± 2.4	5.6 ± 1.6	$F_{2,9} = 1.39$
Survival (%)	7.9 ± 3.8	13.1 ± 4.1	6.7 ± 1.2	$F_{2,9} = 1.07$
Total recruitment (#)	210 ± 96	262 ± 74	204 ± 68	$F_{2,9} = 0.16$

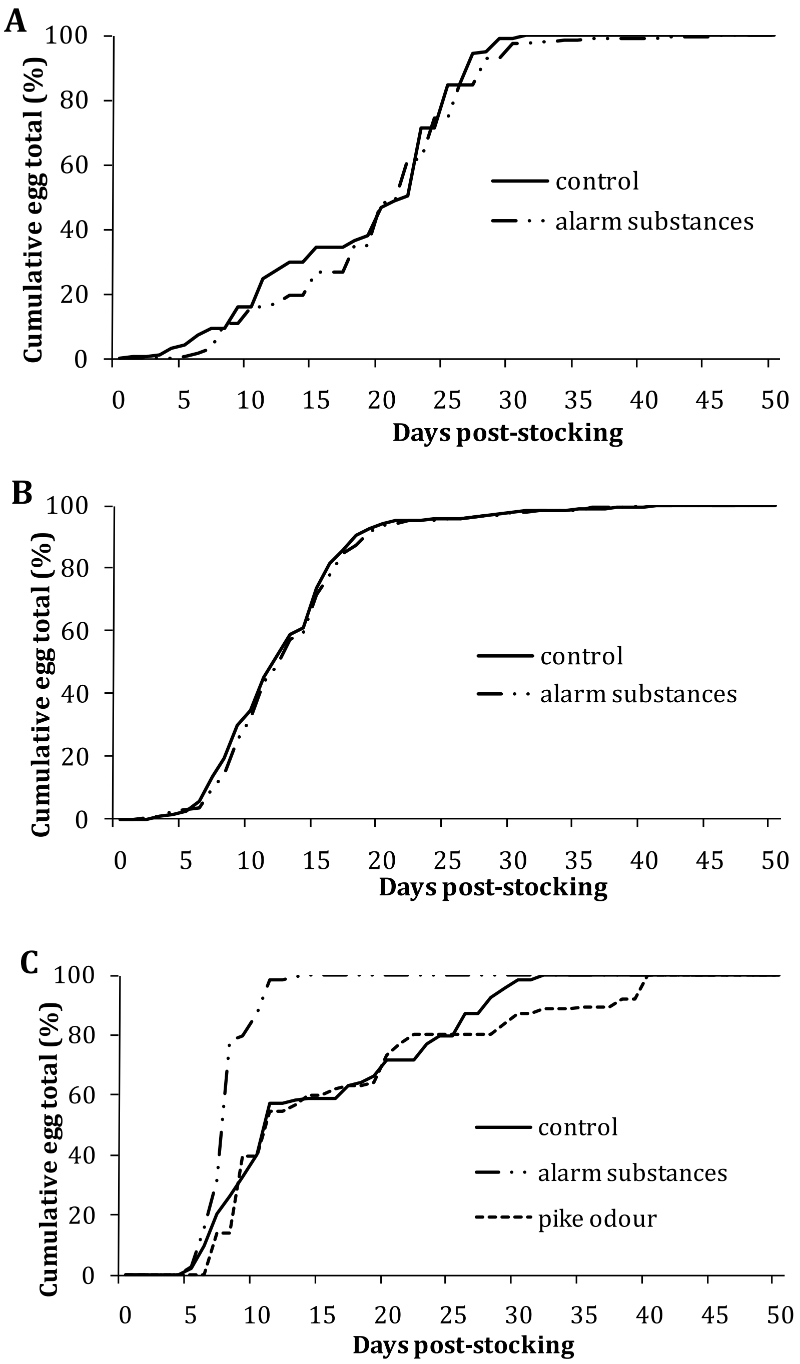

Figure 2. Cumulative egg production over the spawning season, comparing water controls to alarm substances at (A) 1cm^2:20,000L in the 2007 pond experiment (n=2 per treatment), (B) 1cm^2:10,000L in the 2008 pond experiment (n=3 per treatment) and (C) 1cm^2:10,000L and pike odour in the cattle trough experiment (n=4 per treatment). Data are averaged across ponds and cattle troughs respectively.

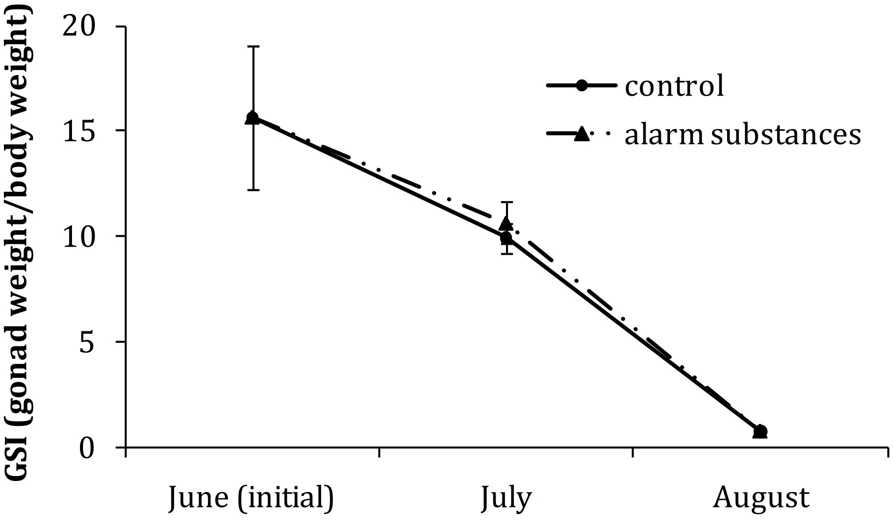

Figure 3. Comparison of female gonadosomatic index (GSI; mean ± SE) between fish exposed to alarm substances at 1cm^2:10,000L or water control. Initial measurements were taken from 16 female fish from the Rochester Lake population that were not stocked in the pond experiment. Fish stocked into the ponds were from Rochester Lake. July and August measurements are each taken from a subset of 6 female fathead minnows stocked in the 2008 pond experiment.

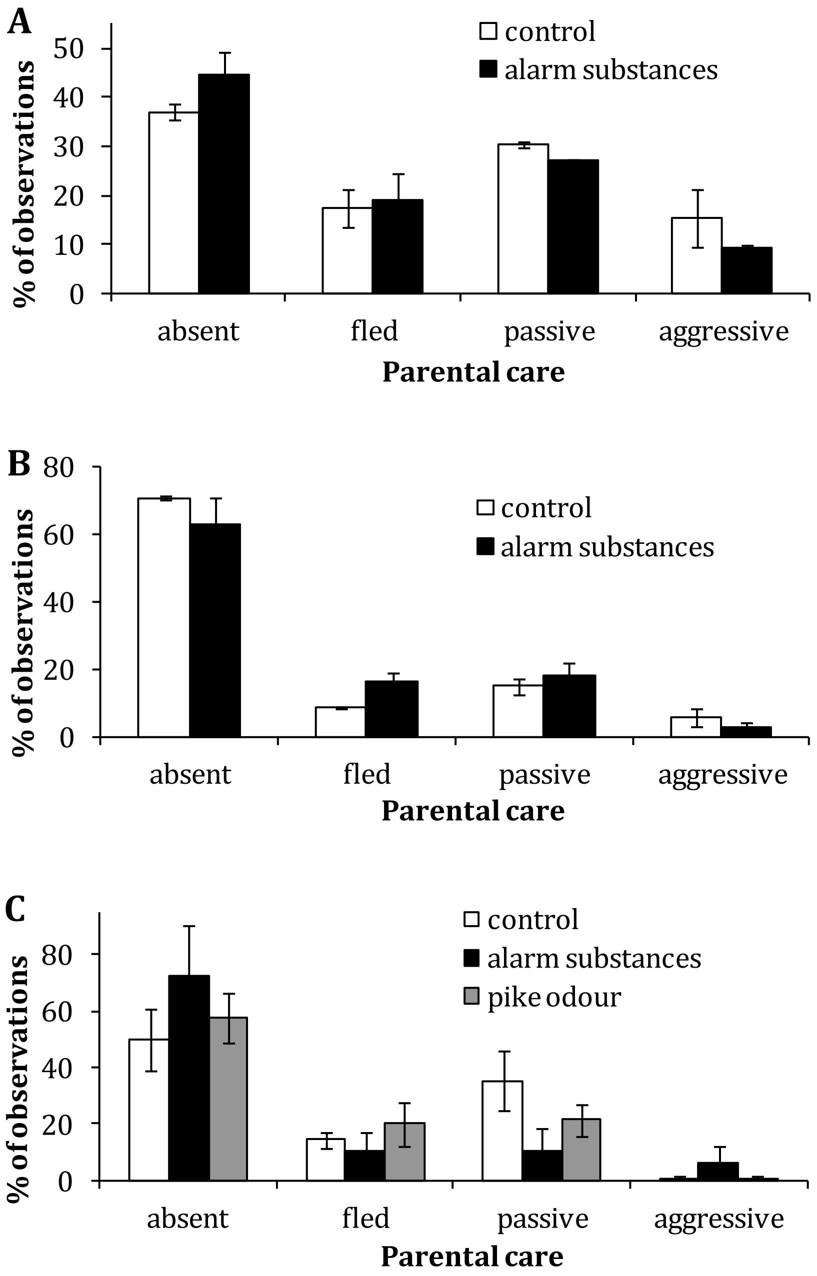

Figure 4. Male parental care (mean ± SE) observed during nest monitoring, comparing water controls to alarm substances at (A) 1cm^2:20,000L 2007 in the pond experiment (n=2 per treatment), (B) 1cm^2:10,000L in the 2008 pond experiment (n=3 per treatment) and (C) 1cm^2:10,000L and pike odour in the cattle trough experiment (n=4 per treatment). Data are percentages of observations at each level of parental care averaged across the ponds and cattle troughs. Parental care levels are defined as absent – male not seen, fled – male seen on approach, but not during nest monitoring, passive – male nearby and aggressive – male actively repelling observer.

Discussion

Alarm substances and pike odour are chemical cues that are associated with an increase in predation risk. In the laboratory, fathead minnow have been found to respond to alarm substances and pike odour by reducing conspicuous behaviours and seeking protection (Lawrence and Smith 1989, Chivers and Smith 1998). Reduction in feeding, growth and reproduction has also been observed in a variety of fishes in the laboratory setting (Jachner 1997, Jachner and Janecki 1999, Pollock et al. 2005).

These responses to predatory chemical cues were expected to have population-level effects on reproduction and recruitment at the larger scale of my experiments. Exposure to alarm substances or pike odour was expected to reduce male courting, nest guarding and parental care behaviours, negatively impacting the number and size of nests, and hatching success. Females were expected to reduce their susceptibility to predation risk by producing fewer eggs and laying them earlier in the breeding season. Reduced foraging and increased energy expenditure caused by a perceived increase in predation risk was expected to decrease growth rate, body condition and survival of adult fathead minnow. Finally, a reduction in reproduction was expected to recruit fewer but larger young-of-the-year into the population. In the pond experiments, neither the 2007 nor the 2008 alarm substances treatment regime produced an effect at the population-level. Similarly, population-level effects were consistently absent when fish were exposed to either alarm substances or pike odour in the cattle trough experiment, with the exception of reproductive timing in the alarm substances treatment.

How much parental care is enough?

Contrary to expectations, conspicuous male breeding behaviours were not reduced in the alarm substances treatment for either the cattle trough or pond experiments. In the laboratory, exposure to alarm substances consistently increases time spent in shelter. For example, male fathead minnows with established nesting territories will abandon nests in favour of shelter when exposed to alarm substances in the laboratory (Jones and Paszkowski 1997a). The addition of alarm substances in my larger-scale field experiments; however, did not change the frequency a male was present at the nests during nest monitoring. In my experiments, it is often difficult to see a dark breeding male when he is motionless and positioned against the black nesting board. Although there was little alternative shelter around the nesting boards, the boards themselves may have provided some means of shelter.

Males that are better able to maintain a good nesting territory and court females will have greater reproductive success (Unger 1983, Sargent 1988). There was no difference in the frequency that males were present at the nest, or the level of nest-guarding behaviours displayed by males with exposure to alarm substances. Males may need to take greater risks to successfully reproduce because the consequences of neglecting a nest may be higher in a natural environment than in the laboratory setting. In the cattle troughs and ponds, unlike many short-term behavioural experiments conducted in the laboratory, males may need to be present at the nest to defend against conspecifics and other egg predators and/or compete for nesting territories regardless of the risk of predation. In addition, heterospecific egg predators will not necessarily respond to alarm substances released from fathead minnow and may capitalize on a male that seeks shelter (Mathis and Smith 1993b). With so many direct threats to their eggs, males may be more willing to accept the additional risks associated with alarm substances by remaining at their nests and actively guarding them against predators.

Once male fathead minnows reach sexual maturity, they are likely to invest all their reserved energy into reproduction, as in northern populations males generally only have one season of breeding opportunity (Becker 1983, Candolin 1998). The presence of alarm substances did not affect the willingness of males to invest in reproduction, as there was no change to the size of nests or the number of batches acquired. The average date of nest establishment was the same between the alarm substances and water control treatment. This is in contrast to the faster breeding found in convict cichlids (Pollock et al. 2005). The addition of alarm substances also did not change the average duration of the nests.

Although males were able to establish and acquire the same number of eggs, infection rates were significantly higher in the alarm substances addition in the 2007 pond experiment. Male dorsal pads may contain anti-microbial properties and actively rubbing eggs can provide some protection against fungal infection (Smith 1978, Hellio et al. 2002, Knouft et al. 2003). Males also appear to actively remove infected eggs from their nest (McMillan 1972). An increase in infection rate in the alarm substances treatment suggests that although males may be present at the nest, they were less active in maintaining their nests. Male fathead minnow that were presented with a caged predator, northern pike, rubbed their nests less frequently than those presented with a wooden model (Jones and Paszkowski 1997b). In contrast to the 2007 pond experiment, however, exposure to alarm substances in the 2008 pond experiment and the cattle trough experiment did not increase infection rates, despite the more concentrated and frequent addition of alarm substances. Infected eggs may remain in the nests longer if males are reluctant to remove infected eggs. Male guarders would have had more time to remove infected eggs in 2007 than 2008, since nest monitoring was generally completed in the morning in 2008, but required the entire day in 2007. This may be reflected by the overall infection rate in 2008 being 5 times higher than in 2007.

Changes to reproductive behaviour may have been more subtle than could be measured at the larger scale of my field experiments. However, hatching success is largely dependent on male parental care (Divino and Tonn 2008). The addition of alarm substances did not alter the hatching success or the rate of nest failure in my experiments. Regardless of the sensitivity of my monitoring, any change in male breeding behaviour that may have resulted from the addition of alarm substances was irrelevant at the population scale.

Female reproductive investment

In environments where the risk of predation is high, the probability of future reproduction is likely lower and as a consequence current reproduction becomes more important to life time reproductive success (Candolin 1998). Fathead minnow populations in northern Alberta generally have only one or two summers in which to reproduce, making current reproduction even more important (Danylchuk and Tonn 2006). The addition of alarm substances, a signal of increased predation risk, in the cattle trough and pond experiments did not suppress the production of eggs. This is reflected in the lack of difference in GSI seen in the 2008 pond experiment. Similarly, production of eggs was found to be unchanged in convict cichlids when exposed to alarm substances (Pollock et al. 2005) and sand gobies in the presence of a predator (Forsgren and Magnhagen 1993). This contrasts with mammalian females, which generally avoid or suppress reproduction when the risk of predation is high (Fuelling and Halle 2004, Creel et al. 2007). Foraging limitation is often the underlying cause of the latter reproductive suppression. The addition of alarm substances, however, did not appear to limit the foraging of fathead minnows, as the average mass and survival of both females and males did not differ among treatments in either the cattle trough or pond experiment. Furthermore, Pollock et al.

(2006b) found that fathead minnow with lower body condition responded equally to alarm substances as an individual in better condition. Although females had poorer body condition at the end of the summer in the cattle trough experiment than the pond experiment, number of eggs laid per female was similar once accounting for the differences in stocking dates between the experiments.

In the cattle trough experiment, eggs were laid earlier in the season when fish were exposed to alarm substances, as compared to the water control. Although fewer convict cichlid pairs had multiple breeding attempts when exposed to alarm substances (Pollock et al. 2005), fathead minnows in the cattle trough experiment produced the same number of egg batches, but in quicker succession, than control fish. Greater synchrony of eggs at the population level may provide better protection for young by swamping predators (Ims 1990). In addition, females would be gravid for a shorter period of time, potentially decreasing their risk of predation (Sih 1994). However, studies have shown that eggs with less maternal investment and faster development produce young with swimming impairments (Ojanguren 1996, Evans et al. 2007). Although earlier reproduction would allow young more time to forage and grow, there was no difference in the length or mass of young among treatments at the end of the summer in the cattle trough experiment.

However, this pattern of earlier, more synchronous reproduction was not seen in the pond experiment. The increased size and more complex habitat structure in the ponds likely provided a safer, less stressful environment by reducing visibility and providing more places to hide. In addition, as the absolute number of fish increases, females are able to form larger schools, which can provide a measure of security against predation when searching for potential mates and foraging (Wisenden et al. 2003, Pollock et al. 2006a). In both the cattle trough and pond experiments, young-of-the-year exposed to alarm substances were of equal quality and quantity to their control counterparts. Any changes observed in reproductive effort or synchrony did not appear to affect the recruitment of young into the population.

Pike odour

Overall, reproduction and recruitment in the pike odour treatment were more similar to the water control than to the alarm substances treatment. Unlike the latter, reproductive patterns in the pike odour treatment did not differ from the water control in the cattle trough experiment. Similarly, the addition of pike odour did not affect recruitment. Alarm substances indicate that there is an active predator in the area, as they are only released when there is an injury (Pfeiffer 1963) and serve as a high priority, short-term cue. Pike odour in contrast is likely a lower level chronic cue that produces heightened awareness, but not necessarily a cessation of conspicuous activities. Rapid cessation of conspicuous activities and seeking protection are appropriate short-term defensive responses, but inappropriate for longer periods of time. Response to alarm substances can persist for over 24 hours (Jones and Paszkowski 1997a); however, fathead minnows will often return to near normal swimming patterns within 10 minutes of exposure (per obs). Pike odour is likely to be continually released and therefore present in the environment most of the time. Pike odour may be perceived as an environmental cue with individuals responding to subtle changes witnin the chemical cue. Prey species have been found to modify their response to pike odour based on the size, density, proximity, and starvation state of the pike (Jachner 1997, Kusch et al. 2004, Ferrari et al. 2006a). Such changes to behaviour may not be expected to greatly affect reproduction or recruitment.

The fathead minnows used in the cattle trough experiment were naive to northern pike, as Rochester Lake does not contain this predator. Although the pike odour is detected by naive fathead minnow (Mathis and Smith 1993a, Ferrari et al. 2006b), they may not respond strongly because pike odour is not recognized as a predator. This problem may be resolved by training fathead minnow to recognize pike odour as a threat prior to experimentation; for example by pairing pike odour with alarm substances. Alternatively, it would be interesting to use fathead minnow that co-exist with northern pike to test for reactions to alarm substances and pike odour.

Scaling up - the addition of competition and environmental variation

The chemical components of pike odour and alarm substances have not been identified and there is currently no protocol to directly measure the concentration of either from the environment. The concentration of alarm substances an individual would experience in a high risk environment has also not been estimated; however, concentrations of alarm substances used in laboratory-based studies are often too high to be feasible at larger, more natural scales. For example, Pollock et al. (2005) used daily doses at a concentration of 1 cm^2: 20 L to determine if alarm substances had an effect on reproduction and growth of convict cichlids. Scaling this to a pond of 100,000 L would require 5,000 cm^2 of epidermis per day. Given that I was able to produce approximately 5 cm^2 of skin from a female fathead minnow, 1,000 fish would be required for a single treatment in one pond. The breeding season in my pond experiments lasted 51 days in 2007 and 44 days in 2008, with alarm substances added to 2 and 3 ponds in 2007 and 2008 respectively. Treating the ponds following the protocols from Pollock et al. (2005) would have required 102,000 donor fish in 2007 and 132,000 in 2008. Higher concentrations of alarm substances may lengthen the time individuals respond behaviourally and produce stronger reproductive and recruitment reactions. Conversely, fathead minnows do respond behaviourally to concentrations as low as 1 cm^2: 296,000 L in the laboratory (Ferrari et al. 2005), a much lower concentration than what was added to the ponds.

The frequency of exposure to alarm substances in a risky environment is also difficult to estimate. The frequencies used in my study were based on estimates of fathead minnow encounter rates with northern pike (Jones and Paszkowski 1997b), combined with the constraints of producing alarm substances at the pond scale. Maximum consumption rate of fathead minnow by YOY northern pike at 20 °C is 0.113 g (fathead minnow)/g(northern pike)*day (Bevelhimer et al. 1985). This equates to approximately 11 adult fathead minnow consumed per day by a northern pike of 400 g, and agrees with studies of stomach contents showing that northern pike can have a number of forage fish in their stomachs (Seaburg and Moyle 1964, Lawler 1965). Fish may therefore detect alarm substances in the environment as frequently as several times a day, rather than daily or the once every few days frequency, which I used. Increasing the frequency of exposure might better mimic the natural regime and provide a stronger test of alarm substances on fathead minnow populations. However, increasing the frequency would lead to similar issues as those of using higher concentrations.

Furthermore, the concentration and frequency of pike odour regularly experienced by fathead minnows co-existing with pike is unknown. The concentration I added to the cattle troughs was used in the literature to elicit a response from fathead minnow by Ferrari et al. 2006a. Having pike odour continually present in the system may be more realistic than regular pulses every few days. Pike odour could be continually present in the system by designing a system to circulate water past a pike prior to entering the cattle trough or by maintaining the pike in part of the cattle trough with a visual barrier that allows water to pass freely (Jones et al. 2003, Dzikowski et al. 2004).

Behavioural responses in the laboratory are typically measured immediately before and after the addition of a chemical stimulus. In contrast, behavioural responses in my study were measured at least 15 to 20 hours after the chemical stimuli were added. By then, fathead minnows may no longer have been responding behaviourally to the alarm substances or pike odour, although Jones and Paszkowski (1997a) found that fathead minnows continued to respond to chemical stimuli 24 hours after exposure. The lack of behavioural changes may have been due, at least in part, to the degradation of the alarm substances and pike odour. A good signal of danger should spread quickly to individuals at risk and decay relatively rapidly so the environment does not become saturated. Wisenden et al. (2009) found that individuals from various species will respond to alarm substances that had been aged for 3, but not 6 hours. Alarm cues from wood frog (*Lithobates sylvaticus*) larvae became ineffective in eliciting behavioural changes after 2 hours in the field (Ferrari et al. 2008), suggesting that decay rates are likely quicker in the natural environment.

The effects of alarm substances are stronger in environments that are small and contained. The volume that is avoided by fish or the active space from 1 cm^2 of epidermis is estimated to be 58 m^3 (Lawrence and Smith 1989). So, a small aquarium can be completely inundated by alarm substances within seconds (Smith 2000). Similarly, the cattle troughs are likely small enough that alarm substances released from any point would quickly circulate throughout the entire volume of water. Unlike the aquarium or cattle trough setting, however, the size and habitat structure of the ponds likely resulted in a heterogeneous distribution of alarm substances in the ponds. Wisenden (2008) found that traps labelled with 2 cm^2 of alarm substances were avoided by fish at a distance of 2 m, but not 8 m in small lakes. It has been well documented that prey use predator odours to avoid areas of high predation risk (Kats and Dill 1998). A heterogeneous environment would have areas of higher and lower predation risk that may dampen the effect of alarm substances at the population-level.

Conclusion

Given the complexities of natural environments relative to small aquaria, it is probably not surprising that there is debate about how alarm substances function in the natural environment (Magurran et al. 1996, Irving and Magurran 1997, Wisenden 2004, Friesen and Chivers 2006). The responses to alarm substances during an 8 or 10 minute observation period in a small aquarium can hardly predict the outcome of exposure in ponds over the course of days and weeks. Reproductive changes in the cattle trough experiment were minor; spawning occurred earlier in the presence of alarm substances, but the number of nesting attempts was unchanged. Furthermore, in the pond experiment I consistently detected no change to behaviour and, more importantly, no change to reproduction in the presence of alarm substances. There was no trade off between growth and reproduction or the abundance and quality of young produced with the addition of alarm substances in the cattle trough and pond experiments. Similarly, there were no changes in behaviour, reproduction or recruitment to the addition of pike odour. Overall, there was no effect of alarm substances and pike odour on fathead minnow at the population level.

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