LEARNING TO RECOGNIZE AND GENERALIZE THE SIGHT OF PREDATORS AND NON-PREDATORS: DOES TURBIDITY IMPAIR RECOGNITION?

A Thesis submitted to the College of Graduate Studies and Research in Partial Fulfillment of the Requirements for the Degree of Master of Science in the Department of Biology University of Saskatchewan Saskatoon

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DEDICATION

I would love to dedicate this thesis to my family

- My parents, who always support and encourage me, and without them I am worthless.
- My wife, for her patience and unyielding love.
- My little angels, Ghadah and Wid.

Without all of you, I would never keep going.
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LIST OF ABBREVIATIONS

AC…………….. Alarm Cues
ASC……………. Alarm Substance Cells
BT……………… Brook Trout
BT₀…………….. group of minnows pre-exposed to Brook Trout
BT₁…………….. group of minnows pre-exposed to Brook Trout in clear water
BT₂…………….. group of minnows pre-exposed to Brook Trout in turbid water
“bt”…………… group of minnows conditioned to brook trout sight + AC
cm……………… centimetre
E……………….. group of minnows pre-exposed to Empty tank
E₁……………… group of minnows pre-exposed to Empty tank in clear water
E₂……………… group of minnows pre-exposed to Empty tank in turbid water
FL……………… Fork Length
L……………….. Liter
M………………. Muscle layer
m……………….. meter
MANOVA…….. Multivariate analysis of variance
MC……………… Mucous Cell
Me……………… Melanophores
mg…………….. Milligram
min…………….. minute
mL…………….. Milliliter
NTU…………….. Nephelometric Turbidity Units
RT……………… Rainbow Trout
“rt”…………… group of minnows conditioned to rainbow trout sight + AC
s………………. second
Sc……………… fish scales
vs……………… Versus
YP…………….. Yellow Perch
“yp”…………… group of minnows conditioned to yellow perch sight + AC
ABSTRACT:

To be successful, individuals that are susceptible to predation have to optimize the trade-offs between predator avoidance and other fitness related activities such as foraging or reproduction. One challenge for prey is to identify which species pose a threat and should be avoided, and which species should be ignored. The goal of this study was to investigate whether minnows can generalize recognition of predators and non-predators using visual cues. I conducted experiments in both clear and turbid conditions to test whether the level of turbidity affects the quality of visual information available to the prey and hence the ability of prey to generalize. Latent inhibition and learned irrelevance are mechanisms of learning that can be used by prey to recognize stimuli as non-risky. Repeated exposure to an unknown stimulus in the absence of risk leads to the stimulus being categorized as non-risky. Fathead minnows were pre-exposed to the sight of brook trout or control water to provide minnows the opportunity to learn to recognize the trout as a non-predator. Following this the fish were conditioned with alarm cues (AC) to the sight of each predator paired and then their responses to the sight of brook trout, rainbow trout, and yellow perch were tested either in clear or turbid water. In clear water, minnows conditioned to recognize one of the trout species generalized their response to the other species. However, when the minnows were pre-exposed to the sight of a brook trout, they were inhibited from subsequently recognizing the sight of brook trout as threat and generalized this non-predator recognition to the sight of rainbow trout but not to yellow perch.

In turbid water, however, minnows that were pre-exposed to the sight of brook trout had impaired responses to all predators while those pre-exposed to water showed an intermediate intensity anti-predator response toward each predator. Overall, my results demonstrate that minnows were able to distinguish between predators and non-predators in the clear environment but turbidity influences the visual information used by minnows and hence impaired the minnow’s ability to recognize and generalize the sight of predators and non-predator species.
CHAPTER 1: Introduction

The daily routine for many animals consists of searching for food or mates while at the same time avoiding predators. Failing to obtain a meal means they will go hungry and failing to find a mate means unsuccessful reproduction (Lima & Dill 1990). The day’s shortcomings of such activities may have minimal effect on lifetime fitness over the long term. On the other hand, other failures, such as failing to avoid predators, are unforgiving and greatly decrease future fitness (Lima & Dill 1990). Predation is an important selective force over evolutionary time that shapes behavioural, morphological and life history defences of prey animals (Edmunds 1974; Harvey & Greenwood 1978; and Sih 1987). Also, predation has been recognized as an important factor in the evolution of sociality in both breeding and non-breeding seasons (Bertram 1978; Pulliam & Caraco 1984). Many prey have been forced to alter their reproductive strategies to avoid predation.

Species that are susceptible to predation have developed various techniques to prevent detection or capture by predators. For some prey, standing and fighting a predator might be an essential defensive tactic while fleeing may be a better choice for others to survive (Dent et al. 1980). Means of defense among animals take various forms whether it is behavioural, morphological, or chemical. According to Silverstein et al. (2008), high speed is enough for some prey to avoid capture. Antelope that can sprint up to 40 miles per hour, can get away from their enemies relatively easily. However, speed alone may not be enough. Rabbits are very fast, but in order to escape an equally fast fox they must hop in a zigzag to confuse the fox (Silverstein et al. 2008).

Camouflage is one of the most commonly observed morphological anti-predator responses. Some species can change color to suit the season. Animals that use this technique have fur, feathers, or skin that match their habitat. Desert animals for example are light brown or tan to match the sandy or rocky landscape (Scott 1994). The zebra is hard to spot in the tall grass because of its stripes. In the Arctic, animals change their color to blend with the habitat when the season changes. The arctic fox coat is brownish gray in the summer, but white in winter to help it sneak up on prey in snowy landscapes. Insects and spiders use camouflage too. A crab spider, for example, changes its color depending on the flower color that it is sitting on. If the flower is
yellow, the spider turns yellow which serves the spider as both predator and prey (Silverstein et al. 2008; Scott 1994). Moreover, tree frogs have a bright green color to hide among the leaves, while the brown color of snowshoe hare of North America hides it among rocks in summer but in winter it grows a new white coat to camouflage against snow. According to Scott (1994), in breeding season, male ducks are brightly coloured to attract a mate which also makes them easy to spot by predators. On the other hand, female ducks usually have a mottled brown color to hide them during dangerous times, as when they have to sit on their nest with limited chance of escaping if they were spotted by a predator.

Prey may use another morphological defense technique such as fighting back using their claws, fangs, or spines. When hedgehogs, for example, become frightened their bodies are curled into a ball, which make their spines stand straight and become painful to touch and this discourages predators (Silverstein et al. 2008).

Some species that cannot fly, run, or hide have another technique to defend themselves against predators, which is becoming inedible or poisonous. Chemical weapons are also a common way to avoid being eaten. Many caterpillars, for instance, do not appear to taste good to other animals. Birds that attempt to eat one will drop it soon and not attack the same type again. Another example is the poison arrow frog that produces one of the most deadly chemicals known; this frog is harmless unless it been bitten by a predator (Silverstein et al. 2008; Scott 1994). Additionally, many insects have bright colors, bold patterns, or bad smells. Tiger moths, monarch butterflies, and ladybugs use all three ways to announce their poisons. Their bodies are covered with inedible chemicals that would make predators sick or even kill them. Millipedes do not announce their poisons but they use different compounds of poisons with different enemies. They produce camphor, the same chemical people use in mothballs. Some millipedes produce a chemical that makes the predator fall asleep when they eat it. Skunks also, do not need to run from predators, they aim their rear-end at the predator and spray it with a terrible smelling chemical that teaches predators to stay away from skunks.

Mimicry is another common behaviour for escaping predation. Some non-poisonous species mimic other poisonous ones in order to mislead predators. Two kinds of orange- and-black butterflies taste bad, but many other orange- and-black ones do not taste bad. Birds usually leave them alone because they cannot tell the difference between them (Silverstein et al. 2008). Species can miss feeding opportunities or expend too much energy if they considered all
other organisms as predators. Hence, the best way for animals to not lose time or waste energy is to differentiate between predators and non-predators.

The goal of behavioural ecologists that study risk assessment is to understand the decision-making rules that govern the daily activities of animals, including when and where to forage, and when to hide from other species. Being successful and safe during daily activities requires prey to optimize their ability to distinguish between predators and non-predators (Ferrari et al. 2008; Gall & Mathis 2010). Conversely, the inability of individuals to differentiate between predators and non-predators may cost prey foraging and mating opportunities, or at worst, even its life (Lima & Dill 1990; Ferrari & Chivers 2006; and Ferrari et al. 2008). Due to temporal variation in predator activity, some periods might be more risky for foraging. Hence, prey animals are predicted to assess the level of risk and select the appropriate time to forage (Lima & Dill 1990).

Many studies have mentioned the ability of animals to alter their feeding behaviour and decisions under predation risk (Lima & Dill 1990; Abrahams & Healey 1993; and Anholt et al. 2000). Some of these changes involve temporal changes in feeding activity, while others are spatial. A preferred habitat from a foraging perspective, may be fraught with risk at the same time, thus the forager has to balance between avoiding predators and foraging. Caldwell (1986) showed that herons reschedule their foraging time to more secure periods to avoid hawks. Also, when dipteran parasitoids are present, ants *Pheidole titanus* do not appear on feeding grounds (Feener 1988). Moreover, fathead grunts deferred their foraging migration time to be safe from a model lizardfish (Helfman 1986).

The level of light also induces many prey to alter their feeding decisions. As demonstrated by many studies, some prey avoid appearing during high level of light and instead forage in periods when the level of light is less to be more secure from predators. For instance, Clarke (1983) found that nocturnal foragers frequently reduce their activities when the light of moon is too bright. Additionally, by bright moonlight, the surface feeding of bannertail kangaroo rats is decreased (Lockard & Owings, 1974a, b). Also, in bright moonlight, Price et al. (1984) and Bowers (1988) have reported that another kangaroo rat, *D. merriami*, increases its relative use of cover. That also happens with deer mice, *Peromyscus maniculatus*, and older field mice, *P. polionotus*, which minimize their foraging under bright moonlight (Clarke 1983; Wolfe & Summerlin 1989). According to Watanuki (1986), after foraging trips, leach’s storm petrels,
Oceanodroma Leucorhoa, avoid returning to their colonies in moonlight so they would not be exposed to predators. Morrison (1978) found that the fruit bat, Artibeus jamaicensis, also avoids feeding or searching when the moon is full.

Additionally, light level appears to affect many diurnal foragers, especially during crepuscular periods when light level changes rapidly (Lima & Dill 1990). For example, dark-eyed juncos, Junco hyemalis, could be at a high level of risk of predation in the dim light of early morning (Lima, 1988a, b). In addition, juncos could feed in a very dim light under two conditions: when there is available relative cover; or, when energy reserves are threateningly low (Lima 1988a). In contrast, Clark and Levy (1988) imply that pelagic planktivorous fishes experience low predation risk in dim light. Hence, the decision of when to feed may be affected by the prey's ability to avoid predator attack when light level changes (e.g., Lima 1988a).

Some prey animals might be forced to change their habitats and patches not only because of reduced foraging opportunities, but also due to the predation risk (Lima & Dill 1990). Many freshwater organisms, such as the crayfish, Orconectes propinquus, (Stein & Magnuson 1976), backswimmers, Notonecta hofSmanni (Sih 1980, 1982), sunfish, Lepomis spp., (Werner et al. 1983; Mittelbach 1984), minnows, Campostoma anomalum, (Power & Matthews 1983; Power et al. 1985), dace, Rhinichthys atratulus, (Cerri & Fraser 1983), creek chub, Semotilus atromaculatus, (Gilliam & Fraser 1987), guppies, Poecilia reticulata, (Abrahams & Dill 1989), and threespine stickleback, Gasterosteus aculeatus, (Fraser & Huntingford 1986) were found to be able to trade-off between predation avoidance and foraging in abundant habitats and patches. For example, the habitat used by larval tiger salamanders, Ambystoma tigrinum, was found to be impacted by predaceous beetles, Dytiscus, (Holomuzki 1986). The salamander larvae feed preferentially in vegetated shallows in both daylight and night in the absence of beetles. However, when the beetles are active or appear around the shallows, the salamander larvae switch to deeper pelagic areas, resulting in a reduction of feeding. Likewise, Harvey et al. (1988) indicated that introduction of largemouth bass, Micropterus salmoides, but not smallmouth bass, M. dolomieu, could cause stoneroller minnows, Campostoma anomalum, to shift into shallow habitats, and this difference might depend on activity levels in the two predators. Magnhagen (1988a) has demonstrated that pink salmon, Oncorhynchus gorbuscha, reduce their use of profitable open-water feeding habitats when they can see potential predators in an adjacent aquarium. Their shift to a safer vegetated habitat, where food is unavailable, depends on their
food intake: the impact was markedly less in hungrier fish. Also, similar results have been reported when two gobiid fish species, *Pomatoschistus minutus*, and, *Gobius niger*, choosing between open and vegetated habitats with and without food, respectively (Magnhagen 1988b). Furthermore, in the presence of bluegill sunfish, larval dragonflies, *Odonata: Anisoptera*, may move into pond bottom litter (Pierce 1988).

The above studies addressed the ability of many prey animals to alter their feeding behaviour due to the risk of predation. That also gives evidence of some ability to recognize their predators. Thus, the capability of prey to assess the extent of predator’s risk level is a prime prerequisite for trade-offs between when and where to forage on one hand, and when to hide on the other hand. Prey should be able to recognize predators as a threat in order to show adaptive responses (Ferrari et al. 2009). Prey that are able to determine the level of predation threats will maximize their fitness by devoting sufficient time to related activities such as reproduction and feeding (Ferrari et al. 2006; Roberts 2011). Helfman (1989), Chivers et al. (2001), and Ferrari et al. (2006, 2009) suggested that to optimize their fitness, prey individuals ought to deal with their predators according to the level of risk (threat-sensitive avoidance hypothesis). A number of studies have tested and examined this hypothesis many times in a wide range of taxa, including freshwater isopods (Holomuzki & Short 1990), mayflies (McIntosh et al. 1999), crustaceans (Wahle 1992), amphibians (Kats et al. 1994; Anholt et al. 1996; Puttlitz et al. 1999; Mathis & Vincent 2000; and Amo et al. 2004), and fish (Williams & Brown 1991; Hartman & Abrahams 2000; Chivers et al. 2001; Golub & Brown 2003; and Ferrari et al. 2008). For example, the intensity of the anti-predator response in small pacific treefrog tadpoles, *Hyla regilla*, to caged salamander, *Ambystoma macrodactylym*, was higher than that observed for the bigger tadpoles (Puttlitz et al. 1999). Similarly, Wahle (1992) found that when small American lobsters, *Homarus americanus*, encountered predatory sculpins, *Myoxocephalus aenaeus*, they were more likely to seek refuge than larger ones. Ferrari et al. (2008) also reported that when fathead minnows conditioned to recognize the odour of brown trout as a high risk, they subsequently revealed an anti-predator response to the odour of both brown trout and rainbow trout, but not to yellow perch. On the other hand, if the odour of brown trout was represented as a low risk, the minnows showed an anti-predator response to brown trout odour only. The previous examples show the ability of many taxa to optimize their fitness and other related activates according to the level of threat.
Fish have received a great amount of attention in the area of predator avoidance. Recent work has shown that many vertebrate species including fish can learn to recognize the sight and odour of predators (Curio et al. 1978; McLean et al. 1996; Griffin et al. 2001; Woody & Mathis 1998; Mathis & Smith 1993; and Brown & Smith 1998). However, we still know little about the specific morphological or chemical cues prey fish use to differentiate between species that represent danger and those that do not (Ferrari et al. 2007, 2008). Therefore, the primary aim of this study was to investigate whether minnows can recognize the sight of predators and non-predators and subsequently generalize their recognition to related species. In addition, this study also addressed if some environmental factors such as turbidity can affect minnows’ ability to generalize their recognition of predators and non-predators.

**Recognizing predatory species:**

Learning to recognize predators has been demonstrated in different taxa including invertebrates and vertebrates (Ferrari et al. 2006). Prey in permanent aquatic habitats often encounter a variety of predator and non-predator species, and the ability to differentiate between the two is an essential prerequisite to increase fitness (Gall & Mathis 2010; Ferrari et al. 2008). Several studies have indicated that some species innately possess the sense to identify their potential predators, while others must have experience to recognize their predators (Ferrari et al. 2008, 2009; Ferrari & Chivers 2009). According to Gall & Mathis (2010), if there is a long evolutionary history between predator and prey, typically innate predator recognition occurs. The ‘innate’ predator recognition has been tested and validated in a wide range of taxa, including mammals, birds, amphibians, and fish (Kiesecker & Blaustein 1997; Berejikian et al. 2003; and Fendt 2006). For example, Göth (2001) found that Australian brush-turkey, *Alectura lathami*, that hatched independently of nest-mates and lived solitary showed an obvious anti-predator reaction when they were exposed to a living cat or dog, a rubber snake, and a raptor silhouette. Additionally, Veen et al. (2000) illustrated that the Seychelles warbler, *Acrocephalus sechellensis*, from both predator-free populations and populations that experience egg predation, have the same response to predators indicating that predator recognition is innate. Berejikian et al. (2003) demonstrated that juvenile Chinook salmon that never had been exposed to predatory stimuli exhibited an innate anti-predator response to northern pike minnows odour, *Ptychocheilis oregonensis*, regardless of whether the salmon came from a population that existed in sympatry.

Other aquatic organisms including amphibians, fishes, and some of invertebrates were found to be able to learn the sight or the odour of a novel predator if they were simultaneous paired with chemical cues from injured conspecifics (Ferrari & Chivers 2009). Ostariophysan fish (minnows, catfishes, suckers, carps, piranha, and electric eels) are the second largest superorder of fish, representing 27% of all fish species and 64% of freshwater species (Nelson 1994). Ostariophysans have a significant feature, which is the existence of specialized epidermal club cells (Figure 1.). Some non-Ostariophysian like perch, *Perca flavescens*, also possess these club cells with similar tissue features (Smith, 1992). When the club cells are damaged by a predator, they release a substance known as alarm cue (AC). As a result, other individuals from the same species or genus can detect the presence of the predator and thus show an anti-predator response (Chivers & Smith, 1994). AC can also mediate predator learning. Fathead minnows are able to learn to recognize unknown predators in the presence of AC.

![Figure 1.](image-url)  
*Picture was taken from Chivers et al. 2008 (http://rspb.royalsocietypublishing.org)*
Pairing AC with predator sight or chemical stimuli (odour) is one common approach in aquatic species to learn to recognize novel predators, and this learning mode directly helps prey to learn both visual and chemical features of unknown predators (Chivers & Smith 1994, 1996). For example, in one study, fathead minnows were pre-exposed to pike odour, and AC. Subsequently, the minnows showed an anti-predator response when they were exposed to the pike odour only (Chivers & Smith, 1994). Furthermore, fathead minnows that were conditioned to the sight of a natural predator, northern pike, with AC exhibited a significant anti-predator response when they were later exposed to the sight of pike only. Likewise, minnows that pre-exposed to the sight of a non-piscivorous exotic goldfish and AC showed an anti-predator reaction when they were subsequently exposed to the sight of the goldfish (Chivers & Smith, 1994).

Despite the fact that much research has indicated the ability of prey to generalize predator recognition, biologists and ecologists know very little about the specific characteristics that individuals use to recognize predators. Recently, a number of studies have demonstrated that prey can use the sight or the odour of known predator to recognize novel, closely-related species of predators. This is known as generalization of predator recognition (Ferrari et al. 2008; 2009). Predators that are closely related often share similar foraging behaviours in general. For instance, carnivorous species will require some particular adaptations such as behavioural, morphological, and physiological features to capture, handle, eat, and digest their prey. Among taxa, these adaptations are various, but closely-related species often share the same adaptations. Hence, it is expected from prey to generalize their recognition of a specific predator to closely-related novel predators (Ferrari et al. 2007).

The ability of prey to generalize recognition of a known predator will decrease their susceptibility to be captured or eaten by a novel closely related species. Thus, the benefits of being able to generalize the recognition of predators might appear equal to the benefits of innate recognition of predators (Ferrari et al. 2008).

Primary studies have empirically tested for visual generalization of predator recognition in mammals. In one study, Griffin et al. (2001) illustrated that naïve tammar wallabies, *Macropus eugenii*, showed no response to models of foxes, cats, or goats. However, when they have been taught to recognize foxes as a threat, they generalized this information to the animals that have similar outer attributes like cats, but not goats. Likewise, black-tailed deer, *Odocoileus*
*hemionus colombianus*, showed an anti-predator response to model cougars, their natural predator, and generalized that to novel predator model tigers (Stankowich & Coss, 2007). Recently, several studies have emerged and highlighted the generalization of predator recognition in different taxa, using both chemical or visual stimuli. Minnows that were conditioned to recognize the odour of a lake trout as a threat generalized their recognition to other related species, brook trout and rainbow trout, but not to distantly related species, i.e. predatory pike and non-predatory sucker (Ferrari et al. 2007). Woodfrog tadpoles, *Rana sylvatica*, that were conditioned to red-bellied newt odour with injured conspecific cues later on showed an obvious anti-predator response not only to newt odour, the reference predator, but also to novel tiger salamander odour (Ferrari et al. 2009). Also, when fathead minnows were taught to recognize the sight of brown trout as a risk in clear water, they displayed a high anti-predator response when they were subsequently exposed to a closely-related rainbow trout but not to yellow perch, which has a different exterior shape (Ferrari et al. 2010). In addition, when juvenile trout were conditioned to recognize the odour of pumpkinseed sunfish as a predation threat, subsequently, they responded to the odours of both pumpkinseed and longear sunfish, which belong to the same genus (Brown et al. 2011). These examples indicated that prey must label specific characteristics of a known predator to learn to recognize a novel predator. Once again, from the phylogenetic perspective, we expect that predators from the same species, genus, or closely related may share similar diet and feeding habits, and that presumably makes them similar, but not completely identical, for prey, visually or chemically (Ferrari et al. 2007, Brown et al. 2011).

**Recognizing non-predatory species:**

Failing to recognize predatory species implies an increase of predation risk while responding to non-predatory individuals may cost prey a loss of some activities such as reproduction and foraging (Gall & Mathis 2010; Chivers & Smith, 1994; and Ferrari et al. 2008). Recognizing the sight or the odour of non-threatening species would save prey time/energy from unnecessary escaping or hiding. The ability of prey to recognize non-predators is an important factor to balance predator avoidance and fitness activities (Ferrari et al. 2008). Research in the area of recognizing non-predatory species is still in its infancy, and has not been given enough
attention by biologists and ecologists. To date, few studies have looked at prey’s ability to recognize non-predator species (Brown et al. 2011).

A well-known mechanism that has retarded the formation of the learned association between alarm cues and an unknown stimulus (predator odour or sight) is called latent inhibition. When prey are exposed to the odour or the sight of a novel species for a number of days in the absence of risk reinforcement, they fail to learn to recognize it as threatening during a learning paradigm with alarm cues, in other words, they have already learned that it is a non-predator (Acquistapace et al. 2003). Latent inhibition has been demonstrated in different taxa including fish and amphibians. For instance, when virile crayfish, *Orconectes virilis*, have been exposed to the odour of goldfish for two h over three consecutive days, they failed to subsequently learn to recognize the cue of the goldfish as a danger (Acquistapace et al. 2003). As well, fathead minnows were incapable of recognizing the brook charr, *Salvelinus fontinalis*, as a threat when they were pre-exposed to the charr odour (Ferrari & Chivers 2006).

Similarly to the way prey species can generalize their recognition of predators to novel related species, Ferrari et al. (2009, 2011) have shown that minnows and embryonic amphibians can also chemically generalize their recognition of non-predators. According to Brown et al. (2011) latent inhibition was used to teach juvenile rainbow trout that pumpkinseed sunfish, *Lepomis gibbosus*, odour is harmless. The trout did not learn to recognize pumpkinseed or longear sunfish odour as potential threats during subsequent predator learning trials. Moreover, because of the embryonic pre-exposure to the odour of tiger salamander, woodfrogs tadpoles were unable to learn to recognize the odour of salamanders as a threat. They also generalized their latent inhibition to related red-bellied newts (Ferrari & Chivers 2011).

To date, only two studies have demonstrated the ability of prey (fish and amphibians) to generalize their chemical recognition of non-predators. No studies have demonstrated the ability of prey to generalize the sight of non-predators, or if some environmental factors, such as turbidity, may affect this ability.

**Turbidity: Causes and Effects**

Turbidity is the cloudiness or haziness of a fluid caused by suspended solids. Eutrophication and other anthropogenic activities are a serious environmental concern, one of which is increasing the level of turbidity (Davies-Colley & Smith 2001; Schwartz et al. 2008).
Turbidity often causes alterations in community composition, and leads to a decline in fisheries (Davies-Colley & Smith 2001; Bilotta & Brazier 2008; and Liljendahl-Nurminen et al. 2008). Several studies have shown that turbidity strongly affects the relationships between predators and prey (Gregory 1993; Bonner & Wilde 2002; Lehtiniemi et al. 2005; and Zamor & Grossman 2007). It disrupts the transfer of visual information, and consequently the response of prey to predators, but also of predators to prey. In some studies, it was shown that in high turbidity situations, prey response to predators is decreased (Vogel & Beauchamp 1999; Quesenberry et al. 2007). Abrahams & Kattenfeld (1997) and Reid et al. (1999) found that as turbidity increased, piscivores were less likely to eat small prey. Moreover, other studies have indicated that a prey’s anti-predator response is reduced in high turbidity conditions (Vogel & Beauchamp 1999; Quesenberry et al. 2007). Hartman & Abrahams (2000) suggested that minnows might perceive that turbidity obscures them from the sight of potential predator. An example for that is a study indicating that the intensity of minnows’ anti-predator response towards brown trout in turbid water was less than their reaction in clear water (Ferrari et al. 2010). Increased turbidity may lead some prey to change habitats (Swenson 1978; Matthews 1984; and Miner & Stein 1996), reduce school cohesiveness (Vandenbyllaardt et al. 1991), or reduce shelter using (Gradall & Swenson 1982; Johnson et al. 1988; Gregory 1993; and Snickars et al. 2004), apparently because turbidity reduces predation risk.

Increased turbidity leads to decrease foraging opportunities for many species (Gardner 1981; Johnston & Wildish 1982; Barrett et al. 1992; Gregory & Northcote 1993; and Benfield & Minello 1996) and that might be as a result of decreased reactive distance (Vinyard & O’Brien 1976; Gregory & Northcote 1993). Additionally, increased turbidity disrupts prey detection; resulting in a decrease in foraging efficiency (Cezilly, 1992). Zamor & Grossman (2007) found that the reactive distance and prey capture success of rosyside dace, Clinostomus funduloides, is decreased by 50% at a level of turbidity as low as nine nephelometric turbidity units (NTU).

Restrictions that could affect the ability of prey fish to generalize their visual recognition of predators and non-predators have not been well examined. Therefore, the main objectives of this research are: (1) to test the ability of fathead minnows to generalize their recognition of predator species visually (2), to test whether fathead minnows that are conditioned to recognize brook trout, Salvelinus fontinalis, as a non-predator will generalize this recognition to the similar looking rainbow trout, Oncorhynchus mykiss, and (3) to test the influence of suspended solids
(turbidity) on minnows’ ability to exhibit generalization of predator and non-predator recognition.
CHAPTER 2: Methods

Experimental overview

The experiment consisted of three phases. In phase 1, minnows were exposed to either a brook trout (group BT₀) in an adjacent tank for one hour twice a day for three days, or to an empty tank (group E). This procedure should allow the trout exposed minnows to learn, through the process of latent inhibition, that the trout do not represent a threat. In contrast, the minnows that did not experience repeated exposure to the predator in the absence of risk would not have learnt the predator as non-risky.

In phase 2, both groups of fish from stage 1 were conditioned to recognize one of three different fish as a predator: brook trout, rainbow trout or yellow perch, by pairing chemical alarm cues with the predator sight.

In phase 3, we tested each of the groups of minnows to the visual stimulus of each of the three fish species. This resulted in 18 treatment combinations in a 2 x 3 x 3 design (2 levels of pre-exposure crossed with three conditioning groups crossed with exposure to three test fishes). The experiment was conducted in clear water and was repeated under conditions of reduced visibility by adding bentonite to the tanks containing the predators during the testing phase. Given that generalization of non-predator recognition with visual cues had never been attempted in any predator-prey systems, it was not justified to run the clear and turbid trials at the same time. If the fish failed to exhibit generalization of the non-predator under clear conditions, then it would have been an excessive waste of animals to conduct all 36 treatments as a single experiment.

Test Subjects:

In this study, minnows, Pimephales promelas, represent prey, while brook trout, Salvelinus fontinalis, and rainbow trout, Oncorhynchus mykiss, which are from the same family, play the role of potential predatory species. We also use yellow perch, Perca flavescens, which has a different morphological shape compared to trout, as an unknown but potential predator.
**Fish collection and maintenance:**

In this study, 720 minnows, 10 brook trout, 10 rainbow trout, and 10 yellow perch were used. Minnows were collected from Feedlot pond in October 2010 by using Gee’s improved minnows traps (Tackle Factory, Fillmore, NY, U.S.A.). We could not collect the entire amount of minnows in one trip only because the lake was freezing, so five return trips were made to the pond.

The minnows were housed in a 1200 liter tank (Figure 2.) and fed daily with fish flakes (Nutrafin basix, Rolf C. Hagen, Inc., Montreal, Quebec, Canada). According to Chivers & Smith (1994, 1995); Brown et al. (1997); and Ferrari et al. (2005) minnows from this pond are naïve to species used in my experiments and have never been exposed to any of these predators.

Brook trout and rainbow trout were obtained from a small farm called REISTER’S TROUT in Alliance, Alberta in July 2011. The two species were housed separately in 1200 liter tanks, and fed daily with trout chow.

In August 2011, perch were collected from Blackstrap Lake using seine nets. The perch were housed in a 1200 liter tank, and fed live minnows twice a week. All fish were housed in dechlorinated tap water at 10-13°C under a 14:10 h light: dark cycle.

**Stimulus collection:**

**Minnows skin extract:**

AC plays an important role in attracting the prey’s attention to the presence of actively foraging predators. To make AC, 10 fathead minnows were used (fork length, FL: mean ± SD = 5.22 ± 0.48 cm). In accordance with the Canadian Council on Animal Care, the minnows were killed by a blow to the head. Then, we removed the minnows’ skin fillets from both sides, and placed them in chilled distilled water. Skin fillets were homogenized using a Polytron homogenizer, and the solution was strained through glass wool. We diluted the total of the skin extract 23.1 cm² to obtain a final concentration of 1 cm² of skin per 20 L of water. Afterwards, the solution was frozen at -20°C in 20 mL aliquots until needed.
Experimental Set-up:

The experimental tanks used for the pre-exposure, conditioning and testing phase were similar. Pairs of 37-litre tanks (50 x 30 x 25 cm) were set beside each other such that the long side of the tanks faced each other separated by a removable barrier. Each tank contained a gravel substrate, an air stone, and were filled with dechlorinated tap water (Figure 3). During pre-exposing and conditioning phase, prey’s tanks contained a 2 m long injection tube attached to the air tube (Figure 4a). The predator tank was similar except it lacked the injection tube and shelter object.
In the testing phase, the prey’s tank was not equipped with a stimulus injection tube, but a 10 x 20 cm ceramic tile mounted on three 3.5 long cylindrical glass legs was placed as a shelter (Figure 4b.). During this phase, the predator tank was divided into thirds by a plastic divider placed along the long axis of the tank, and the predator was placed in closest part to the prey tank. When the predators were placed in this section of the tank they had a restricted ability to move towards or away from the prey. Their alignment in the tank ensured that all of the minnows were presented with comparable visual information across trials (i.e, they had a lateral view of the predator).

To remove any potential of reflection and to provide a high visual isolation from other tanks, each pair of tanks was wrapped on the outer and back sides with black plastic cardboard (Figure 5.).

**Figure 4a.** Picture shows the installation of injection tube to the air tube during conditioning phase in the prey tank. **b.** Picture shows inserting a shelter in the prey tank during the testing phase.

**Figure 5.** Picture shows the set-up of the experimental tanks.
**The Experimental Design:**

In the beginning, minnows were divided into two large groups, BT\textsubscript{0} which were pre-exposed to brook trout sight and E which were pre-exposed to an empty tank. After, each group was split again into three smaller groups that were assigned as “bt”, “rt”, and “yp”. Then each of these groups was conditioned to recognize a predator, and later they were divided equivalently to be tested to the visual stimulus of each predator (Figure 6.).

![Flowchart shows the steps of the experimental design.](image)

Following the completion of the original experiment, the entire experiment was repeated under conditions of reduced visibility during the testing phase. The experiment steps of both groups BT\textsubscript{1} and E\textsubscript{1} were conducted in clear water, while groups BT\textsubscript{2} and E\textsubscript{2} were conducted in turbid water (Figure 7.). By dividing the groups in this manner, we achieved two main goals: (1) the effect of pre-exposing to the visual stimulus of the predator on prey’s behaviour; (2) the effect of turbidity on prey’s ability of recognize and generalization.
Experiment 1: Recognizing the sight of predators and non-predators in clear water

Objectives:
1- Testing the ability of group BT$_1$, which was pre-exposed to brook trout sight in clear water, to recognize brook trout as a non-predator, and generalize that to rainbow trout but not to yellow perch.
2- Observing the anti-predator response of group E$_1$, which was pre-exposed to controlled water, to the visual stimulus of brook trout, rainbow trout, and yellow perch.

(i) The pre-exposure phase:

In this step, minnows were exposed either to brook trout sight “BT$_1$” or water “E$_1$” (control). Here we taught /conditioned group BT$_1$, to recognize the sight of the brook trout as non-predator. One-day prior to the trial 10 minnows were placed in the prey tank and fed.
Two-hours prior to exposure, a brook trout (mean standard length ± SD = 20.5 ± 2.4 cm) was placed in the predator tank, and the minnows were fed again. The pre-exposure phase began when the barrier that separated the two tanks was removed twice for one hour a day, and was repeated for the next two days (Figure 8.). We assumed that the frequent pre-exposure to the sight of the brook trout, in the absence of any attack, would habituate the minnows to the presence of the brook trout later. Using the similar set up, group E₁ was exposed to a tank filled with clear water only (Figure 9.).
(ii) The conditioning phase:

Here both groups, BT$_1$ and E$_1$, were divided into three equal groups, “bt”, “rt”, and “yp”, whereas this time, each group was exposed independently to the sight of brook trout, rainbow trout, or yellow perch in the presence of AC. On day four, and two hours prior to start conditioning, we placed brook trout, rainbow trout, or yellow perch in the predator tank and fed the minnows. To remove any stagnant water from the stimulus tube, 60 mL of tank water was withdrawn and discarded, and we then withdrew and retained an additional 60 mL of water.

The conditioning phase was started when we removed the barrier to expose the minnows to the predator sight, and injected 25 mL of conspecific alarm cues AC at the same time, followed by the 60 mL of retained water. The divider was placed back after giving the minnows 60 s to observe the predator (Figure 10a & b.). Two hours after the end of conditioning, minnows were transferred to another 37 L tank filled with clean tap water and fed preparing for the testing phase.

Due to the first encounter of the sight of the yellow perch in this phase and the different exterior shape of the yellow perch, we expected that minnows would recognize the yellow perch as a threat, especially with the presence of the AC.

Figure 10a. The conditioning phase before injecting AC.

b. The conditioning phase after injecting AC.
(iii) The Testing phase:

24 hours after the conditioning phase, testing trials were conducted. As clarified above, the prey testing tanks were not equipped with an injection tube but contained the 10 x 20 cm ceramic tile as a shelter. Two hours before testing a single minnow was placed in the prey tank and fed while a brook trout, a rainbow trout (mean standard length ± SD = 22.4 ± 2.3 cm), or a yellow perch (mean standard length ± SD = 20.3 ± 2.6 cm) was set in the predator tank. Testing contained two periods of observation, eight min of pre-exposure and eight min post-exposure. We were observing two main antipredator behaviours that are well-documented of a single minnow, increasing shelter use and decreasing of time moving/swimming (Chivers & Smith 1994). As indicated in previous studies, these behaviours are exhibited by minnows when they encounter a predator or in the presence of AC (Chivers & Smith, 1998). Therefore, the number of seconds spent under shelter and spent moving/swimming were measured before and after removing the barrier. The order of testing was randomized and the observer was blind with respect to the treatments.

Experiment 2: Recognizing the sight of non-predatory in turbid water

Objectives:
1- To examine the influence of turbidity on group’s BT2 ability, which was pre-exposed to brook trout sight in clear water, to recognize and generalize the sight of a non-predatory fish.
2- To detect whether turbidity affect the ability of group E2, which was pre-exposed to controlled clear water, to differentiate between the visual stimulus of predators and non-predators.

The procedure of this experiment in the pre-exposure and conditioning phases followed the same protocol of Experiment 1. However, the testing phase was conducted in turbid water by adding 4.5 g of bentonite in the predator’s tank (0.12g/L) to make ~31.5 NTU of turbidity (~20 cm secchi depth, Shoup & Wahl 2009) (Figure 11.). This amount of turbidity is lower than that minnows may encounter in the wild (Hartman & Abrahams, 2000). The amount of bentonite was injected into the predator tank 2 h prior to the exposure stage. Similarly to Experiment 1, minnows were observed for 8 min before and after the exposure period, and results were recorded. In both environments, clear
and turbid, trials in each group of all conditions consisted of 20 replicates for a total of 720 trials, and each minnow was tested once only.

![Figure 11](image1.png)

**Figure 11.** Picture shows the setting up of testing phase in turbid water.

**Behavioural Bioassay:**

The minnows’ response was observed and recorded according to two major behaviours; time spent swimming and, time spent under shelter. As indicated in previous studies, these behaviours are exhibited by minnows when they encounter a predator or in the presence of -AC (Chivers & Smith, 1998). Using shelters, which are usually set in examination tanks, helps minnows to hide from the sight of predators (Figure 12.).

![Figure 12](image2.png)

**Figure 12.** Picture shows a minnow’s anti-predator reaction by using a shelter to hide in.
**Statistical analysis:**

For both shelter use and time spent moving, we computed a change in behaviour from the pre-stimulus baseline (post-stimulus value minus pre-stimulus value). These differences were used as raw data in our analysis. Because trials in clear and turbid water were not performed over the same period, we analyzed the data separately. Both analyses were similar: to test the effect of pre-exposure (brook trout vs. water), conditioning (AC+BT vs AC+RT vs AC+YP), and testing cues (BT vs RT vs YP) on the change in shelter use and time spent moving, we performed a 3-way MANOVA. The MANOVA approach was used because the two behaviours are not independent (fish that score high on shelter use tend to score low on time spent moving). Following 3-way interactions, we split the analysis by conditioning, and thus investigated the effect of pre-exposure and testing cue on the behaviour of minnows that underwent different conditionings.
CHAPTER 3: Results

Minnows exposed to predators in clear water:

Our results indicated that the behaviour of minnows were affected by all 3 factors (3-way interaction: Pillai’s Trace: $F_{8,684} = 5.5$, $P<0.001$). For minnows conditioned to recognize a brook trout as a predator, both pre-exposure and testing cues influenced their anti-predator responses (interaction: $F_{4,228} = 10.3$, $P<0.001$). Specifically, we found that when minnows were pre-exposed to water, they displayed a strong anti-predator response to the sight of trout, but not that of yellow perch ($F_{4,114}=9.3$, $P<0.001$) (Figure 13.). Minnows did not seem to differentiate the two trout species (Tukey post-hoc tests: $P=0.16$ and $P=0.59$ for shelter use and time moving, respectively). However, minnows pre-exposed to brook trout did not respond to the sight of any of the 3 species ($F_{4,114}=1.5$, $P=0.20$) (Figure 15., 16.).

Figure 13. Picture shows a minnow behaves normally after exposing to yellow perch sight.

The responses of minnows conditioned to recognize a rainbow trout as a predator were also affected by pre-exposure and testing cues (interaction: $F_{4,228} = 11.9$, $P<0.001$). Similar to the
previous case, minnows pre-exposed to water responded to both trout with similar intensity (Tukey post-hoc test: P=0.083 and P=0.92 for shelter use and time moving, respectively) but not to yellow perch (F_{4,114}=9.3, P<0.001). However, minnows pre-exposed to brook trout did not respond to yellow perch or brook trout (P=0.99 and P=0.58 for shelter use and time moving respectively), but responded to rainbow trout (F_{4,114}=2.8, P=0.028) (Figure 15., 16.).

The responses of minnows conditioned to recognize yellow perch were affected by testing cues (F_{4,228}=34.5, P<0.001), but not by pre-exposure (F_{2,113}=2.9, P<0.06), nor was there an interaction between the two factors (F_{4,228}=0.4, P=0.82). Minnows responded to yellow perch (Figure 14.), but not to either trout (Tukey post-hoc tests: both P=0.9) (Figure 15., 16.).

**Minnows exposed to predators in turbid water:**

Our results indicate that the responses of minnows were affected by pre-exposure, conditioning and cues (conditioning x cue: Pillai’s Trace: F_{8,684} = 3.8, P<0.001; pre-exposure x conditioning: F_{8,684} = 3.6, P=0.006). The responses of minnows conditioned to recognize brook trout as a predator were affected by pre-exposure (F_{2,113} = 16.0, P<0.001), but not by cue (F_{4,228} = 1.2, P=0.30), or a cue by pre-exposure interaction (F_{4,228} = 0.7, P=0.58). Their reactions were not
significantly different to the sight of the three fish, however, minnows pre-exposed to water displayed an anti-predator response while those pre-exposed to brook trout showed a slight anti-predator response (Figure 17., 18.).

A similar pattern was observed for minnows conditioned to recognize rainbow trout as a predator (pre-exposure: $F_{2,113} = 14.3$, $P<0.001$; cue: $F_{4,228} = 0.7$, $P=0.58$; interaction: $F_{4,228} = 1.3$, $P=0.28$) (Figure 17., 18.).

Minnows conditioned to recognize the sight of a yellow perch were not affected by pre-exposure ($F_{2,113} = 1.4$, $P=0.25$), nor by a cue by pre-exposure interaction ($F_{4,228} = 0.3$, $P=0.85$). Minnows displayed an anti-predator response to the sight of perch (cue: $F_{4,228} = 7.2$, $P<0.001$; Tukey post-hoc tests: both $P<0.001$), but not to the sight of either trout species (both $P>0.9$) (Figure 17., 18.).
Figure 15. Mean ± SE change from the prestimulus baseline in shelter use for minnows exposed to one of three fish species (brook trout, rainbow trout, or yellow perch) maintained in clear water.
Figure 16. Mean ± SE change from the prestimulus baseline in time spent moving for minnows exposed to one of three fish species (brook trout, rainbow trout, or yellow perch) maintained in clear water.
Figure 17. Mean ± SE change from the prestimulus baseline in shelter use for minnows exposed to one of three fish species (brook trout, rainbow trout, or yellow perch) maintained in turbid water.
Figure 18. Mean ± SE change from the prestimulus baseline in time spent moving for minnows exposed to one of three fish species (brook trout, rainbow trout, or yellow perch) maintained in turbid water.
CHAPTER 4: Discussion

In this Discussion, I will address several issues that have emerged from our results, explore the influence of turbidity in the second experiment, and present some recommendations for future studies.

The results of the first experiment indicated that minnows were able to recognize the sight of both predators and non-predators and generalize their recognition to the related species. Also, the results demonstrated that the minnows were able to differentiate between predatory and non-predatory species visually. In addition, these results support previous studies that illustrated the ability of minnows to recognize predator species by using visual cues and generalize that to other related species (Ferrari et al. 2010).

Our results show the role of pre-exposure to the sight of potential predator, and its importance on the mechanism of latent inhibition. Minnows that were pre-exposed to the sight of brook trout, group $BT_1$, subsequently failed to recognize brook trout and the related species rainbow trout as predators. Their use of shelter and time spent moving pre- and post-exposure were not drastically different. It is worth mentioning that in a previous study, pre-exposure to predator’s odour also performed a similar function on the mechanism of latent inhibition. Juvenile trout that were pre-exposed to a pumpkinseed sunfish odour subsequently were inhibited from identifying the odour of pumpkinseed as a threat, although it was paired with alarm cues (Brown et al. 2011). The results also support an earlier research study which suggested that minnows would never exhibit an anti-predator response towards any unknown species unless there was a previous experience (Chivers & Smith 1993). In the experiment, the minnows had not shown an anti-predator reaction to the sight of yellow perch until they were conditioned to the yellow perch sight plus AC. In addition, the results showed that besides the ability of minnows to generalize their recognition of related species, they were able to differentiate between them. Minnows that were pre-exposed to brook trout and conditioned to rainbow trout sight plus AC showed a reaction when they were revealed to the rainbow trout sight but not to the brook trout sight. This could be clear evidence of minnows’ ability to use some information concerning the morphological exterior shape to distinguish between trout species, even though they share a number of mutual characteristics. Ferrari et al. (2010) indicated that brown and rainbow trout
share enough similarities that enable minnows to generalize between them, but they also are
different enough for minnows to discriminate them. Therefore, we would say whenever related
species have common morphological features, prey would be more capable to generalize. In one
study (Ferrari et al. 2010), it was illustrated that when minnows were conditioned to recognize
the sight of brown trout as a predator, subsequently they showed a significant anti-predator
response toward both trout types, brook and rainbow.

Our results also support the outcomes of a previous study, which suggested that minnows
do not exhibit an anti-predator response towards any predator because of its size or odour only,
but most probably because of a prior experience of it. When the odour of northern pike, *Esox
lucius*, was paired with AC, minnows could recognize the pike odour as a threat when they were
revealed to its odour only (Chivers & Smith 1994a). Similarly, predator-naïve minnows that were
conditioned to alarm substance plus visual stimulus, the sight of northern pike, expedited a
significant anti-predator response when they later were exposed to the pike’s sight only (Chivers
& Smith 1994b). In the experiment, we noticed that when we conditioned minnows to recognize
the sight of brook or rainbow trout as a threat, later they showed a significant reaction to both
tROUT species, but not the perch that has different color and appearance. Conversely, when we
conditioned minnows to identify yellow perch as a predator, afterward they exhibited a scared
reaction towards the perch’s sight only, but not the trout.

The second experiment followed the same process of experiment one except for the
testing phase, which was set in turbid water. As mentioned earlier, one of the main goals of this
study was to put one of the natural environmental constraints such as turbidity under scrutiny,
and examine its direct impact on the ability of prey to identify predators. Increased turbidity in
the environment is a result of many anthropogenic and eutrophication activities (Davies-Colley &
Smith 2001; Schwartz et al. 2008). As mentioned in one study, the level of turbidity in the
environment usually is fluctuating due to various changes such as sediment additions, sediment
re-suspension, or phytoplankton density (Nellis et al. 1998; Dirnberger & Weinberger 2005;
Chow-Fraser 1999; Anthony & Downing 2003; Parkos et al. 2003; and Cozar et al. 2005). The
influence of turbidity can be seen from a number of aspects. As demonstrated in several studies,
turbidity strongly affects the relationships between predators and prey (Gregory 1993; Bonner &
Wilde 2002; Lehtiniemi et al. 2005; and Zamor & Grossman 2007) and their ability to forage and
reproduce (Sweka & Hartman 2001; Shoup & Wahl 2009; and Grosse et al. 2010). One study
suggested that anti-predator behaviour is directly influenced by turbidity (Shoup & Wahl 2009). The transfer of visual information is disrupted by turbidity, resulting in confusing responses for both players and interactions, prey to predators, and predators to prey. Sweka & Hartman (2001) indicated that turbidity is one of the main causes that affect preys’ ability to identify predators, which lead them to reveal a confused or a weak reaction. That happens presumably because of the morphological shape of the predator, which does not appear with full information, and the image prey acquired in previous experience (Ferrari et al. 2010). In other words, we may say that turbidity impedes the efficiency of visual information individual used to identify predator. As a result, we can say that the intensity of anti-predator response might appear higher or lower according to the level of turbidity at that moment. Also, increased turbidity directly disturbs prey’s capability in decision making whether towards a known or unknown predator, resulting in wasting opportunities in activities such as foraging or mating.

The results of the second experiment clarify that minnows’ anti-predator reaction was affected by turbidity. In general, the ability of minnows to differentiate between predators and non-predators was weaker here. When we conditioned minnows to identify brook trout or rainbow trout as predators, afterwards they presented a relatively similar reaction to all of the three predators. However, when minnows were conditioned to recognize yellow perch as a threat, the intensity of their anti-predator response towards the perch sight was significantly higher than their response towards both trout species. The reason seems to be some morphological features that perch possess such as colour, which was noticeable for the minnows even in the turbid condition.

The mechanism of pre-exposure phase also is influenced by turbidity as shown by the data collected. By comparing the results of minnows that were pre-exposed to water $E_2$ with those pre-exposed to brook trout $BT_2$ we found that there was an obvious reaction towards the predators for both of them; however, it was lower in the second group. The intensity of anti-predator response of minnows that were pre-exposed to water $E_2$ towards all of the predators was extremely close, which means that turbidity affected minnows’ ability to recognize and generalize. On the other hand, a group of minnows that were pre-exposed to brook trout $BT_2$ displayed a reaction that was lower than what had been shown by group $E_2$. That means turbidity did not completely disrupt the role of the pre-exposure phase but caused some confusion. Generally, the results of the second experiment demonstrated that minnows’ reaction in turbid
water was associated with caution and fear, presenting a slow action in making the decision, preferring staying close to the shelter, or staying in the farthest spot from the predator’s tank. This may be supported by another hypothesis which is that generalization of a predator is more common than generalization of a non-predator.

After browsing the effects of turbidity on our results, a question might be raised here, are the visual cues enough for prey fish such as minnows to identify their predators? As indicated in many studies, most fish rely on vision to recognize predators. However, in other conditions when the visibility is extremely poor due to some factors such as turbidity, industrial waste, or sediment action, auditory or olfaction senses would be the alternatives (Wootton, 1994). In their natural environment, minnows live in a level of turbidity that is higher than the one we used in this experiment. Consequently, the full dependence on visual stimuli by prey might be considered as an adventure and a risk in the presence of such natural phenomena. Hence, we may say that it is better to depend on some chemical stimulus, such as predator’s odour, to be more efficient and certain to determine your opponent in environments where visibility is extremely poor.

The impact of turbidity is not limited to visual stimulus only, but it can be seen on chemical stimulus too. Even though chemical stimulus seems to be an appropriate alternative when the visibility is poor, it can be influenced by turbidity too. The impact of some anthropogenic activities on water chemistry cannot be ignored or underestimated. As shown in some research, the influence of these activities on water chemistry also affects the ability of prey to identify predator and vice versa. For example, Leduc et al. (2004, 2006, 2007) demonstrated that acidic milieus (PH < 6.4), which are mostly a result of acid rain or industrial waste, deactivate the role of AC in salmonid fishes resulting in the lack of predator recognition. Furthermore, some of the anthropogenic activities alter the level of the pH in predator’s odour, and thus influence prey’s ability to identify predators. In one study, when a juvenile trout was acclimated to identify the odour of a predator at pH= 6 or 7, later it expressed the same reaction in the same levels of the pH only (Smith et al. 2008).

Beside the previous studies that demonstrated the ability of prey mammals, amphibians, and fish to recognize predator and non-predator species, our study also may add more evidence to the ability of prey fish to distinguish visually between the sight of predators and non-predators. As I mentioned before, the research concerning predator recognition in this field is still relatively
unstudied by biologists and ecologists, as little is known about the information prey use to recognize predator. However, somewhat better progress has been made regarding the influence of some environmental obstacles on this information. We encourage more in depth future studies in this field. In order to support the idea of the impact of different degree of turbidity on the reaction of the prey, recommendations have been made for future studies. One of the suggestions is to test prey ability to recognize the sight of non-predator in different levels of turbidity, and to gain more knowledge in the main factors that turbidity conceals, and hence inhibits prey from recognizing predators. This study might raise some questions concerning the transfer of the information that prey used to recognize predatory species. Can minnows transfer the information they acquire by experience to other naïve individuals? In other words, if minnows were conditioned to recognize the sight or the odour of a trout as a predator, can the minnows pass this piece of information to other fathead minnows? If we conditioned one minnow to recognize the predator as non-predator, and another minnow to recognize the same species as predator, which minnow will affect the other’s decision? What if we taught minnows to recognize a predator as a high priority risk, will the minnow’s reaction in the different levels of turbidity be the same or will minnows use turbidity as an implement to assess the level of risk? Moreover, we still know little about the type of visual information that minnows use to recognize their predator. In other words do minnows focus more on the colour or other morphological attributes to recognize a predator? A suggested experiment for such a situation is to condition minnows to recognize a predator and test them later to the sight of a non-predator which has been somehow coloured to look like the predator.
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