Sensory compensation and its influence on predator detection:
the interaction between chemical and visual information

By:

Eric J. Hartman

A Thesis submitted to the Faculty of Graduate Studies
of the University of Manitoba in partial fulfillment
of the requirements of the degree of

MASTER OF SCIENCE

Department of Zoology
University of Manitoba
Winnipeg, Manitoba

THE UNIVERSITY OF MANITOBA

FACULTY OF GRADUATE STUDIES

*****

COPYRIGHT PERMISSION PAGE

Sensory Compensation and its Influence on Predator Detection: The Interaction Between Chemical and Visual Information

BY

Eric J. Hartman

A Practicum submitted to the Faculty of Graduate Studies of The University of Manitoba in partial fulfillment of the requirements of the degree of

MASTER OF SCIENCE

Abstract

The ability to detect a predator is very important to prey, allowing them to assess the risk of predation and determine the appropriate course of action. Fathead minnows, *Pimephales promelas*, have the ability to detect predators through visual, chemical and possibly mechanosensory cues. The predominant chemical cue used by minnows is alarm substance, a chemical released when the skin of a minnow is damaged. Previous research assumed that alarm substance was a pheromone designed to alert other members of the shoal of danger. If individuals sensed alarm substance, they were expected to react regardless of the context. Recent studies have created a controversy by demonstrating the response to alarm substance is dependent on the context (i.e., the level of risk) in which it is encountered. I propose that the response to alarm substance is not only determined by the level of risk, but also by the availability of information through the other senses.

I developed a sensory compensation model that assumes the concentration of alarm substance necessary to generate an antipredator response decreases as the predation risk and the turbidity level increases. Animals are predicted to be more willing to respond to alarm substance in a high risk or low visibility encounter. The model predicts that minnows would not respond to alarm substance at low risk levels in low turbidity but would in high turbidity. Two experiments were conducted to test the predictions of the model by manipulating the level of risk. The first experiment modified risk sensitivity with hunger levels and the second altered risk of predation through the availability of cover.

The results provide strong support for the sensory compensation model. When visibility was reduced or when the level of risk was increased, minnows were more willing to respond to

alarm substance. The results demonstrated that fish are capable of adjusting their sensitivity to chemical cues based on the level of risk and the availability of visual information. This indicates that minnows in turbid water may have the ability to modify their behavior in an effort to decrease their mortality rates.

Acknowledgments

Obviously, I would first like to thank my supervisor, Mark Abrahams, because without him, this project never would have been possible. His direction, advice and support opened my mind to more possibilities and made this project a better effort. He also helped keep me motivated when my mind wandered to other pursuits.

I would also like to thank the members of my committee, Drs. Darren Gillis and Ted Wiens for comments on the thesis and advice on different directions of further research. Dr. Bob MacArthur deserves thanks for lending me his digitizing tablet, which I should have used in the first place. I would also like to thank Dr. Ross McGowan for putting up with me stealing stuff from his lab, borrowing his pump and invading his freezer.

The grad students here in the dept. of Zoology helped with this project, either through comments on the project or making life here more enjoyable. I would like to thank Marten Koops for conversations which helped me better understand my project and Tonia Robb for making me feel smart.

I would finally like to thank my fiancee, Susan Robertson, for supporting me and putting up with me during my Masters studies.

During my Masters studies, I was financially supported by a Harold E. Welch Memorial scholarship and support was also provided from an NSERC Research Grant to Mark Abrahams.

Table of Contents

List of Figures

List of Tables

Materials and Methods

Results

List of Appendices

Introduction

1. Importance of detecting predators: risk of predation and its ecological importance

Failure to perform an activity is rarely as serious as failing to avoid a predator. Encountering a predator may result in death, potentially eliminating all future reproductive success (Lima and Dill 1990). To deal with predators, prey have evolved various adaptations including cryptic and warning coloration and protective armor (Abrahams 1994, 1995; Alcock 1993; Andraso and Barron 1995). These adaptations provide a set morphological defense against predators because predation pressure varies little over evolutionary time (Lima and Dill 1990). During an animal's lifetime, the risk of predation can vary significantly over small time scales. Prey should be sensitive to the risk of predation and modify their behavior to complement any morphological adaptations.

The probability of death as a result of an encounter with a predator can be described by three parameters: the encounter rate between predator and prey, the probability of death given an encounter, and the time spent vulnerable to an encounter (Lima and Dill 1990). An encounter occurs when the distance between the predator and prey is less than the radius of the larger of the two detection distances. If the prey detects the predator first, the prey has the ability to assess the encounter and decide on the appropriate response (Ydenberg and Dill 1986). If the predator detects the prey first, the prey has a much greater probability of death (Lima and Dill 1990). The probability of detection of prey by predators should be determined by both the behavior of the predators and their prey. Martel and Dill (1995) demonstrated that juvenile coho salmon (*Oncorhynchus kisutch*) decreased their activity levels when threatened with a visual predator stimulus. This resulted in reduced success of the predator which indicated a situation where prey

behavior may be more important. An animal can also reduce its risk of predation by reducing the amount of time spent in areas of numerous predator-prey encounters or by increased feeding close to protective cover (McLean and Godin 1989; Lima and Dill 1990). The magnitude of these responses will be positively correlated with their mortality rate (Abrahams 1995) but these behaviors can be expensive because they eliminate the ability to perform other essential activities (Reissen 1992). Dashing to cover or reducing activity can result in reduced mating activity, lower offspring investment or reduced foraging (Holomuzki and Short 1988). By enhancing the ability to detect predators, prey will be able to perform more essential activities such as locating food or mating.

2. **How to detect predators**

The ability to detect predators is very important for prey. This ability benefits prey in several ways. Predator detection aliows the prey to assess the situation and respond appropriately given the costs of each possible course of action (Ydenberg and Dill 1986). If it will be an encounter with a high risk of predation, the prey can respond with the appropriate anti-predator behavior. Also, correctly identifying individuals which are not predators allows prey to avoid wasting energy and time on unnecessary anti-predator responses (Gelowitz *et al.* 1993). To detect predators, fish can acquire information directly or indirectly by monitoring the behavior of others (Fuiman and Magurran 1994, Åbjörnsson *et al.* 1997, Koops 1998).

2.1 *Visual*

Motion detection and the use of peripheral vision may be important in initially detecting the presence of a predator (Fuiman and Magurran 1994). Once the predator is detected, prey can

determine the best course of action (Ydenberg and Dill 1986) and many species approach potential predators (Pitcher *et al.* 1986). Magurran (1990a) found that predator inspection behavior and predator awareness led to reduced attack rates by the predator. Jachner (1995) showed that a species of cyprinid (*Alburnus alburnus* L.) did not respond to the sight of a live predator placed in a tank separate from the test aquarium. As it was satiated throughout the experiments, the predator did not display any interest in the potential prey (Jachner 1995). Experiments indicate that through predator inspection behavior, fish can assess the identity of the predator and the probability it will attack (Magurran and Girling 1986; Magurran and Pitcher 1987; Magurran 1990b). Magurran and Girling (1986) introduced stalking pike models (*Esox lucius*) of varying detail to groups of European minnows (*Phoxinus phoxinus*). After visual predator inspections, the minnows reacted most significantly to the most realistic model. By recognizing potential predators, prey eliminate the possibility of wasting energy reacting to non-predators (Magurran and Girling 1986; Gelowitz *et al.* 1993).

2.1.1 Public information - the behavior of others

Public information is acquired from the behavior of other animals (Valone 1989) and an individual can respond to danger by observing the behavior of animals in adjacent areas (Verheijen 1956; Magurran and Higham 1988; Gerkema and Verhulst 1990; Suboski *et al.* 1990). Verheijen (1956) performed experiments where visual induction of the fright reaction occurred between groups of minnows in two separate tanks. The alarm substance was introduced into one tank and those fish displayed the fright reaction. The fish in the adjacent tank observed the reaction and reacted in the same manner. This has also been demonstrated with zebra danios (*Brachydanio rerio*; Suboski *et al.* 1990) and with European minnows responding to visual alarm

stimuli (Magurran and Higham 1988). This phenomenon is not limited to fish as it has also been shown in other social animals. Gerkema and Verhulst (1990) showed that common voles, *Microtus arvalis*, exposed to predator models displayed escape responses. Neighboring voles, who could not see the model, also responded provided they could see the model-exposed voles.

2.2 *Chemical*

Recognition of predators through chemosensory cues has been demonstrated for a wide variety of vertebrates (Weldon 1990). Prey have been found to react to the presence of chemical stimuli from their predators (brook stickleback: Gelowitz *et al.* 1993; crayfish: Blake and Hart 1993; *Daphnia*: Pijanowska 1997; dytiscid beetle: Åbjörnsson *et al.* 1997; fathead minnows: Chivers and Smith 1994a, b, 1995; Mathis and Smith 1993a, b; geckos: Dial and Schwenk 1996, Schwenk 1993; mayflies: Peckarsky 1980; rats: Heale and Vanderwolf 1994)). Fathead minnows (*Pimephales promelas*) can learn to avoid areas frequented by their predators with the use of chemical cues in their predators' diet (Mathis and Smith 1992a; Brown *et al.* 1995). Even predator-naive prey have the ability to recognize the chemical stimulus of a predator when that predator had consumed conspecifics (Mathis and Smith 1992a; Brown *et al.* 1995). Fathead minnows can also use chemical stimuli to discriminate natural shoal-mates from unfamiliar conspecifics (Brown and Smith 1994; Chivers *et al.* 1995). Individuals that associate with familiar shoal-mates demonstrate increased shoaling efficiency or cohesiveness (VanHavre and FitzGerald 1988) and therefore have enhanced predator evasion ability (Magurran and Pitcher 1987).

2.2.1 Alarm Substance

Alarm substance, also called Schreckstoff, was first discovered by Karl von Frisch (1938) with European minnows. It was later determined that the alarm substance is produced in the epidermal club cells and is released when the skin is ruptured, as occurs during a predator attack (Pfeiffer 1962; 1963a,b). The primary function of this substance is believed to be to warn conspecifics of the presence of a predator (Pfeiffer 1963a; Smith 1992; Fuiman and Magurran 1994). Upon detecting alarm substance, the reaction is characterized by one or more of the following behaviors: hiding, freezing, dashing, area avoidance, or increased shoaling (Levesley and Magurran 1988; Magurran 1990a; Smith 1992; Krause 1993a).

2.2.1.1 Characteristics of the alarm substance cells and alarm substance

Alarm substance cells are found in the middle to basal zones of the epidermis. These cells lack any external pores, they do not come in contact with any blood vessels, and they do not reach the outer surface of undamaged epidermis (Smith 1982). These characteristics suggest that alarm substance is normally stored or retained in the cells without the possibility of being voluntarily released externally or being secreted internally for another function (Smith 1982).

Alarm substance is a colorless, non-fluorescent substance (Pfeiffer 1982). It is slightly soluble in water but is extremely unstable in water (Pfeiffer 1982) where it becomes inactive within a very short period (Smith 1982). Pfeiffer (1982) determined that hypoxanthine-3(N)-oxide elicited a response similar to alarm substance but the chemical structure of alarm substance is still not known. The mechanism of the reception of the chemical is also not well known but is thought to be mostly olfactory (Pfeiffer 1982, Chivers and Smith 1993). Fish that do sense the

chemical respond with increased movement away from the source area and increased bradycardia (Pfeiffer 1982). Exposure of black tetras to the alarm substance of *Phoxinus* resulted in enhanced optical alertness (Pfeiffer and Riegelbauer 1978; Pfeiffer *et al.* 1985). The amount of alarm substance released during a predator attack is not known but it has been determined that only a very small amount is necessary to elicit a response (Smith 1982).

Breeding fathead males lose the ability to produce alarm substance yet they still retain the ability to respond to alarm substance (Smith 1976). The reason for losing the cells is not known but it is hypothesized that they are lost because of the males' breeding behavior. Male fatheads make pits in the substrate defining their territory. The pits are made by rubbing their bodies against the substrate. Alarm substance is released with little effort (Pfeiffer 1962) and the action of creating and maintaining a territory would cause the release of alarm substance (Smith 1976). This would be counterproductive because the presence of alarm substance would cause potential mates to flee the area (Smith 1976).

2.3 *Other Sensory Systems*

Other sensory systems that may be important in detecting predators are the mechanosensory and the auditory systems. The movements of a predator cause vibrations that can be perceived by free neuromasts, canal neuromasts, and the inner ear (Fuiman and Magurran 1994). These systems are sensitive to the low frequencies which are characteristic of the swimming movements of a predator. The Weberian apparatus of ostariophysans and the auditory bullae of clupeids provide these fish with an even lower threshold and a broader dynamic range than unspecialized fish. The swim bladder is an important component of the mechanosensory system. It converts variation in sound pressure into fluid displacements inside the body which can initiate the startle response

and possible avoidance behavior (Canfield and Eaton 1990). The scope of the lateral line and its associated mechanosensory cells is about one body length (Schellart 1992) which may make it more effective in regulating shoaling (Partridge and Pitcher 1980) and evasion behavior (Fuiman and Magurran 1994) than if it were more discrete.

3. **Factors affecting predator detection and prey response**

3.1 *Visibility*

For minnows, visual detection of a predator is a very important component of its anti-predator adaptations as it is with any animal, but as Mathis *et al.* (1993) point out, reliance on visual information may be limited when the visibility is poor. Underwater visibility is highly variable. Visibility at noon in sunny weather can range from 0.2 to 80 meters (Schellart 1992). Factors which can significantly influence visibility include turbidity, wavelength, direction of sight, depth in water, bottom depth, time of day, weather conditions, and the time of the season (Schellart 1992).

3.1.1 Turbidity

Turbidity is a common feature of many lakes and reservoirs and it is often highly variable (Miner and Stein 1993). Turbidity provides cover for prey which suggests that the impact of predation may be reduced. Turbidity also allows predators to get closer to their prey before being detected suggesting predator benefit. Vandenbyllaardt *et al.* (1991) observed that fathead minnows formed looser schools and increased swimming activity when they were in turbid water. Gregory (1993) found that juvenile chinook salmon (*Oncorhynchus tshawytscha*) demonstrated a lower magnitude and shorter duration of response to a predator in turbid water and Gregory and

Northcote (1993) showed that juvenile chinook salmon increased their foraging rates at increased turbidity levels.

Though turbidity may provide cover, encounters that do occur between predators and prey are more likely to result in mortality because of ineffective anti-predator behavior (Vandenbyllaardt *et al.* 1991; Abrahams and Kattenfeld 1997). The presence of turbidity serves to reduce the reactive distance of prey to predators (Figure 1; Gregory and Northcote 1993; Miner and Stein 1996) mainly due to the hyperbolic relationship between turbidity and visual range (Figure 2; Vinyard and O'Brien 1976; Confer *et al.* 1978; Wright and O'Brien 1984; Aksnes and Giske 1993). Abrahams and Kattenfeld (1997) found that fathead minnows in turbid water had lower detection distances which reduced the effectiveness of any anti-predator behavior. This is because an attacking predator may be detected only after it is inside the distance at which the prey would have fled when in clear water (Ellis 1982). In clear water, prey have the ability to detect the predator and determine its course of action by weighing the costs of fleeing against the costs of remaining. When the costs of remaining exceed the costs of fleeing, an animal should leave the area (Ydenberg and Dill 1986). Abrahams and Kattenfeld (1997) also found that a perch predator preferentially preyed on smaller minnows in clear water but that size preference disappeared when the water was turbid. This suggested that the larger minnows lost their enhanced ability to evade predators when the water became turbid (Abrahams and Kattenfeld 1997). In a turbid situation, there are no trade-offs to determine between fleeing and remaining because prey do not have the ability to assess the encounter and therefore prey should flee as soon as the predator is detected (Ydenberg and Dill 1986). When visibility is low, the presence of the alarm substance should allow prey to detect predators earlier than would be possible by using visual input.

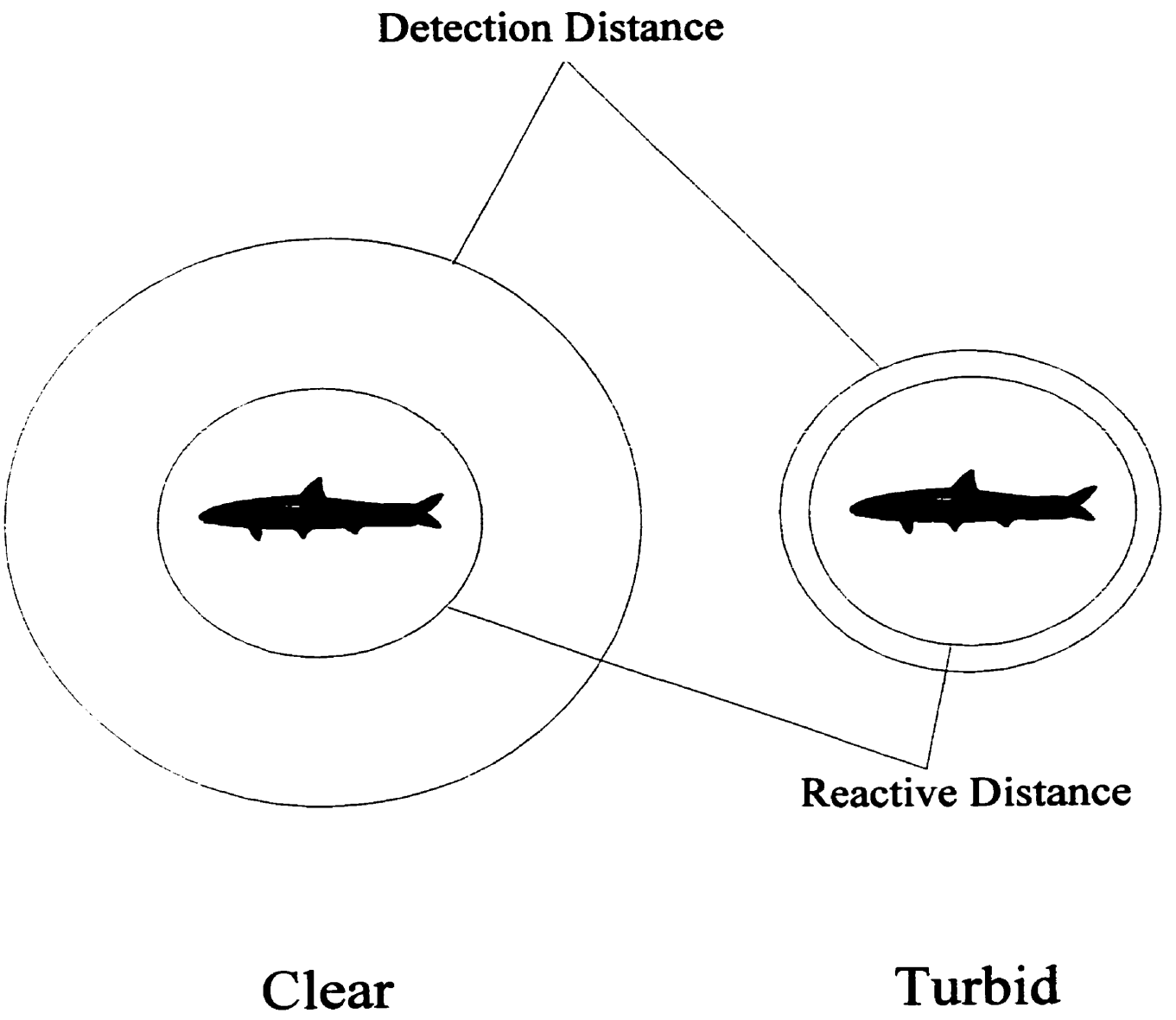


Figure 1. The effect of turbidity on the reactive distance (distance at which an individual reacts to either prey or a predator) and the detection distance (distance at which a predator or prey can be initially detected) (from Kattenfeld 1995).

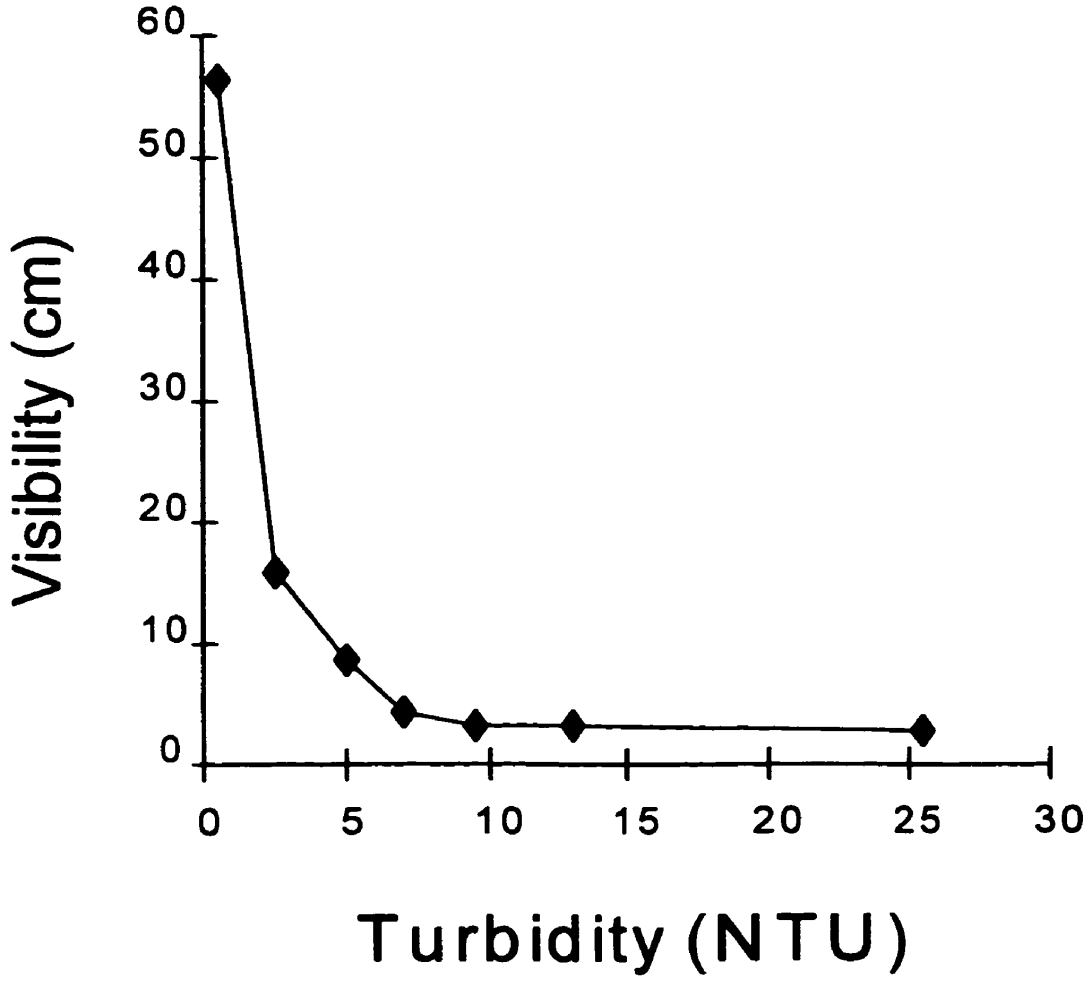


Figure 2. Turbidity causes the visual range to decrease exponentially. This was determined by placing a perch model in the test apparatus and recording its visibility with a Sony Hi8 video camera (CCD-V99). Turbidity was increased by adding approximately 0.80 grams of bentonite to 1100 ml of aquarium water for each turbidity level. To get a level of 13 Nephelometric Turbidity Units (NTU), an additional 1.13 g was added and 25.5 NTU required 4.50 g of bentonite. Turbidity was measured with a Hach Model 2100A Turbidimeter. Visibility was measured as the distance from the edge of the tank to the nose of the perch model.

Variations in brain forms of teleosts are more clearly correlated with their habitats than in other vertebrates (Davis and Miller 1967; Kishida 1979). The brain and sensory apparatus determine the position and range of a species' potential niche (Kotrschal and Junger 1988). Van Staaden *et al.* (1995) found a strong negative correlation between habitat visibility and the size of the olfactory apparatus for cichlids of the Great African Lakes. Fish from areas of reduced visibility had larger olfactory apparatus which suggests an increased reliance on the use of chemicals to receive information from the environment. Huber and Rylander (1991) examined three clear water and three turbid water minnow species and discovered that brain structures associated with vision were much more developed in the clear water species. Parts of the brain thought to be associated with olfaction were more developed in the turbid water species. Huber and Rylander (1991) suggested that the importance of olfaction increases in species which inhabit turbid water. Another study by Huber and Rylander (1992) looked at more species of cyprinids and concluded that different species rely on different sensory modalities. The dominant sensory system used by a species correlates with the physical parameters of its preferred habitat (e.g., turbidity level; Huber and Rylander 1992).

3.2 *Some parameters that influence the response to predators*

3.2.1 Confinement

Previous laboratory experiments have demonstrated that minnows respond to the alarm substance in clear water in the absence and in the presence of a visual predator stimulus (Verheijen 1956; Pfeiffer 1962, 1963a; Magurran and Girling 1986; Lawrence and Smith 1989; Mathis *et al.* 1993; Brown and Smith 1996; Irving and Magurran 1997). Magurran *et al.* (1996) proposed that the confined nature of the aquaria increases the level of risk of predation a minnow senses. This

results in the minnows being more prepared to react to an alarm stimulus. When the size of the test apparatus is increased, the reaction to the alarm substance has been found to be significantly reduced (Irving and Magurran 1997). Irving and Magurran (1997) suggested this was a result of decreasing the level of risk of predation.

3.2.2 Hunger

Many experiments have shown that hunger levels modify critical reactive distances by decreasing their sensitivity to risk (Magnhagen 1988a, b; Gregory 1993; Gregory and Northcote 1993). As the hunger level increases, prey are more willing to risk exposure to a predator because the benefits of continued foraging exceed the costs of fleeing from a predator (Ydenberg and Dill 1986). This general pattern of behavior is predicted by state-dependent optimization models (Mangel and Clark 1986; McNamara and Houston 1986). This model is supported by experiments demonstrating that hungry fish are more likely to inspect a potential predator (Godin and Crossman 1994; Magurran 1990a). Krause (1993b) has shown that hungry fish are more willing to take up outside and leading edges of schools. These positions increase their contact rate with food (Bumann *et al.* 1997). Hungry fish are also more willing to risk exposure to a predator in order to obtain access to better quality food patches (Magnhagen 1988a, b; Dill and Gillett 1991).

Other experiments have shown that the hunger level of the fish exposed to an alarm substance affects the magnitude of the fright reaction (Smith 1981; Brown and Smith 1996). As the hunger level increases, the reaction to the alarm substance becomes weaker until the reaction disappears. The presence of alarm substance represents a high risk of predation and hungry fish are more willing to accept that risk. Brown and Smith (1996) found that even though naive

fathead minnows deprived of food for 48 hours did not respond to the introduction of the alarm substance, they were able to determine that alarm substance signified a threat. These fish responded to alarm substance with the typical fright reaction when they had resumed normal feeding.

3.2.3 Cover/Safe areas

In aquatic communities, structural complexity of the environment modifies the type and intensity of predator-prey interactions (Savino and Stein 1989). Structurally complex microhabitats tend to reduce a prey's encounter rate with potential predators (Endler 1986; McLean and Godin 1989) and can impair the predator's attack success. Numerous studies have discovered that predators become less effective as the structural complexity of the environment increases (Glass 1971; Savino and Stein 1982, 1989; Coull and Wells 1983; Anderson 1984). Savino and Stein (1989) found fathead minnows became more difficult to capture as plant density was increased. Highly structured habitats also reduce the ability of predators to detect or encounter prey (Lima and Dill 1990). The presence of a structured habitat represents a lower risk of predation for the prey when compared with less structured habitats (Savino and Stein 1989; Rangeley and Kramer 1998). When faced with a predator, prey commonly move toward or into cover to reduce their risk of predation (Gotceitas and Colgan 1987; Abrahams 1995; Rangeley and Kramer 1998).

4. **Benefit of alarm substance and controversy over the use of chemical signals**

4.1 *Benefits of alarm substance*

4.1.1 Secondary benefit: kin selection?

Since its discovery, alarm substance has been considered a signal designed for communication between conspecifics (Pfeiffer 1963a; Smith 1992; Fuiman and Magurran 1994). To release

alarm substance, the signaler must already be injured; therefore, the release of alarm substance only appeared to benefit the receivers by signaling the presence of danger (Pfeiffer 1963a; Smith 1992). This benefit to the receiver without benefiting the sender suggested that the sender gained via kin selection (Williams 1992). Naish *et al.* (1993) determined that European minnow school members did not share the same maternal mtDNA lineages but Smith (1997) pointed out they could not exclude kin selection because the results did not eliminate the possibility of kin subgroups within larger shoals.

There is evidence of responses to alarm substances from distantly related genera and families and evidence of inter-order signaling (Schutz 1956; Mathis and Smith 1992b). Pfeiffer and Lemke (1973) and Pfeiffer (1978) demonstrated that giant danios (*Danio malabricus*) reacted strongly to the alarm substance in the skin of European minnows. Big eye shiners (*Notropis boops*) reacted to their own alarm substance but also reacted as significantly to the alarm substance from several other cyprinids (Smith 1982). This indicates that species can use information from other species to detect a dangerous situation.

4.1.2 Primary benefit: predator attraction

Mathis *et al.* (1995) found that both pike and predaceous diving beetles (Dytiscidae) were attracted to fathead minnow extract and the arrival of a secondary predator or predators significantly increased the prey's probability of escape (Chivers *et al.* 1996). This occurs when the secondary predator tries to interrupt the primary predator, either by trying to take the prey away or by attempting to eat the primary predator (Mathis *et al.* 1995; Chivers *et al.* 1996).

Similar mechanisms have been proposed as the function of distress calls in birds and mammals (Högstedt 1983) and bioluminescence in dinoflagellates (Abrahams and Townsend 1993).

4.2 *The controversy*

Recent experiments have demonstrated the fright reaction elicited by the alarm substance became less significant as the environment became more natural (Magurran *et al.* 1996; Irving and Magurran 1997). Irving and Magurran (1997) conducted experiments in a fluvarium that contained part of the Mill Stream in England. The European minnows were exposed to conditions that more closely resembled their natural habitat while still being confined in the fluvarium. The minnows showed a response to the alarm substance but the reaction was weak when compared with the reactions they had displayed in the lab. Magurran *et al.* (1996) performed experiments in the River Frome with European minnows. These experiments were conducted in a completely natural setting and the minnows failed to change their behavior or leave the area when exposed to alarm substance.

This has initiated a debate as to whether alarm substance is a signal or a cue to conspecifics (Magurran *et al.* 1996; Henderson *et al.* 1997; Smith 1997). These can be distinguished based on the function of the information being transferred (Koops 1998). A signal is defined as a transfer of information that has been shaped for this purpose by natural selection (Seeley 1989; Dusenbery 1992). This means both the information generated by the transmitter and the interpretation by the receiver are shaped by evolution and both the sender and the receiver benefit from the signal (Koops 1998). Smith (1997) argues that alarm substance was designed specifically to alert other members of the shoal and the senders benefit through kin

selection. It was proposed that a reaction to alarm substance should always occur regardless of the environmental conditions involved. The reaction may be immediate or a more long-term behavioral shift (Smith 1997).

Cues are patterns of information that have not been shaped to convey information to the receivers (Seeley 1989; Dusenbery 1992). This suggests that the source of the cue does not experience selection for improved information transfer, but the receivers' interpretation should be subject to natural selection; therefore, only the receiver benefits from the transfer of information (Koops 1998). Magurran *et al.* (1996) and Henderson *et al.* (1997) suggest that alarm substance was not designed for the purpose of communicating danger to other members of the shoal. They propose that alarm substance may have some other purpose but prey species use its presence as a means of detecting a predator. They suggest that fish can sense the alarm substance but the response is mediated by the relative margin of risk of predation of the encounter. Magurran *et al.* (1996) propose that strong reactions occur in the laboratory because fish are in an unnatural environment. Confined fish are more wary and therefore sense a higher level of risk of predation (Magurran *et al.* 1996). This heightened level of risk of predation makes the fish more willing to react to alarm substance. Fish in their natural environment do not react because they are not wary. These fish sense a lower level of risk of predation as a result of increased information about their environment. Information such as familiarity with the area, knowledge of safe areas, and possibly familiarity with individual predators (Magurran *et al.* 1996). Von Frisch (1938) has shown that habituation in aquaria reduces the magnitude of the response to alarm substance. This could be a result of becoming more familiar with the environment which would eliminate the reaction as suggested by Magurran *et al.* (1996).

5. **Sensory compensation hypothesis - a resolution to the debate**

Once prey detect a predator, they must then decide whether they should flee (Ydenberg and Dill 1986). Factors influencing this decision are the costs of flight (e.g., lost foraging opportunities) and the benefits (e.g., the reduced probability of capture). These costs and benefits of fleeing will ultimately determine the distance at which prey respond to a predator (Ydenberg and Dill 1986). Prey use a variety of stimuli to detect predators and I propose they should preferentially use the signal which gives the most reliable information (Figure 3). By using the most reliable signal, prey can maximize their benefits and respond to a predator only when absolutely necessary. A more reliable signal would allow earlier detection of the predator. This would allow the prey more time to determine the appropriate course of action and more assessment time should result in a better choice. Responding to less reliable stimuli when better cues are available would not provide the animal with an optimal flight distance and therefore extra costs would be incurred. In clear water, vision allows prey to detect predators before a response is necessary. The prey still has the ability to assess the encounter and decide on the appropriate course of action (Ydenberg and Dill 1986). As visibility is reduced, the animal is progressively denied information that would allow an adaptive decision to be made. Under these circumstances, relying solely on visual cues increases the probability of death given an encounter (see section 3.1). Various crayfish of the genus *Orconectes* increased their use of mechanoreceptive organs when visual input was reduced at night (Bruski and Dunham 1987).

Sensory compensation has been demonstrated in humans. Subjects deprived of various senses demonstrated increases in sensitivity to alternate senses. Duda and Zubek (1965) demonstrated significant increases in auditory sensitivity after being visually deprived for one

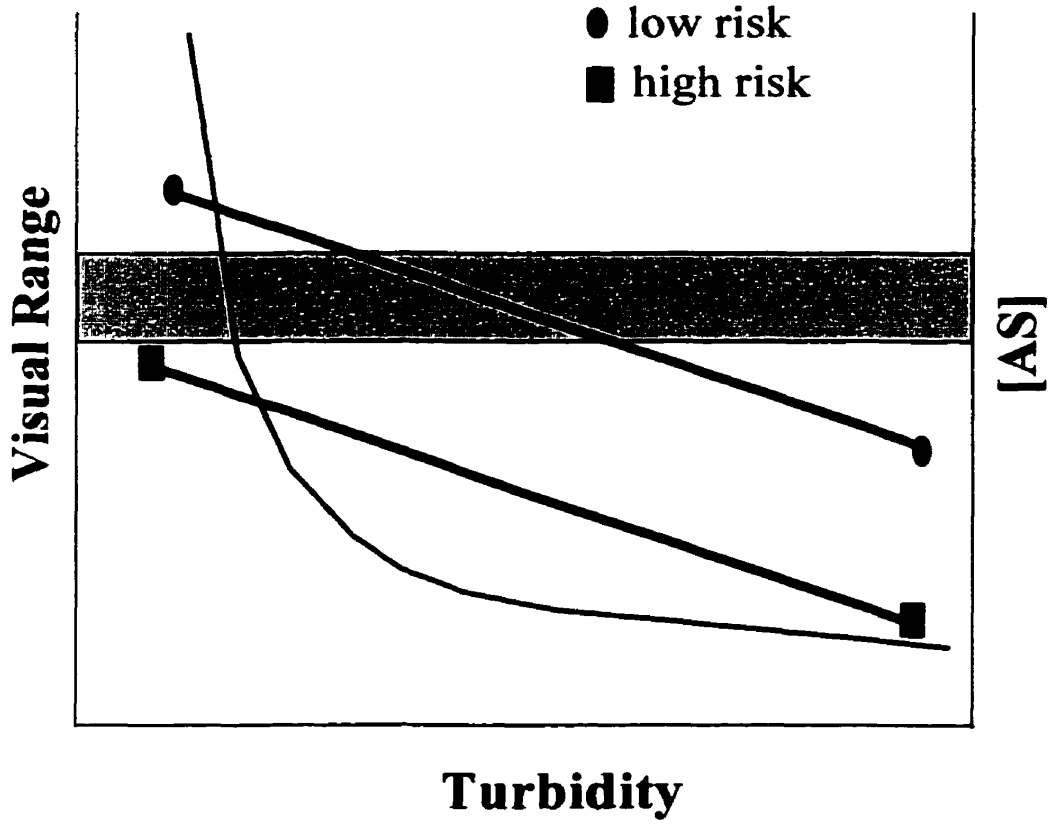


Figure 3. The sensory compensation model. The gray bar indicates the concentration of alarm substance (AS) released for a given situation. The heavy lines represent the concentration of AS necessary to generate an antipredator response which is assumed to be lower (lower line) at higher levels of predation risk. This concentration is also assumed to decrease in response to diminishing visual information due to increased turbidity. When faced with a high risk of predation, minnows should respond to a given concentration of AS in both clear and turbid water. In a low risk situation, the minnows should not respond to AS in clear water but respond to the same concentration in turbid water.

week. Studies by Vernon and McGill (1961), Zubek, Flye, and Aftanas (1964), and Zubek, Flye, and Williams (1964) found that pain sensitivity was increased after a period of sensory deprivation. Zubek (1964) confined subjects under conditions of perceptual deprivation and found that tactile acuity was significantly increased. As similar result was obtained by Biase and Zuckerman (1964) and Zuckerman *et al.* (1964). I propose that prey compensate for a reduction in one sensory input by increasing the use of alternate stimuli to detect predators. In turbid water, increasing sensitivity to alarm substance should compensate for the reduction in visual input (Figure 3). This would allow earlier detection of predators than would be possible if the minnows only used visual information.

Magurran *et al.* (1996) suggested that responses to alarm substance are mediated by the level of risk of predation. Higher risk of predation situations cause prey to be more prepared to respond to the presence of alarm substance. Ydenberg and Dill (1986) demonstrated evidence that the flight distance is directly proportional to the level of risk of predation. With respect to alarm substance, flight distance may be modified by altering the concentration of alarm substance necessary to generate a response. I propose that in a high risk situation, fish will be more sensitive to alarm substance and will show strong reactions (Figure 3). Many previous experiments have demonstrated significant responses to alarm substance in the absence of a visual stimulus (see section 3.2.1). In low risk situation, fish should be less sensitive to the same concentration of alarm substance and therefore should not show significant responses (Figure 3). Magurran *et al.* (1996) and Irving and Magurran (1997) demonstrated that as the level of risk of predation was reduced (by increasing the naturalness of the environment), sensitivity to alarm substance was decreased.

Risk will be manipulated with two parameters. In the first experiment, risk acceptance will be altered with hunger levels. Hungry fish are less sensitive to risk of predation than are well fed fish: therefore, it is assumed that increasing the hunger level will have an effect equivalent to decreasing the actual level of risk. For the second experiment, the actual level of risk will be decreased through the addition of cover to the apparatus.

Materials and Methods

1. Animals

The test subjects for these experiments were wild fathead minnows. The minnows were captured in the fall of 1997 from the University of Manitoba Field Station at Delta Marsh, located at the southern tip of Lake Manitoba. They were collected using minnow traps. The predator for these experiments was a yellow perch (*Perca flavescens*, 169.2 mm, 66.45 g) also obtained from Delta Marsh. This species is commonly observed with fathead minnows in a variety of freshwater environments in North America (Scott and Crossman 1973).

After capture, the minnows were transported to the University of Manitoba Animal Holding facility where they were held in two 200 liter tanks at 12 °C with a 12 hour photoperiod. All fish were fed a diet of frozen brine shrimp (*Artemia salina*). Prior to the experiments, five groups of ten fish were moved to the laboratory for Experiment 1 and each group was held in a 40 liter aquarium at 19.5 °C with a 12 hour photoperiod for the duration of the experiments. For Experiment 2, another five groups of ten fish were moved to the laboratory. While in the holding tanks, the fish were fed a diet of NutraFin flake food. The perch was held in an equally divided 90 liter aquarium. The perch was fed a diet of fathead minnows on a weekly basis. The tank was maintained at approximately 19.5 °C and the photoperiod was the same as for the fathead minnows.

2. Materials

The test apparatus consisted of a square (76 × 76 × 30.5 cm) aquarium (Figure 4) positioned on a light table constructed for these experiments. The light table supported the tank and contained a

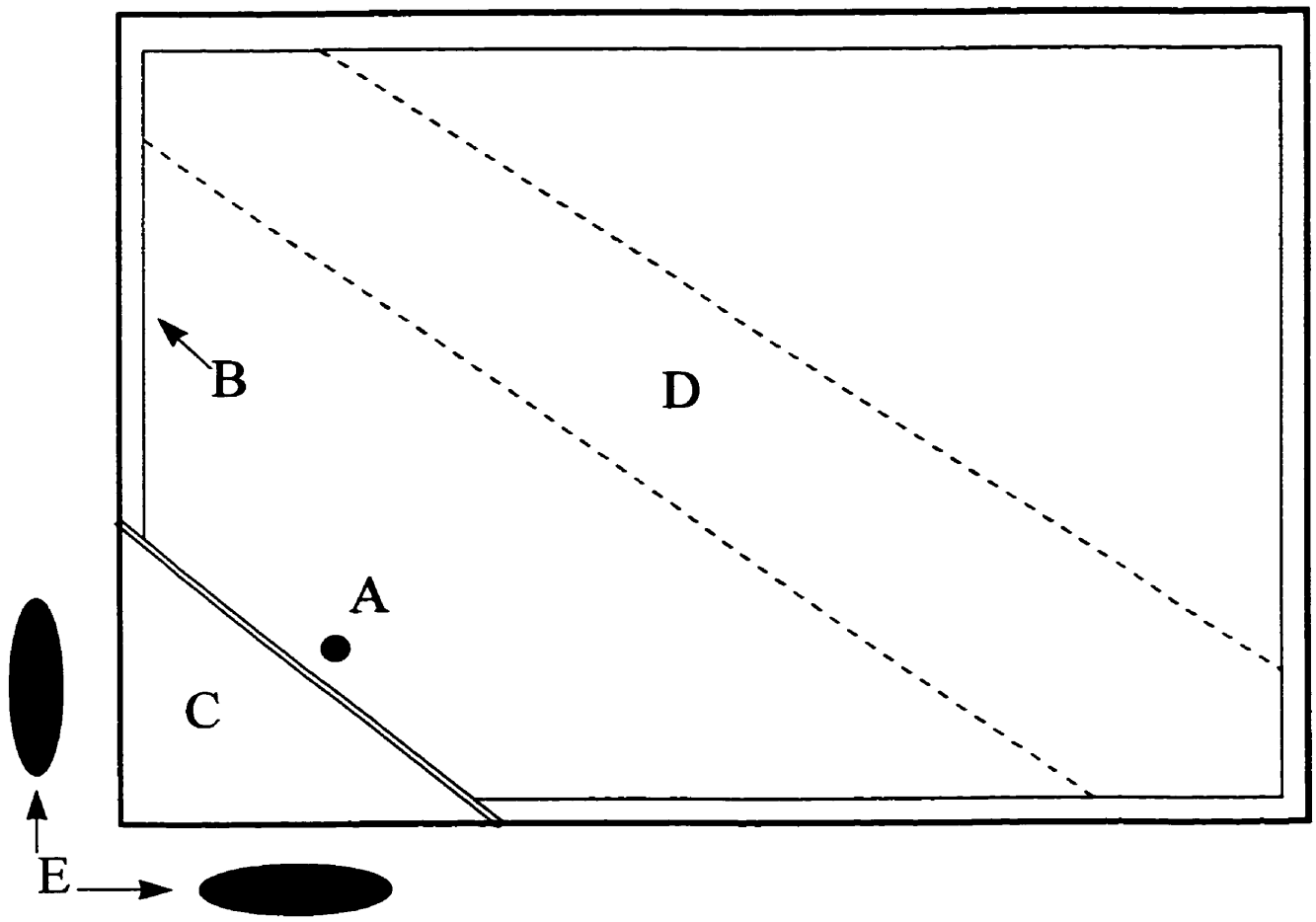


Figure 4. The overhead view of the apparatus which includes the stimulus introduction point (A), the Plexiglas insert (B), the predator area (C), the standardization zone (D), and the halogen lights for illuminating the predator (E).

pane of sandblasted glass which diffused the light from 12 fluorescent bulbs (30 watts) set in six 90 cm strip fixtures placed 25 cm below the tank. The square test aquarium was lined with cardboard packaging material to minimize any external disturbances and to help focus the light from the light table. The entire tank was monitored with a Hi8 video camera (Sony CCD-V801) placed 175 cm above the tank. The test tank had a Plexiglas insert (approx. 70 × 70 × 10 cm) attached to the tank to keep the minnows away from the shadows around the edges caused by the frame that supported the tank. This resulted in a test area which was approximately 69 × 69 cm. The apparatus was filled to a water level of 6.5 cm. This low water level allowed easier observation of the minnows' behavior when viewed from the overhead camera.

A corner of the test tank was partitioned with a piece of one-way glass. This created a triangular five liter area which held the predator. The bottom of this area was covered with a black piece of Plexiglas which blocked light from the light table. Two halogen floodlights were positioned on either side of the predator area. When these lights were turned on, the predator area became visible to the test fish, revealing the predator if it was present. Two lines, 20 cm and 45 cm away from the one-way glass, were drawn on the bottom of the apparatus. Fish were only exposed to the chemical or visual stimulus when they were between these lines. Conducting experiments in this fashion reduced the influence of location on the response to the chemical and visual stimuli.

Water was pumped through the tank at a rate of 10 ml/sec with a Manostat Varistaltic pump and Tygon tubing. The chemical stimulus was introduced into the tank at point A (see Figure 4) by injecting the chemical stimulus into the Tygon tubing.

3. **Stimulus Preparation**

Alarm substance was prepared following the methods of Magurran *et al.* (1996) and Irving and Magurran (1997). It was obtained by humanely killing female and non-breeding male fathead minnows (Experiment 1: mean total length = 64.36 mm ± 2.33 s.e., mean weight = 2.60 g ± 0.33 s.e.; Experiment 2: mean total length = 63.43 mm ● 1.93 s.e., mean weight = 2.41 g ± 0.27 s.e.; Appendix 1) with a blow to the head. Then the minnows were weighed and measured. Breeding males were not used because they seasonally lose their alarm substance cells (Smith 1976). The skin was removed, measured and weighed. It was then ground with a mortar and pestle over an ice bath. Chilled, distilled water was added to produce a dilution of 0.450 cm^2 skin / ml. The extract was stirred vigorously with the mortar and pestle for five minutes and then filtered through glass wool to remove scales and other large particles. Preliminary experiments conducted in a 40 liter aquarium with 4 test fish determined that 3 ml of 0.450 cm^2 skin / ml was sufficient to obtain a reaction from the fish. This extract contained an amount of alarm substance similar in both wet weight and skin area to that used by Mathis and Smith (1992a), Brown and Smith (1996), and Irving and Magurran (1997). This amount of alarm substance added represents approximately 20 % of the amount of alarm substance contained in an average sized fathead minnow.

The control stimulus was prepared following Irving and Magurran (1997) by using muscle from the caudal peduncle of the same fish from which the alarm substance had been extracted. It was prepared as above, using an equal mass of muscle. The final solutions of the alarm substance and the muscle control were divided into 1 ml aliquots and were stored in a freezer at -80 °C until needed. The stimulus was prepared every week, with only enough made

for the trials that would proceed during the following week. Before either chemical substance was used, it was thawed in a Petri dish filled with water for approximately 30 minutes.

4. **Turbidity preparation**

Tests involving clear water had a turbidity level of < 1 NTU (Nephelometric Turbidity Units). For tests requiring turbidity, bentonite was added to generate a turbidity level of at least 20 NTU (Experiment 1: 21.11 NTU ± 0.24 s.e.; Experiment 2: 22.48 NTU ± 0.39 s.e.). This was accomplished by removing 1.2 liters of water from the test aquarium. Seven grams of bentonite were added to this water and then mixed thoroughly. This water was then added to the aquarium and the turbidity was measured before and after each trial.

5. **Experimental procedure**

5.1 *Experiment 1* - The effect of turbidity and hunger on the response to chemical and visual stimuli

Five groups of 10 fathead minnows (mean total length = 57.73 mm ± 1.84 s.e., mean weight = 1.53 g ± 0.18 s.e.) were exposed to conditions involving water clarity (clear or turbid), hunger level (hungry: no food for 72 hours or satiated: fed just prior to the experiments), visual stimuli (predator or no predator), and chemical stimuli (alarm substance or muscle control). The groups of minnows were randomly exposed to all of 16 different possible combinations of the conditions. The water clarity treatment was randomly determined for each week and maintained constant throughout that week while the other three treatments were randomized daily for each group of minnows. Keeping the water clarity constant during the week eliminated any possibility of the clear water becoming turbid. The experiments were performed as a repeated measures experiment. There was a minimum of 48 hours between subsequent exposures to the

alarm substance for each group and each group was only exposed to the alarm substance eight times over a three month period. This design reduced the possibility that these fish would become acclimated to the predator stimulus.

Trials involving hungry fish required that they not be fed for 72 hours. Brown and Smith (1996) found that going without food for 48 hours was sufficient to eliminate the fright reaction but I found that 72 hours without food was necessary to cancel any significant reactions in the test apparatus. Each group of 10 fathead minnows was placed in a 40 liter, food free holding tank 72 hours prior to their trial. Satiated fish were added directly to the apparatus for the acclimation period. The minnows were gently dipnetted from either their holding tank or the food free tank and were placed in the test apparatus 90 minutes prior to the pre-exposure trials. During acclimation, only satiated fish were fed flake food. After 75 minutes, the air stones were removed, the lights were turned on, the pump was started, and the predator (if required) was added to the apparatus. Fifteen minutes after the lights were turned on, the camera began recording for a pre-exposure period of 15 minutes. At the end of the 15 minutes, recording stopped until all of the fish were within the standardization zone. The camera would then begin recording as the chemical and visual stimuli were introduced. To expose the predator area, the halogen floodlights were turned on for two minutes. The chemical stimulus was added directly to the Tygon tubing with a 3 cc syringe. Pre-experiments found that a dyed sample of alarm substance took eight seconds to completely enter the tank from the point of injection into the Tygon tubing. The chemical dispersed throughout the tank within about 20 seconds. The camera recorded for 15 minutes following the introduction of the stimuli.

Following each trial, the fish were removed and the turbidity of the water was rechecked. The apparatus was completely drained and the Tygon tubing flushed with fresh water to remove

any chemical residue. The apparatus was then refilled for the following trial. I conducted no more than three trials in one day (between 0900 hrs and 1900 hrs).

After each group of fish had completed all of the experimental treatments, the minnows were anesthetized with 2-phenoxyethanol (1 ml:1800 ml water). The total length and the wet weight of each individual was measured (Table 1).

5.2 *Experiment 2* - The effect of turbidity and cover on the response to chemical alarm stimuli

Cover was created by cutting a 68 cm x 68 cm sheet of clear Plexiglas and drilling 160 holes staggered 5 cm apart. A 7 cm strand of polypropylene rope was put in each hole. The rope was burnt on one end with a Bunsen burner. The melted end was then flattened to form a plug. This secured the rope in the Plexiglas sheet. This setup provided fish with a significant amount of cover while allowing me to easily observe the fish from above.

The procedure for Experiment 2 was the same as for Experiment 1. Five different groups of ten fathead minnows (mean total length = 68.14 mm ± 2.27 s.e., mean weight = 3.02 g ± 0.34 s.e.) were randomly exposed to eight different environmental conditions involving water clarity (clear or turbid), cover (present or absent), and chemical stimuli (AS or control). The presence of a predator was not necessary for these trials because I only wanted to determine the effect of turbidity and cover on the response to alarm substance.

After each group had completed the treatments, minnows were anesthetized and the wet weight and total length were determined (Table 2).

Table 1. The mean total length and wet weight of each group of fathead minnows used in Experiment 1.

	Total length (mm)		Wet weight (g)	
Group	**Mean**	**Std. Error**	**Mean**	**Std. Error**
1	56.325	1.610	1.490	0.158
2	60.739	1.790	1.659	0.168
3	52.309	1.060	1.152	0.119
4	59.370	2.250	1.641	0.220
5	59.898	2.510	1.729	0.229

Table 2. The mean total length and wet weight of each group of fathead minnows used in Experiment 2.

	Total length (mm)		Wet weight (g)	
Group	**Mean**	**Std. Error**	**Mean**	**Std. Error**
1	69.831	2.200	3.216	0.345
2	68.670	2.570	3.077	0.393
3	69.740	2.200	3.427	0.337
4	64.860	1.560	2.411	0.181
5	67.580	2.800	2.993	0.450

6. **Analysis**

6.1 *Behavior*

6.1.1 Response to alarm stimulus

Dashes were defined as a rapid dart in an apparently random direction. A dashing fish swam with strong S-shaped curves which is characteristic of the Mauthner mediated S-startle response. A freeze was defined as immobility for a period that lasted a minimum of 30 seconds. Hiding occurred in the preliminary experiments when the minnows took refuge under the curved Plexiglas or among the vegetation.

Previous experiments have demonstrated a variety of responses to the introduction of alarm stimuli (Levesley and Magurran 1988; Magurran 1990a; Smith 1992; Krause 1993a). My preliminary experiments determined that in a structurally complex environment, hiding was the most common response. Under these conditions, dashing rarely occurred. In the test apparatus, there were no areas to hide and substrate was not present. Here, the most common responses were dashing and freezing. Minnow reaction was determined using the difference in the number of dashes and freezes between the post-exposure and the pre-exposure period.

6.1.2 Movement and direction changes

Another measure of the behavior of each group was the distanced traveled and the number of times an individual changed direction. These behaviors were analyzed by tracing the movement of one fish onto acetate from the recorded videotape. One fish was randomly selected by numbering the fish from 1 to 10 starting at the top of the screen and proceeding clockwise around the tank. Numbers from 1 to 10 were randomly generated and the corresponding fish was observed for one minute. This was repeated a second time for a total of two minutes of

observation. The first tracing was made at five minutes and the second one was made at ten minutes into the pre-exposure period. The images were scanned and the length of the line was determined using Jandel Sigma Scan v.2.0 (1990). The 25 cm width of the standardization zone was used to calibrate the program. The number of times a minnow switched direction was determined from the scanned tracings. Any change in direction of the line of less than 90^0 was recorded. For the distance traveled and the direction change, significant differences between the treatments involving water clarity and the treatments involving cover were determined using paired t-tests ($\alpha = 0.05$).

There were problems with the videotapes from Experiment 1 due to the video camera; therefore, observations were only made with tapes from Experiment 2.

6.2 *Statistics*

6.2.1 Paired t-tests

Paired t-tests ($\alpha = 0.05$) were used to determine if the behavior of the groups was random. The null hypothesis was that the number of observed dashes or freezes for a given group was random. The alternative hypothesis was that the magnitude of the reaction was not random for each group and was dependent on the treatment.

6.2.2 Approximate randomization

Approximate randomization was used to examine the effects of each treatment on the response. This method was used to test if the response was unrelated to the treatment; therefore, the null hypothesis specified that there was no correlation between the observed reaction and the treatment in which the observation was made. The alternative hypothesis was that the treatment

strongly affects the observed response. This technique was used because computer intensive statistics are based on the resampling of the actual data and therefore do not need the assumptions required for parametric statistics (Adams and Anthony 1996; Manly 1991; Thomas & Poulin 1997).

Test experiments demonstrated that a typical number of dashes during a normal trial without any alarm stimuli resulted in 7.23 dashes (± 1.24 s.e., n = 10) while a stimulus trial resulted in more than 20.23 dashes (± 2.01 s.e., n = 10). These results demonstrated a bimodal distribution. To compare the effects of each treatment, the presence or absence of a response was the important consideration. Therefore, the distribution of the dashes was observed for each experiment and a cutoff was determined. Less than the cutoff was considered no-response (y = 0) and more dashes than the cut off was a response (y = 1). Data in this binary format could not be analyzed using simple parametric tests. For analysis of freezing, the data did not display any clear difference between a response and no-response. Therefore these data were not transformed.

A program by Noreen (1989) was used to determine the difference between the means of the treatments being compared using Microsoft Excel Visual Basic v.7.0a (1996; Appendix 3). The null hypothesis of the randomization was that the dependent variable, the response (dashing or freezing), is unrelated to the explanatory variable, the type of treatment. The alternative hypothesis was that the dependent variable (response) was directly related to the explanatory variable (treatment). To test this, the program calculated the difference of the observed response between the experimental treatment and the control treatment. This value was then compared to the values obtained by shuffling the data (number of shuffles was determined with a maximum variance around the p-value, +/- 0.005 for 10 replications) and recalculating the difference between the means. A calculation of the number of times the random test statistic exceeded the

actual test statistic was used as the p-value ($\alpha = 0.05$). This value represented the number of times a difference greater than the actual statistic would occur.

Results

1. Experiment 1

1.1 *Dashing*

When groups of minnows displayed a response, there were many more dashes observed than during the no-response trials (Figure 5). A no-response trial was defined as a trial in which less than 15 total dashes were observed (Figure 6). A strong or obvious response was a trial in which 15 or more total dashes occurred in response to the addition of a stimulus (Figure 6). The proportion of dashes observed in each one minute post-exposure time period were similar regardless of the level of response (Figure 7). 87.46 % and 85.28 % of the dashes observed during strong and no-response trials occurred within the first two minutes, respectively (Table 3). For all of the treatments combined, fish reacted immediately to the introduction of a stimulus, 67.4 % of the dashes were observed in the first minute of the post-exposure period (Figure 7).

The dashes observed in both the strong and the no-response trials were similar in appearance. A dashing minnow would typically swim in a rapid and exaggerated manner, usually along the edge of the apparatus. When the minnows dashed, they displayed the typical S-start fast response which is controlled by the Mauthner cells. During a strong reaction trial, minnows would usually stop a dash and then perform the same behavior in a different direction. In a no-response trial, dashes did not appear as long in distance and were not repeated as often as the strong response trials.

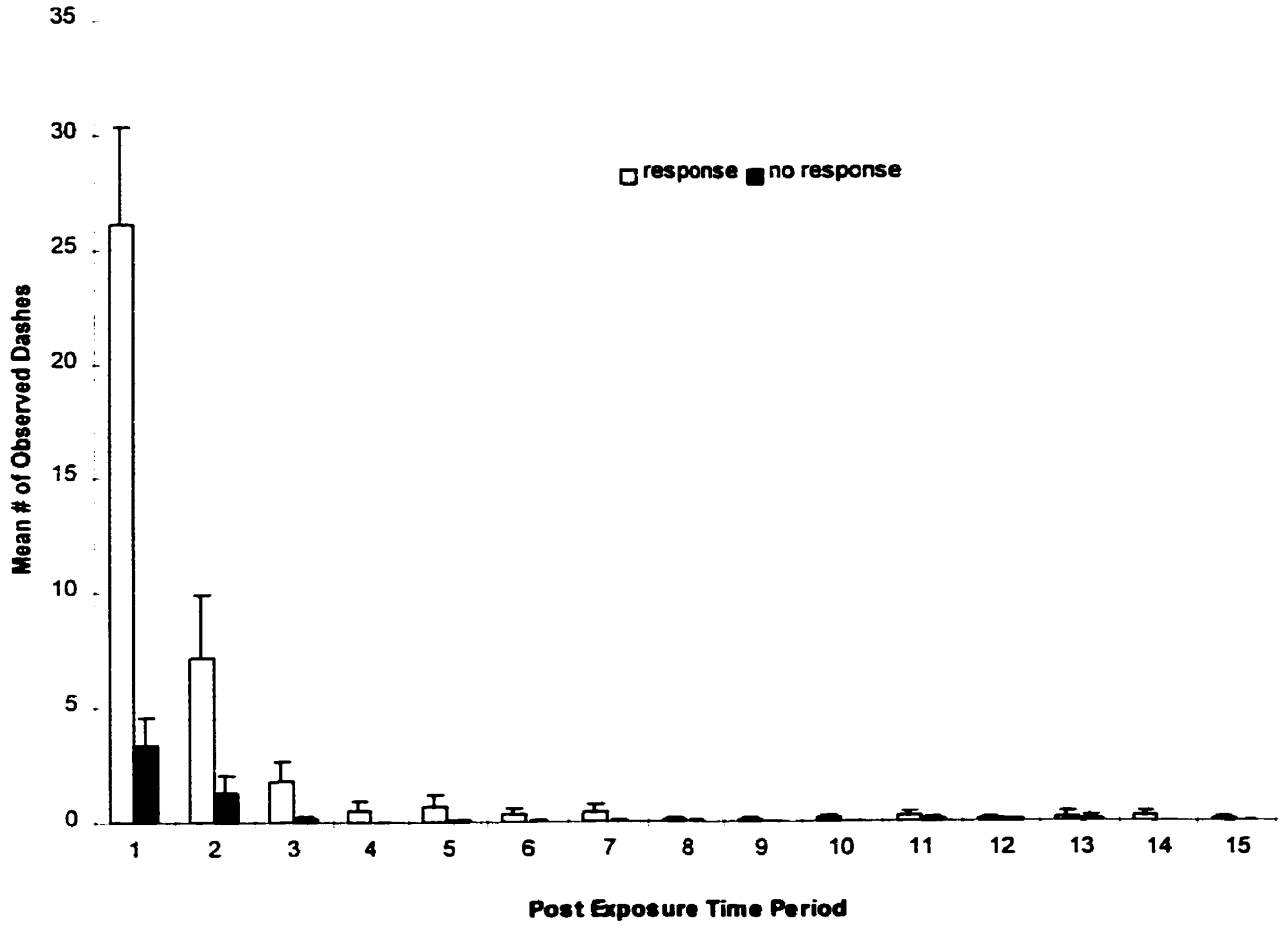


Figure 5. The mean number of dashes (± 1 s.e.) performed by the five groups of minnows during the 15 minute post-exposure period of the 9 response and the 7 no-response treatments of Experiment 1.

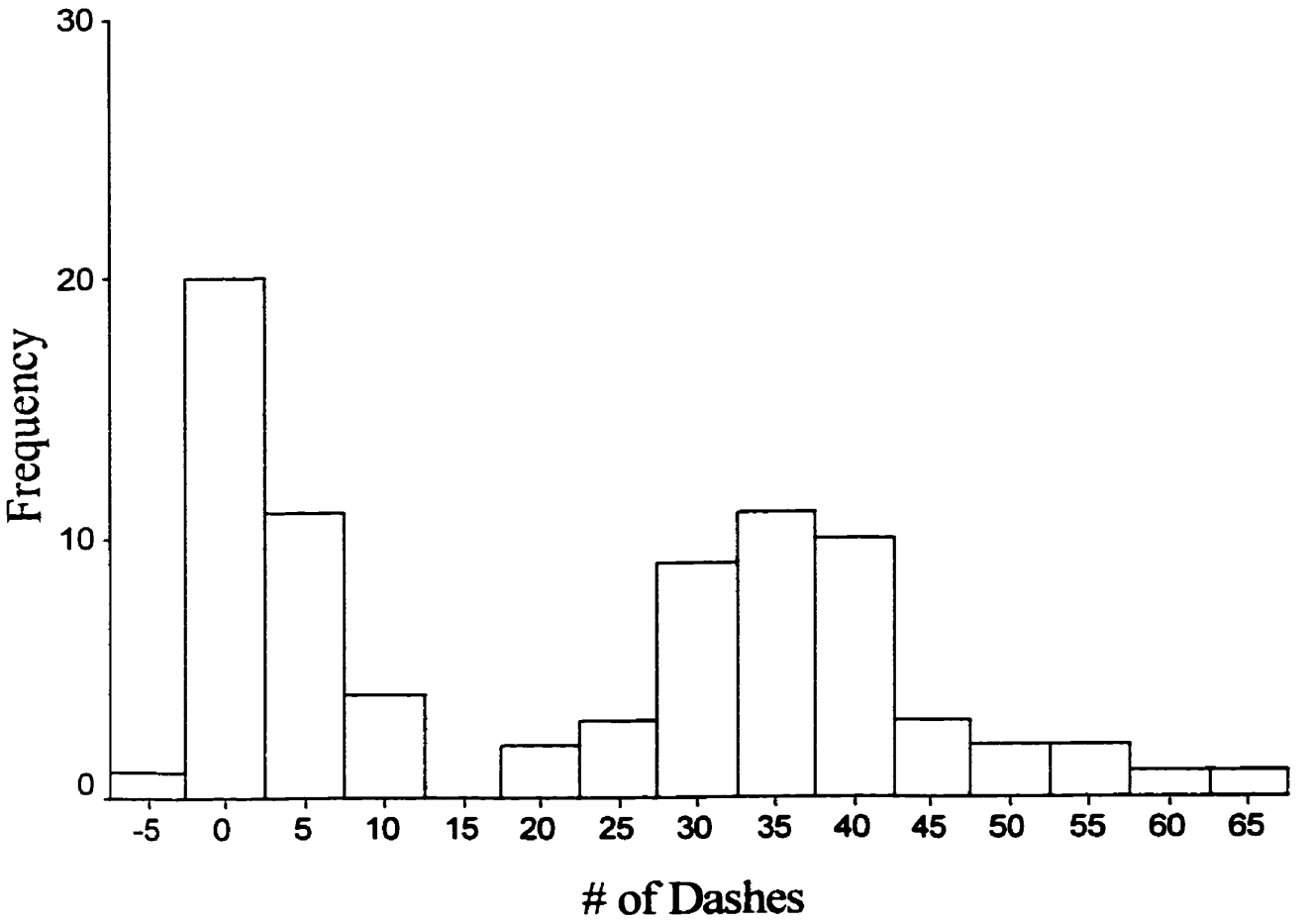


Figure 6. The distribution of the number of dashes [(post-exposure) - (pre-exposure)] observed for all groups of minnows in all 16 treatments in Experiment 1. Note the bimodal distribution with the drop after 10 dashes.

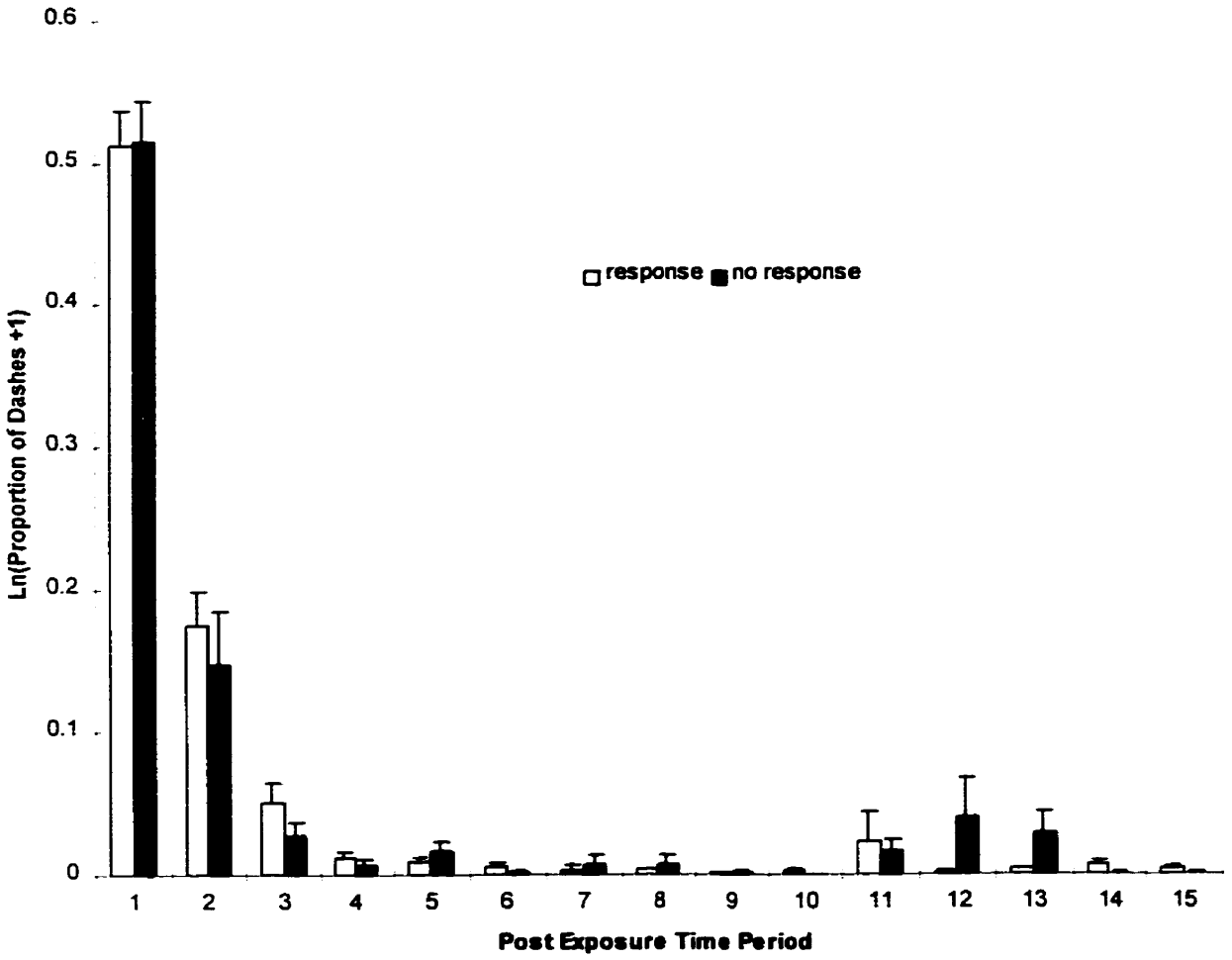


Figure 7. The mean proportion of dashes (± 1 s.e.) observed in each one minute time period of the total 15 minute post-exposure period during the 9 response and the 7 no-response treatments of Experiment 1.

Table 3. The number of dashes observed within the first two minutes of a: A) response trial (> 10 dashes) and B) no-response trial (≤ 10 dashes) in Experiment 1 for each group.

A)

Group	# of trials observed	mean # of total dashes	mean dashes w/in 2 min	% of dashes w/in 2 min
1	10	33.6	26.6	79.17
2	10	35.3	31.6	89.52
3	9	34.0	30.9	90.85
4	10	44.0	40.3	91.59
5	10	33.3	28.7	86.17

B)

Group	# of trials observed	mean # of dashes	mean dashes w/in 2 min	% of dashes w/in 2 min
1	6	3.5	3.2	90.48
2	6	4.8	3.5	72.41
3	7	4.1	4.0	96.55
4	6	4.2	3.3	80.00
5	6	3.8	3.3	86.96

Approximate randomization was used to test the null hypothesis that the variables were unrelated (i.e., a stimulus treatment and the number of dashes). The analysis demonstrated a strong correlation between the number of dashes and the treatments involving the chemical cue(test statistic = 38, $p < 0.01$, 100 replications), the visual cue (test statistic = 38, $p < 0.01$, 100 replications), and the hunger level (test statistic = 22, $p < 0.01$, 1000 replications). There was no significant difference in the number of dashes observed in clear and turbid water (test statistic = -2, $p = 0.68$, 2000 replications). There were significant differences between treatments involving water clarity and chemical cue (test statistic = 25.5, $p < 0.01$, 1000 replications) and there were also significant differences between treatments with water clarity and visual cue (test statistic = 35.5, $p < 0.01$, 100 replications).

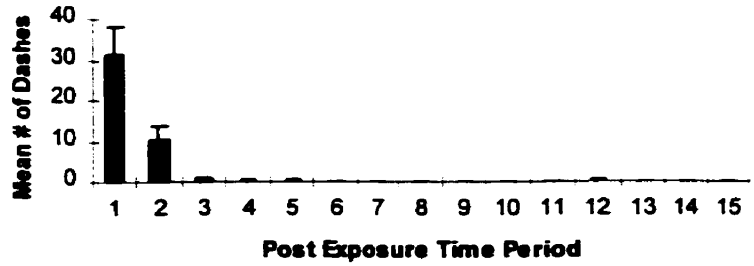
The unique predictions of the sensory compensation model were that minnows would not respond to alarm substance when risk was decreased in low turbidity but would in high turbidity. These treatments are shaded in Table 4 and illustrated in Figure 8. When presented with only alarm substance in clear water, satiated fish displayed obvious responses while the low risk, hungry fish demonstrated significantly fewer dashes ($t_4 = 5.815$, $p = 0.002$, Figure 8). When the water became turbid, hungry fish showed a strong reaction to the addition of alarm substance compared to the addition of the control chemical stimulus ($t_4 = 19.647$, $p < 0.001$). These low risk fish responded to alarm substance with a number of dashes that was not significantly different from the number observed when satiated minnows responded to alarm substance ($t_4 = 1.672$, $p = 0.170$). Increased turbidity caused hungry fish to react to alarm substance with significantly more dashes than they did in clear water ($t_4 = 17.182$, $p < 0.001$, Figure 8).

The behavior of satiated fish in response to alarm substance was not significantly affected by the turbidity of the water ($t_4 = 0.716$, $p = 0.257$). They showed a significant response in both

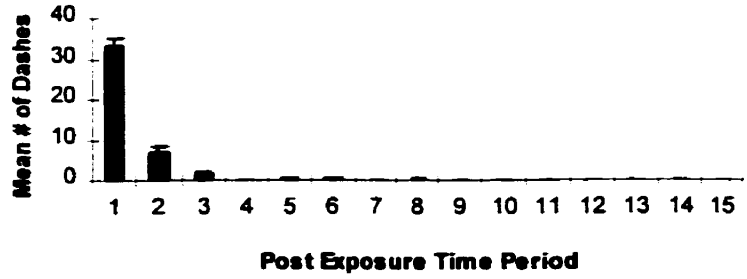
Table 4. The mean number of dashes (± 1 s.e., (post-exposure) - (pre-exposure)) observed for the five groups of minnows in response to the introduction of alarm stimuli in Experiment 1. The results in the shaded cells are consistent with the sensory compensation hypothesis. C+ = alarm substance; C- = muscle control; V+ = predator present; V- = predator absent.

			Turbidity			
			Low		High	
			C+	C-	C+	C-
Hunger	High	V+	44.8 +/- 2.75	35.6 +/- 1.63	30.0 +/- 9.66	4.2 +/- 2.13
		V-	2.0 +/- 0.45	4.4 +/- 2.16	39.8 +/- 2.08	1.2 +/- 0.8
	Low	V+	41.2 +/- 3.93	30.2 +/- 1.02	33.0 +/- 3.35	0.2 +/- 1.28
		V-	40.0 +/- 6.80	4.6 +/- 2.20	35.0 +/- 2.77	1.2 +/- 1.02

A)



B)



C)

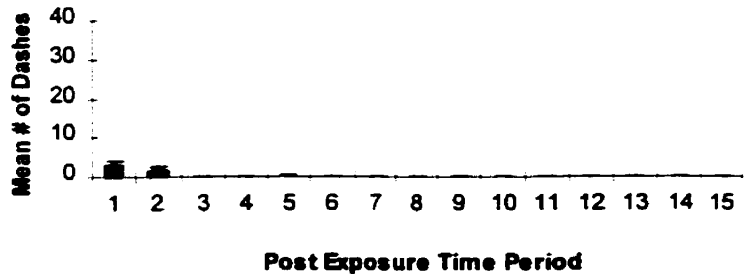


Figure 8. The mean number of dashes (± 1 s.e.) performed by the five groups of minnows in the treatments of Experiment 1 important for support of the sensory compensation model: A) Clear water, no predator, satiated, and alarm substance; B) Turbid water, no predator, hungry, and alarm substance; C) Clear water, no predator, hungry, and alarm substance.

clear and turbid water. In clear water, minnows always displayed strong responses to the presence of the visual predator stimulus. When the water was turbid, minnows displayed significantly fewer dashes in response to the visual predator stimulus than were observed in clear water ($t_4 = 19.117, p < 0.001$). When this visual stimulus was combined with the presence of alarm substance, all groups of minnows again displayed strong reactions to the stimuli (Table 4).

The pattern of observed dashes did not vary significantly between groups over the first two minutes of the post-exposure period (Figure 9, Figure 10). A One-way ANOVA demonstrated that the proportion of observed dashes in time period 1 ($F_{4,75} = 0.697$, $p = 0.569$) and 2 ($F_{4,75} = 0.293$, $p = 0.882$) did not vary significantly between the five groups. The same test indicated no significant differences in the number of observed dashes between each group for periods 1 and 2 ($F_{4,75} = 0.467$, $p = 0.759$; $F_{4,75} = 0.1.535$, $p = 0.201$, respectively). These results indicated that the groups responded in a similar manner in response to the introduction of stimuli. The majority of the dashes that occurred after the first two minutes appeared to be in response to background noise as was observed during the pre-exposure period.

1.2 *Freezing*

Freezing was defined as a thirty second period of inactivity. It was not observed very frequently during these experiments. Trials which were defined as a response trial using dashes did not demonstrate difference in the distribution of freezes when compared to a no-response trial, as defined with dashes (Figure 11). When freezing was observed, it was usually after the initial response of dashes (Figure 12). Only 26.8 % of the freezes performed were observed in the first two minutes with the majority (92.98 %) observed after the first one minute period.

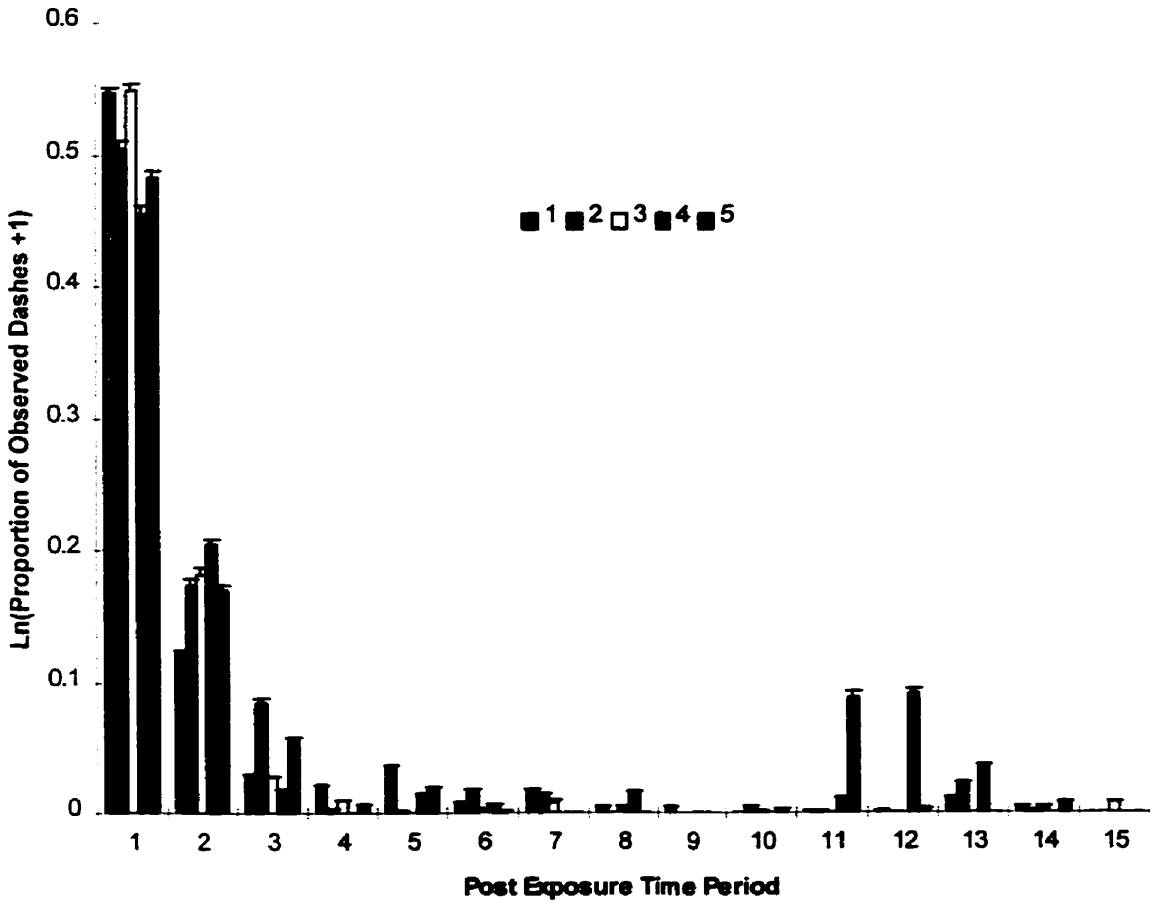


Figure 9. Inter-group variation of the mean proportion of dashes (± 1 s.e.) observed during each one minute time period of the total 15 minute post-exposure period of all of the 16 treatments of Experiment 1.

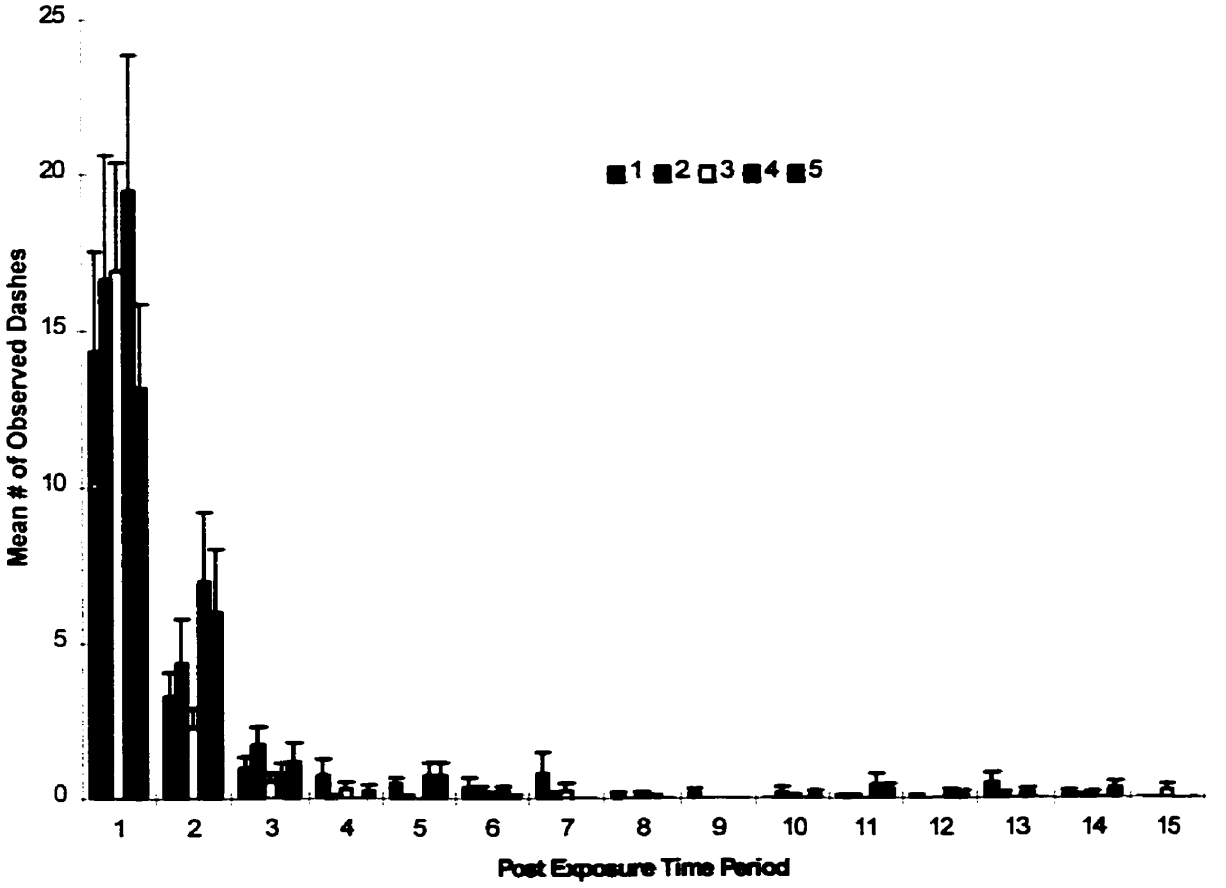


Figure 10. Inter-group variation of the mean number of observed dashes (± 1 s.e.) during the 15 minute post-exposure period of all of the 16 treatments of Experiment 1.

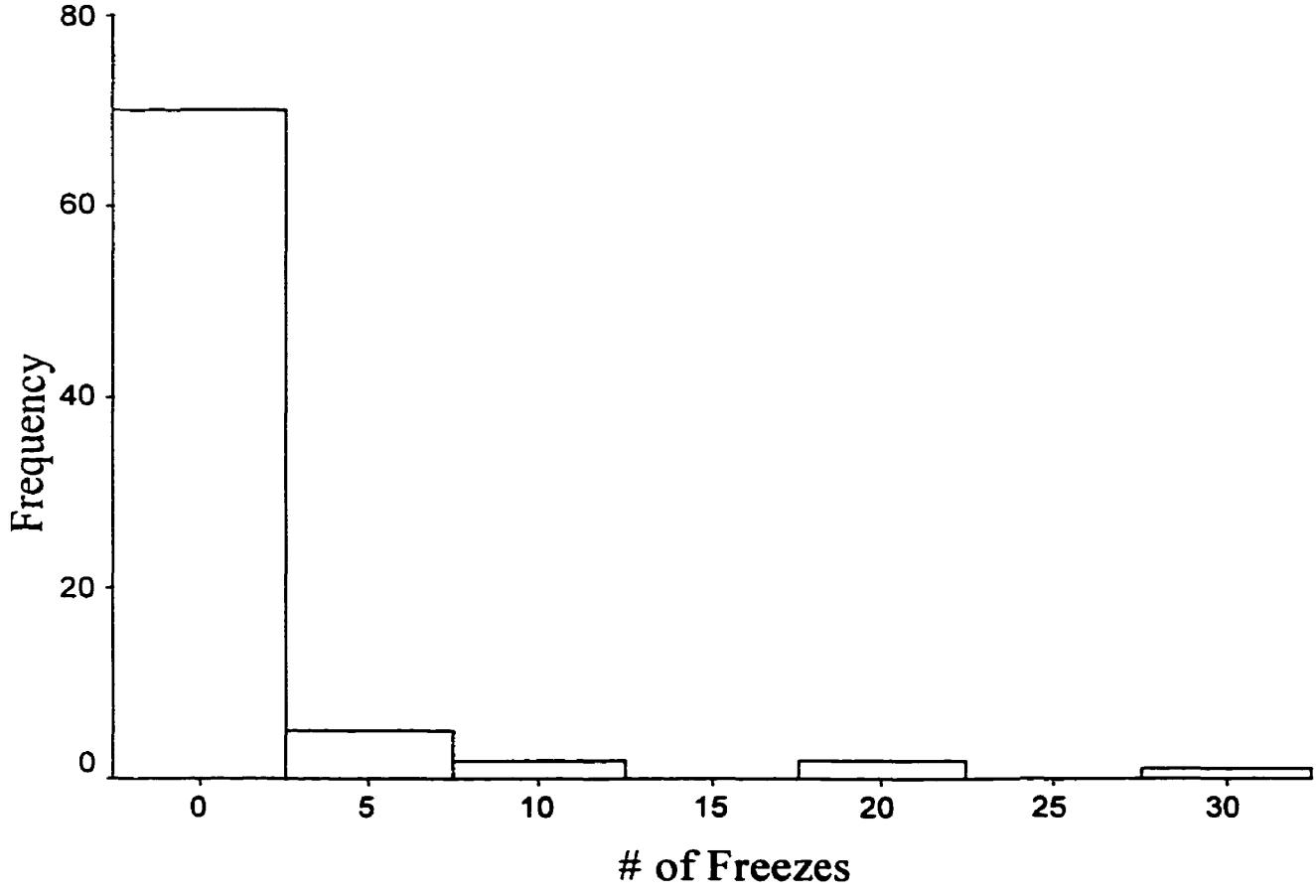


Figure 11. The distribution of the number of freezes [(post-exposure) - (pre-exposure)] observed for all groups of minnows in all 16 treatments in Experiment 1.

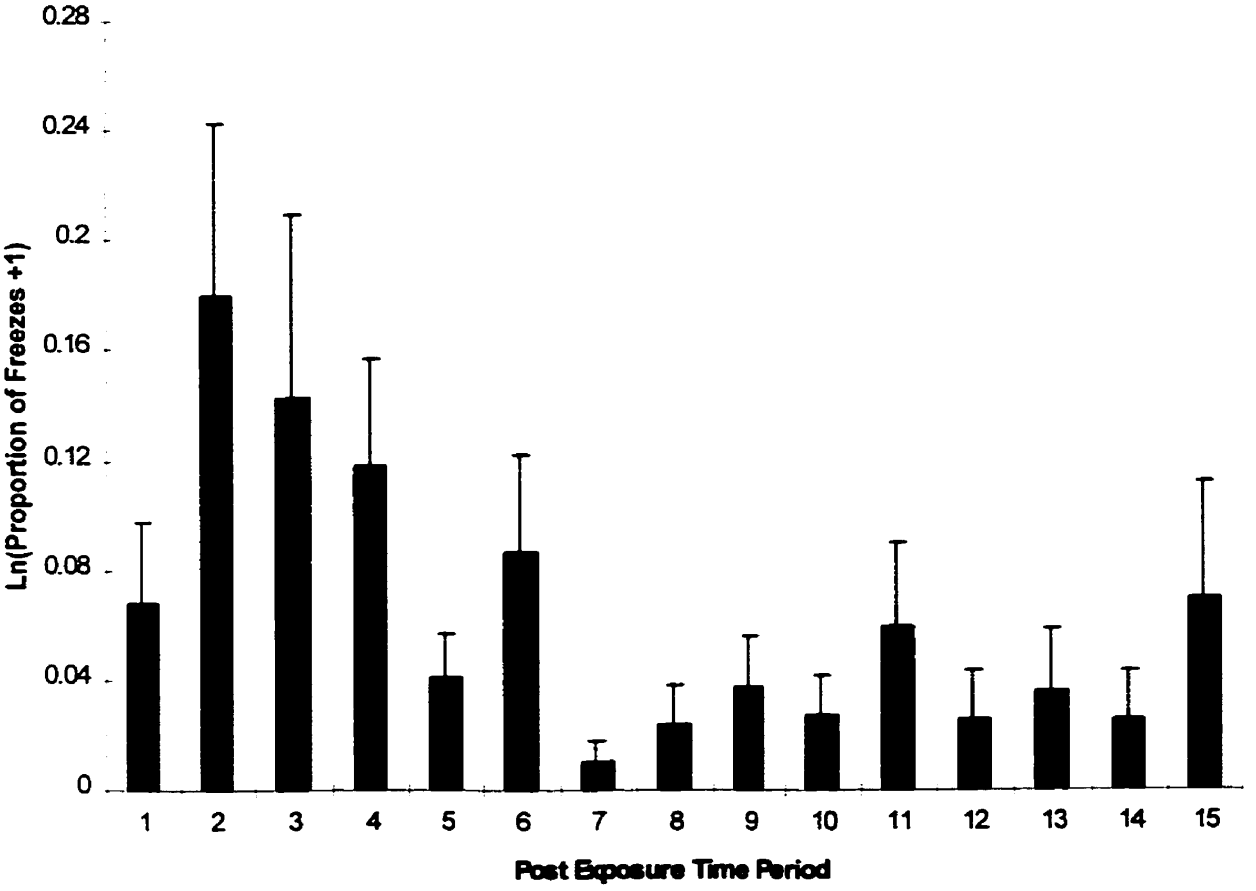


Figure 12. The mean proportion of freezes (± 1 s.e.) observed for the five groups of minnows following the addition of the experimental stimuli in all of the 16 treatments of Experiment 1.

Table 5. The mean number of freezes (± 1 s.e., (post-exposure) - (pre-exposure)) observed for the five groups of minnows in response to the introduction of alarm stimuli in Experiment 1. C+ = alarm substance; C- = muscle control; V+ = predator present; V- = predator absent.

			Turbidity			
			Low		High	
			C+	C-	C+	C-
Hunger	High	V+	2.4 +/- 1.17	2.6 +/- 2.11	0.4 +/- 0.24	0 +/- 0
		V-	0 +/- 0	0 +/- 0	0.4 +/- 0.4	0.2 +/- 0.2
	Low	V+	13.8 +/- 5.25	4.0 +/- 3.51	1.0 +/- 1.26	0 +/- 0
		V-	0 +/- 0	0 +/- 0	0.8 +/- 0.8	-0.2 +/- 0.2

Freezing was more common in clear water and occurred more regularly in the presence of a predator (Table 5). Approximate randomization revealed that turbidity (test statistic = 2.525, $p = 0.009$, 5000 replications) and the presence of a predator (test statistic = 2.875, $p < 0.001$, 2000 replications) strongly influenced the number of freezes performed in response to the alarm stimuli. Hunger and the presence of the chemical cue were not found to have significant effects on the number of freezes. Minnows were more likely to freeze in response to a predator in clear water and this effect was lost when the water became turbid. The groups of minnows did not freeze in response to the presence of alarm substance (Table 5).

The number of freezes performed by each group was variable but was not found to be significantly different between groups with a one way ANOVA ($F_{4,75} = 1.115$, $p = 0.356$). Group 1 appeared to freeze more often than the other groups but the difference was not significant as indicated by the ANOVA, probably as a result of the large standard error (Figure 13). The other groups appeared to be more similar in the number of freezes performed during each time period (Figure 13).

2. **Experiment 2**

2.1 *Behavior*

During the acclimation periods, the behavior of the minnows did not appear to differ much between the cover and no-cover trials. A paired t-test ($t39 = 0.955$, $p = 0.345$) did not detect any significant differences in the distance traveled during one minute observations by fish in cover (676.72 ± 53.55 s.e.) compared to the distance traveled by fish without the presence of cover (726.47 cm ± 45.92 s.e.). The number of times minnows switched directions was not

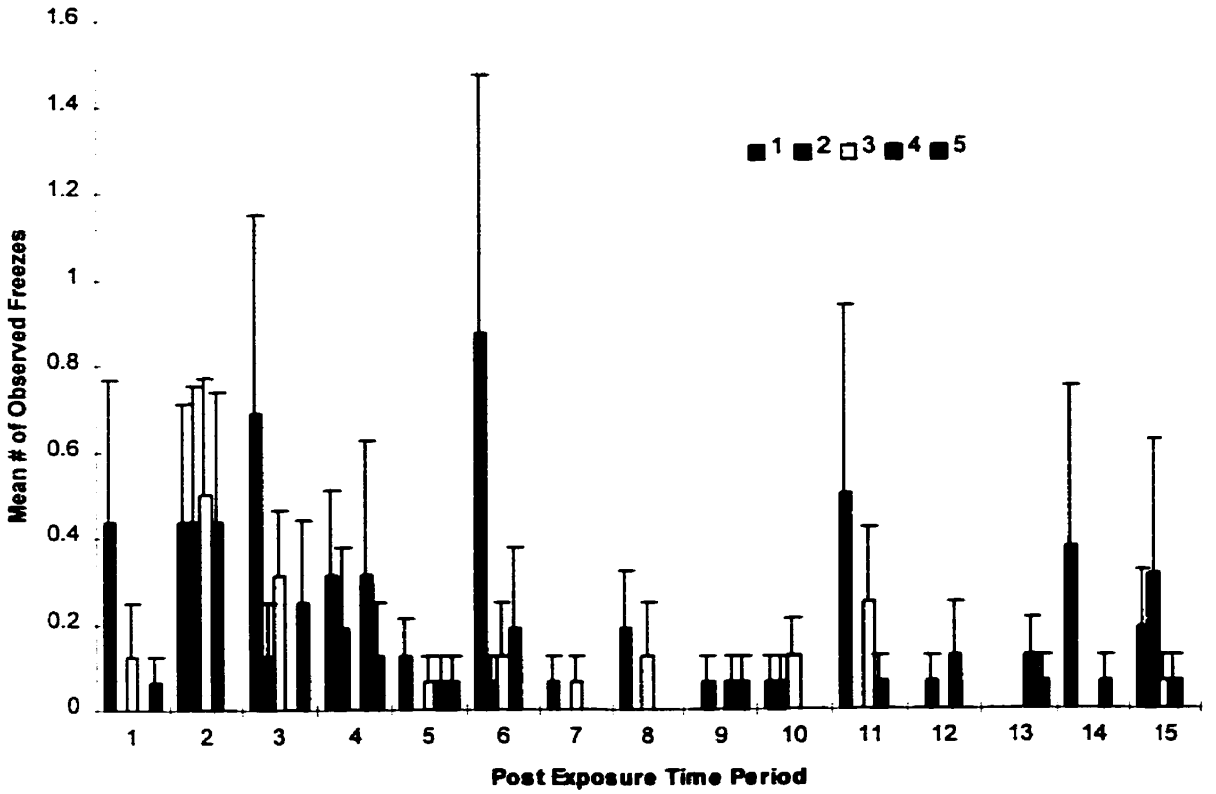


Figure 13. Variation of the mean number of freezes (± 1 s.e.) observed for each group during the 15 minute post-exposure period in all of the 16 treatments.

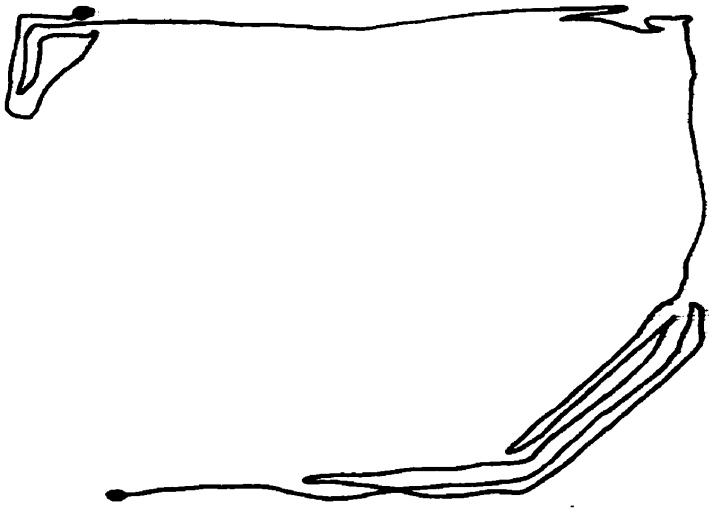
significantly affected by the presence of cover test ($t39 = 1.667$, $p = 0.104$). Without cover, observed minnows changed direction 7.03 times (± 0.85 s.e.) while minnows in the presence of cover switched direction 5.78 times (± 0.56 s.e.). I observed minnows swimming along the edge in both cover and no-cover treatments.

The behavior of the minnows was significantly affected by changing the turbidity of the water. Figure 14 demonstrates typical swimming patterns observed by minnows in clear and in turbid water. In turbid water the fish swam over a larger area than when in clear water. Fish in turbid water moved significantly further and faster (936.46 cm ● 39.01 s.e.) during the one minute observations than fish in clear water (466.73 cm ± 25.63 s.e., $t39 = 9.695$, $p < 0.001$). Fish in clear water swam in irregular patterns, regularly changing direction ($< 90^{o}$ angle) while minnows in the turbid water trials swam in circular patterns with few changes in direction. The fish in clear water changed direction significantly more than did fish in turbid water ($t39 = 9.238$, $p < 0.01$).

2.2 *Dashing*

The overall reaction to AS was reduced when compared with the reactions from the first experiment (Figure 15). The classification of a response and no-response trial was the same as for the first experiment but the cutoff point was different. More than ten dashes were considered a response while ten or less dashes was a no-response trial (Figure 16). The fish reacted rapidly to the addition of a stimulus, with 87.33 % of the total number of dashes occurred within the first two minutes of a response trial and 78.03 % of the dashes observed in a no-response trial occurring in the first two minutes (Table 6). 57.3 % of the dashes occurred in the first minute of the post-exposure period for all trials.

A)



B)

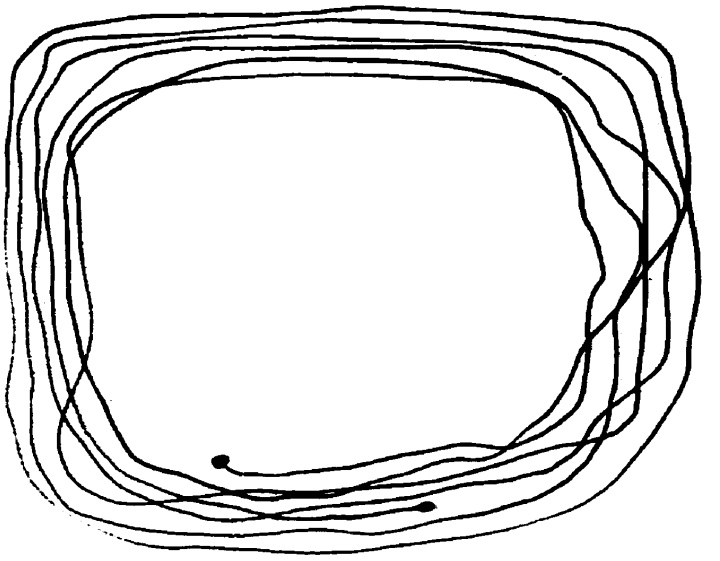


Figure 14. An example of the characteristic swimming patterns observed in A) clear water and in B) turbid water during one minute observations of a single minnow.

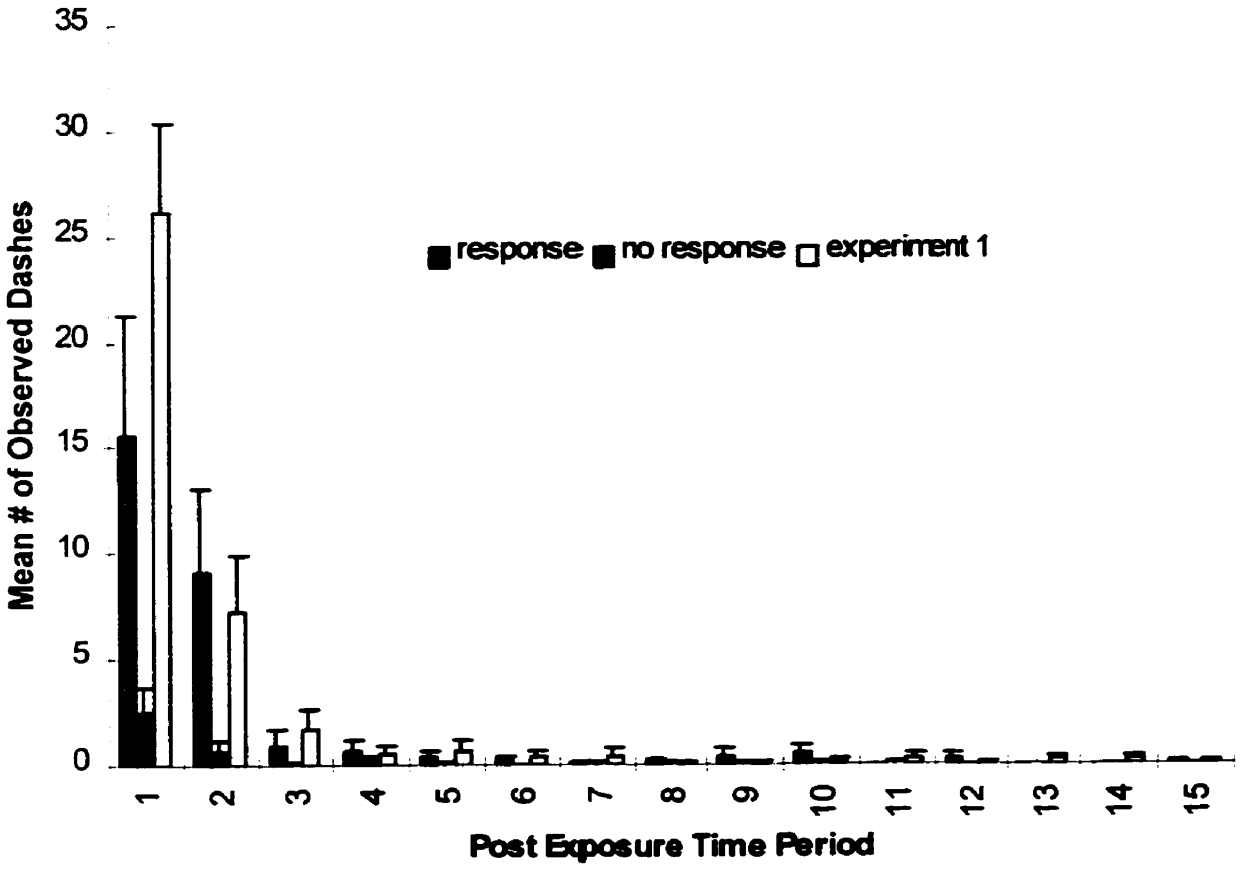


Figure 15. Comparison of the mean number of dashes (± 1 s.e.) observed in the 3 response and the 5 no-response trials (no-response was defined as 10 or less dashes) of Experiment 2. The mean number of dashes (± 1 s.e.) observed during a response trial is also compared to the mean number of dashes (± 1 s.e.) observed in 9 response trials of Experiment 1.

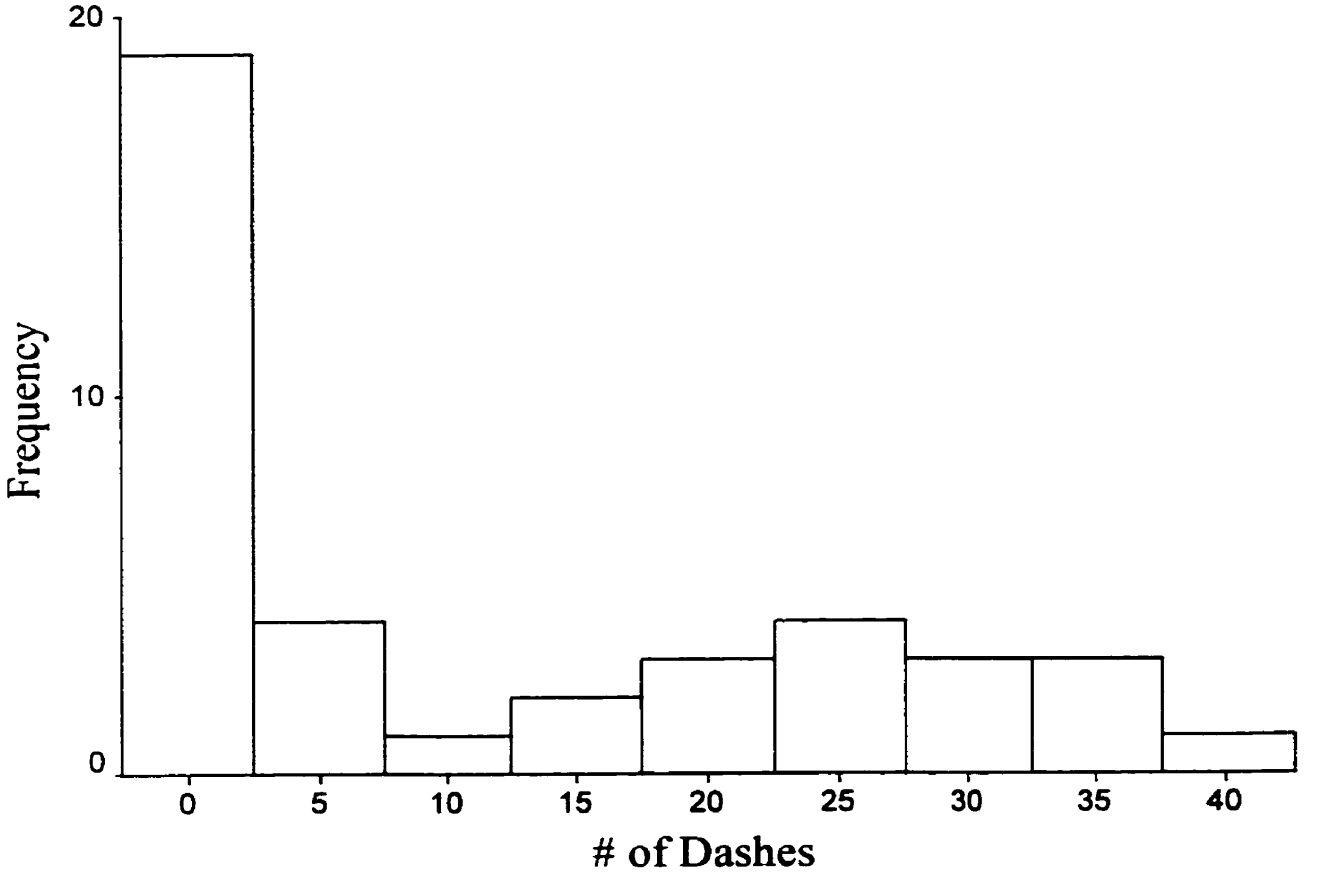


Figure 16. The distribution of the number of dashes [(post-exposure) - (pre-exposure)] observed for all groups of minnows in all 8 treatments in Experiment 2. Note the bimodal distribution with the drop at 10 dashes.

Table 6. The number of dashes observed within the first two minutes of a: A) response trial (> 10 dashes) and B) no-response trial (≤ 10 dashes) in Experiment 2 for each group.

A)

Group	# of trials observed	mean # of total dashes	mean dashes w/in 2 min	% of dashes w/in 2 min
1	3	23.67	18	76.06
2	4	20.75	19.5	93.98
3	4	27.5	27	98.18
4	3	32.3	29	89.69
5	4	23.5	18.5	78.72

B)

Group	# of trials observed	mean # of total dashes	mean dashes w/in 2 min	% of dashes w/in 2 min
1	5	2.6	2.4	92.31
2	4	3.5	1.5	42.86
3	4	1.25	1.0	80.00
4	5	3.4	3.4	100.00
5	4	2	1.5	75.00

The chemical cue had a strong effect on the number of dashes (approximate randomization test statistic = 74, $p < 0.01$, 100 replications). Neither the presence of cover or increasing the turbidity was found to be related to the number of observed dashes but when the treatments were broken down further, water clarity and chemical cue (test statistic = 62.5, $p < 0.01$, 100 replications), and variation in the amount of cover and chemical cue (test statistic = 68.5, $p < 0.01$, 100 replications) were found to be strongly related to the number of dashes observed for each group of minnows.

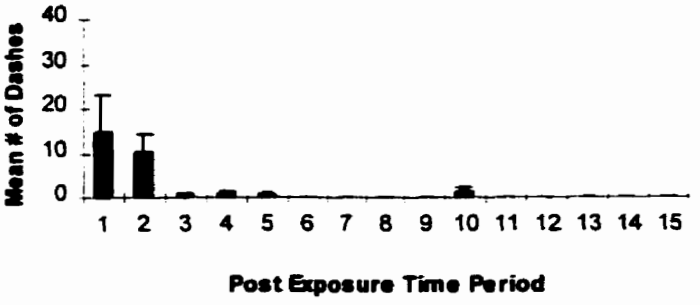
When the water was turbid, minnows always displayed strong reactions in response to the addition of alarm substance regardless of the cover level while the response in clear water varied depending on the cover (Table 7). The addition of cover caused a significant reduction in the number of dashes observed in response to the addition of alarm substance in clear water ($t_4 = 4.258$, $p = 0.006$; Figure 17). Minnows in clear water with cover showed no significant difference between the number of dashes in response to alarm substance or to the muscle control ($t_4 = 1.928$, $p = 0.126$; Table 7). Fish that did not react to alarm substance in cover when the water was clear demonstrated a significant increase in the number of dashes when the water became turbid ($t_4 = 5.202$, $p = 0.004$; Figure 17).

The reaction to the alarm stimulus did not differ significantly between the five groups. Each group displayed a similar proportion of dashes and a similar number of dashes (Figure 18, Figure 19). One way ANOVAs did not demonstrate any significant differences in the proportion (Period 1: $F_{4,75} = 0.614$, $p = 0.655$; Period 2: $F_{4,75} = 1.788$, $p = 0.153$) or number of dashes (Period 1: $F_{4,75} = 0.560$, $p = 0.693$; Period 2: $F_{4,75} = 0.590$, $p = 0.672$) performed by each group in the first two one minute periods of the post-exposure period.

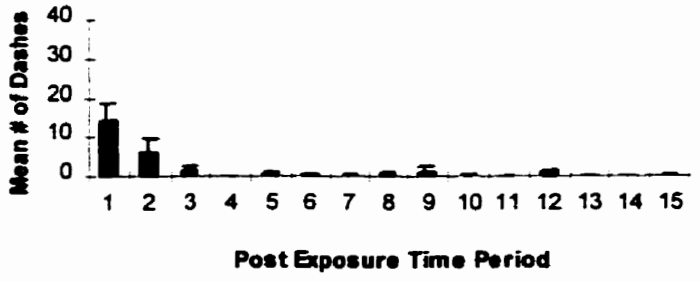
Table 7. The mean number of dashes (± 1 s.e., (post-exposure) - (pre-exposure)) observed for the five groups of minnows in response to the introduction of alarm stimuli in Experiment 2. The treatments which provide support for the sensory compensation hypothesis are shaded. C+ = alarm substance; C- = muscle control.

		Turbidity			
		Low		High	
		C+	C-	C+	C-
Cover	High	6.40 +/- 2.56	0.20 +/- 0.86	24.6 +/- 2.44	0.8 +/- 0.97
	None	27.8 +/- 4.40	1.2 +/- 0.66	30.2 +/- 1.43	-0.6 +/- 0.40

A)



B)



C)

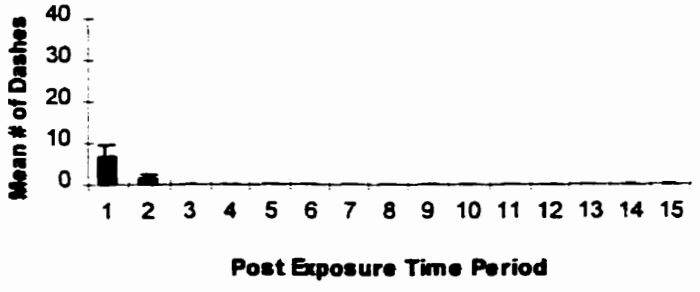


Figure 17. The mean number of dashes (± 1 s.e.) performed by the five groups of minnows in the treatments important for support of the sensory compensation model: A) Clear water, no-cover, and alarm substance; B) Turbid water, cover, and alarm substance; C) Clear water, cover, and alarm substance.

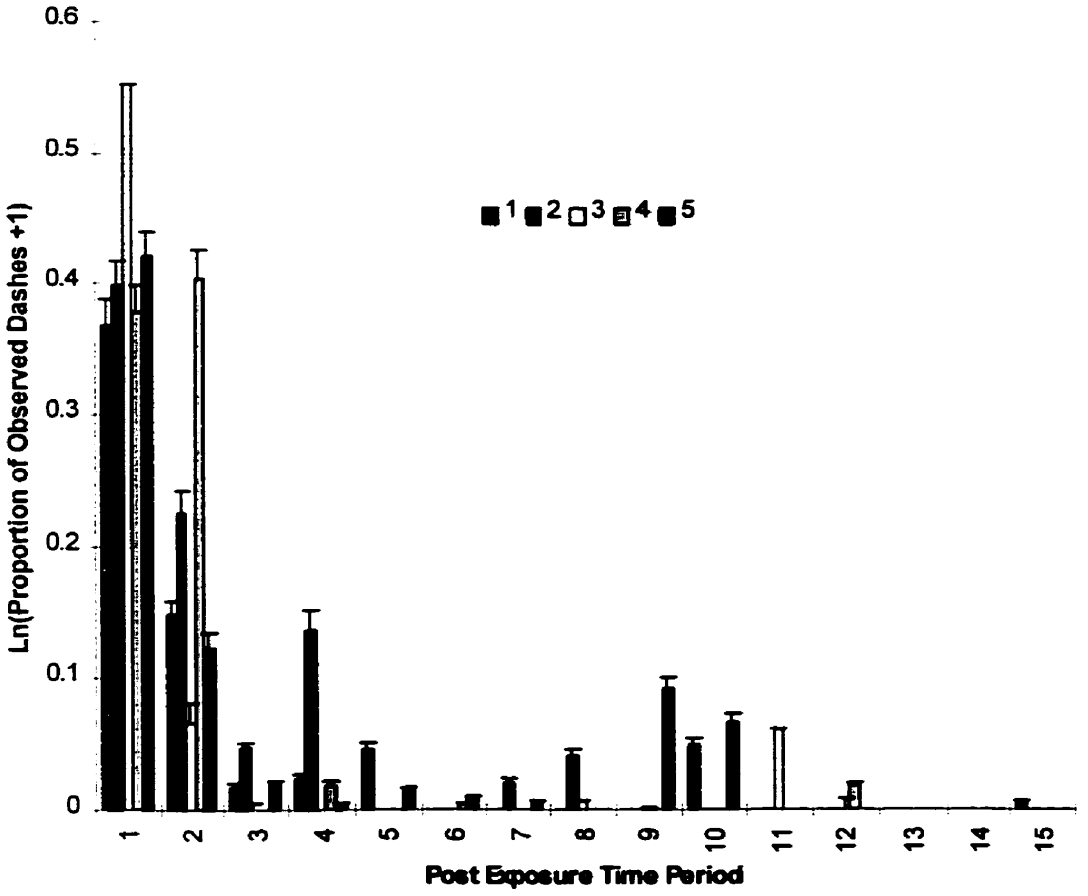


Figure 18. Inter-group variation of the mean proportion of dashes (± 1 s.e.) observed during each one minute time period of the total 15 minute post-exposure period of all of the 8 treatments of Experiment 2.

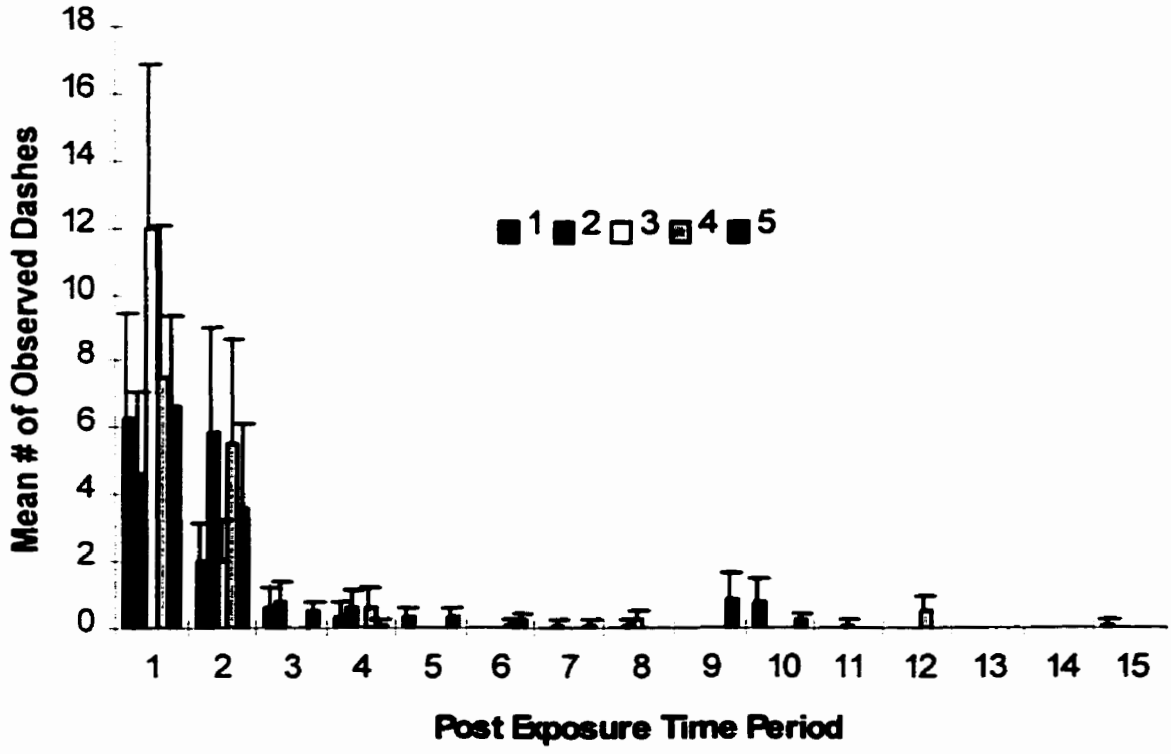


Figure 19. Inter-group variation of the mean number of observed dashes (± 1 s.e.) during the 15 minute post-exposure period of all of the 8 treatments of Experiment 2.

Table 8. The mean number of freezes (± 1 s.e., (post-exposure) - (pre-exposure)) observed for the five groups of minnows in response to the introduction of alarm stimuli in Experiment 2. C+ = alarm substance; C- = muscle control.

		Turbidity			
		Low		High	
		C+	C-	C+	C-
Cover	High	0 +/- 0	0.6 +/- 0.6	0.2 +/- 0.2	0 +/- 0
	None	0 +/- 0	0 +/- 0	0 +/- 0	0.2 +/- 0.2

2.3 *Freezing*

In Experiment 1, freezing was rarely observed in the absence of a predator and this trend was also observed in these experiments (Table 8). In all cases, there were very few observed freezes (Figure 20), and in several treatments, no freezes were observed in any of the groups. There weren't any significant relationships between freezing and any of the treatments, cover (test statistic = 0.15, $p = 0.74$, 2000 replications), clarity (test statistic = 0.05, $p = 1.0$, 100 replications), or chemical cue (test statistic = 0.15, $p = 0.74$, 2000 replications).

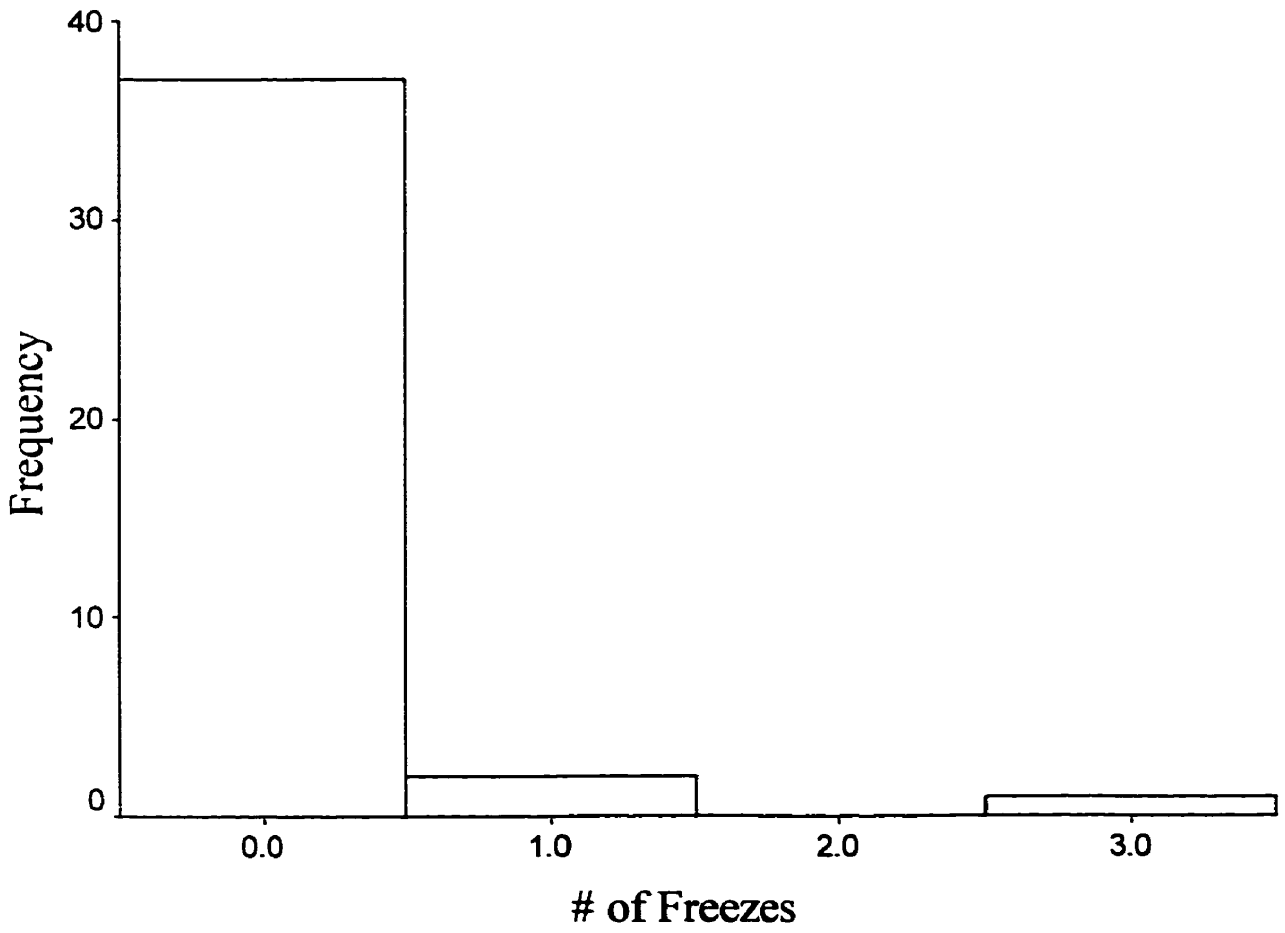


Figure 20. The distribution of the number of freezes [(post-exposure) - (pre-exposure)] observed for all groups of minnows in all 8 treatments in Experiment 2.

Discussion

The sensory compensation model predicted that prey should preferentially use the source of information which most reliably indicates the presence of a predator. This would allow prey to respond to a predator at a greater flight distance. In clear water, visual cues were predicted to be the most important. The model also predicted, as did Magurran *et al.* (1996), that the level of risk will affect the response to alarm substance. The unique predictions of the model involved the effects of changes in visibility as a result of variations in turbidity. Increased turbidity reduced visual input resulting in lower detection distances (see Figure 1). Fish in low risk treatments that did not respond to alarm substance in clear water were expected to respond when the turbidity of the water was increased. This was predicted because fish should respond to decreased visual information by increasing their sensitivity to alternate cues.

The results of my experiments are consistent with this model. In clear water, decreasing the level of risk or increasing risk acceptance resulted in a significant reduction in the number of dashes performed in response to the introduction of alarm substance. Similar results have been observed by Brown and Smith (1996), Magurran *et al.* (1996) and Irving and Magurran (1997). The unique prediction of the sensory compensation model was that fish which did not respond to alarm substance in clear water would demonstrate an increase in sensitivity and respond to alarm substance when the water became more turbid. My results demonstrated that the fish that did not respond in clear water showed a strong response to the alarm substance when the water was turbid. The data indicate that when the water becomes turbid and visual input is reduced, prey increase their sensitivity to alarm substance.

Data consistent with the sensory compensation model have been observed elsewhere. Diving beetles (*Acilius sulcatus*) in a fluvarium did not respond to the scent of a hungry perch

predator in the absence of a visual stimulus during the daytime but with reduced light, the beetles reacted strongly to the predator scent (Åbjörnsson *et al.* 1997). Åbjörnsson *et al.* (1997) do not offer a specific hypothesis for their results but these data are consistent with the sensory compensation hypothesis. The model predicted that when faced with reduced visual input, animals should be more sensitive to alternate inputs to obtain the best information possible. Diving beetles have well developed eyes which suggests they may rely mainly on visual information (Åbjörnsson *et al.* 1997). When visual information is reduced, the beetles demonstrate an increased sensitivity to chemical cues.

Bruski and Dunham (1987) demonstrated that various crayfish of the genus *Orconectes* increased their use of mechanoreceptive organs when visual input was reduced at night. These crayfish are preyed upon by diurnal perch and nocturnal eels (*Anguilla anguilla*). During the day, visual stimuli should be important for detecting the perch but when the light levels are reduced, increased use of mechanoreception would allow the crayfish to better detect predatory eels (Blake and Hart 1993). These data are also consistent with the sensory compensation model in that they demonstrate an increased use of alternate cues (mechanosensory) to detect predators when visual input becomes reduced and visual information is less reliable.

The level of risk strongly influenced the response to cues indicating the presence of a predator. Hungry minnows, which were more willing to accept risk, did not respond to the alarm substance when the predator was absent but they did react when the predator was added. The presence of the visual predator stimulus combined with the proximity of the predator (maximum distance = 80 cm) represented a greater risk of predation. Similarly, mayfly larvae (*Paraleptophlebia adoptiva*) only responded to chemical cues when a predator was visible (Ode and Wissinger 1993). These results demonstrate that the sensitivity to chemical cues in clear

water is mediated by the level of risk. In clear water, chemical cues are not as reliable as visual information; therefore, the sensory compensation model predicts that animals should be less sensitive to the chemical cues. When the level of risk is increased, animals should be more sensitive to all inputs, including chemical. The mayflies may have been less willing to respond to the chemical cue because of a decreased level of risk. Adding a predator increased the risk and a response was observed.

The sensory compensation model provides a resolution to the controversy surrounding the use of alarm substance. Smith (1997) argued that the alarm substance is an alarm pheromone designed to convey information about the presence of a predator. Smith cites previous field observations and field experiments involving the use of traps as his evidence (Von Frisch 1938; Smith 1976; Mathis and Smith 1992a, b; Chivers *et al.* 1995). Many previous laboratory experiments have demonstrated that fish respond to alarm substance without a predator present (Verheijen 1956; Pfeiffer 1962, 1963a; Brown and Smith 1996; Irving and Magurran 1997). As proposed by Magurran *et al.* (1996), my experiments demonstrate it is due to the relative risk of predation. Fish in a confined environment have reduced opportunities of escape. This represents a higher risk situation and therefore fish are more willing to respond to the presence of alarm substance. When I manipulated the risk or risk acceptance associated with a trial by increasing hunger or cover, minnows were less willing to respond to the presence of alarm substance. Therefore increasing the level of risk strongly influences the response to alarm substance. This level of risk may have been a significant factor influencing the responses observed in the field experiments cited by Smith (von Frisch 1938; Smith 1976). In both of these examples, direct observations were made by human observers during the experiments. Minnows in the experiments conducted by von Frisch (1938) responded to the release of alarm substance from an

injured shoal member. I suggest this reaction was observed due to the presence of alarm substance and the presence of the observer which could represent a large predatory stimulus. The presence of the observer would increase the risk of predation associated with the trial and would result in the minnows' being more sensitive to the release of alarm substance. The experiments by Smith (1976) demonstrated similar reactions which again would be due to the presence of the observer combined with the alarm substance.

The trap experiments cited by Smith do not discuss the immediate response of the minnows (Mathis and Smith 1992a, b; Chivers *et al.* 1995). There were significantly more minnows caught in the control traps than in the traps marked with alarm substance. This could have been a result of the confined nature of the traps. A trap may represent a safe area for the minnows to hide but they were not willing to enter a confined area that was scented with alarm substance. This situation would represent a greater risk of predation which would result in fewer trapped minnows.

My model and experiments take the hypothesis proposed by Magurran *et al.* (1996) further by introducing the effect of information availability. Magurran *et al.* (1996) proposed that the response to alarm substance is mediated by the level of risk in which it is encountered. They suggest that alarm substance is a cue and the minnows should only respond when the level of risk is increased. They state that fish should not respond to alarm substance when they are in a natural setting because they sense a low level of risk. They suggest this is a result of familiarity with the habitat. Minnows in natural settings are aware of the potential escape routes and may even be familiar with local predators (Magurran *et al.* 1996). Magurran *et al.* (1996) propose that when the level of risk is increased, the minnows are predisposed to react to the cue. My model predicted and my results confirmed that fish do respond to alarm substance in low risk situations

but only when the availability of visual information becomes significantly reduced. I determined that sensitivity to alarm substance is not only affected by the level of risk but also by the availability of information to the senses. When the primary source of information becomes reduced, minnows are more willing to use alternate cues.

Dashing was the most obvious response to alarm stimuli in my experiments. Other experiments have demonstrated a wide variety of actions in response to encountering a danger stimulus (Levesley and Magurran 1988; Magurran 1990a; Smith 1992; Krause 1993a). When dashes were observed, they occurred directly after the addition of the alarm stimulus.

When freezing did occur, it followed the initial dashes. Freezing was observed in clear water when the predator was present. Initially, dashing would serve to confuse the predator by preventing it from focusing on one individual. Then a lack of motion due to freezing would make the individual more difficult to detect. In my preliminary experiments, hiding and freezing appeared to be the most important components. These experiments were conducted in a 40 liter aquarium which was filled with substrate and an area to hide. In the test apparatus, there was no substrate and there were no areas for the minnows to hide. The lack of a substrate could have limited the number of freezes. A freezing animal relies on a lack of movement to avoid detection (Fuiman and Magurran 1994) and without a substrate, the minnows should be less able to effectively blend with their surroundings. Although there was a lack of substrate, freezing in the presence of the predator in clear water would reduce the possibility of detection (Fuiman and Magurran 1994). Movement increases vulnerability to visually hunting predators (Wright and O'Brien 1984; Lawler 1989; Sih and Moore 1989) and this increased prey movement enables prey detection and initiates attack by many predators (Burghardt 1964; Wodinsky 1971).

Freezing was rarely observed in turbid water in response to the introduction of either alarm stimulus. A response to the predator was not anticipated because of the significant reduction in the visual range. If the animal is unable to detect the predator, a response should not occur, either in the form of dashes or freezes. The lack of freezing in response to the addition of alarm substance poses an interesting question. Werner and Anholt (1993) predict that as the movement of each individual increases, the encounter rate will also increase. In light of this, the absence of freezing in my experiments appears to be counterproductive. If an animal has information that a predator is in the vicinity, movement should decrease to reduce the possibility of an encounter with a predator. A possible explanation for the lack of observed freezes could be the size difference between predator and prey. This difference favors the prey in turbid water because for an individual to become a predator, it must be large enough to consume the smaller prey. As a result, a predator would present a larger image in turbid water when compared to the prey. This could result in the prey having a larger detection distance and would allow the prey to detect the predator first and employ the appropriate antipredator behavior (Ydenberg and Dill 1986). If prey do have the ability to detect the predator first, freezing may not be necessary.

Along with a decrease in freezing, there was a significant increase in movement by the fish when they were in turbid water. During the pre-exposure trials, each group of minnows moved further and faster than when they were in clear water. This result is consistent with other studies involving turbidity. Vandenbyllaardt *et al.* (1991) found that fish formed looser schools and increased swimming activity when in turbid water and Gregory and Northcote (1993) showed that juvenile chinook salmon increased their foraging rates at increased turbidity levels. This increased movement demonstrated in my experiments could have been an effort to increase their contact with food. In clear water, the entire apparatus was visible and the fish should have

been able to determine the availability of food. When the water became turbid, visual range was only a few centimeters (see Figure 2). As a result, the minnows did not have the ability to determine if food was available in the apparatus and they may have increased their movement in an effort to encounter more food.

An interesting result of my experiments was that hungry fish did not respond to the presence of alarm substance in clear water but they did react strongly to the presence of the visual predator stimulus. Many experiments have demonstrated that increasing the hunger level increases the willingness of an individual to risk exposure to a predator (Bumann *et al.* 1997; Dill and Gillett 1991; Godin and Crossman 1994; Krause 1993b; Magnhagen 1988a, b; Magurran 1990a). My experiments demonstrated that fish were less willing to respond to a cue indicating a potentially dangerous situation (i.e., alarm substance) but when faced with a predator, they still displayed a response.

Fish in natural settings should respond to alarm substance when certain conditions are present. When minnows are faced with reduced visual input, they become more sensitive to the presence of alarm substance. Along with turbidity, factors such as darkness or increased depth in the water, visual defects or injury should increase sensitivity to the presence of alarm substance. Blind fish introduced to alarm substance showed stronger reactions to the stimulus than did fish with normal vision (Göz 1941). A high number of predators in the environment will also cause increased sensitivity to alarm substance. More predators would increase the risk associated with the environment as a result of an increased probability of encountering a predator. Levesley and Magurran (1988) demonstrated that populations of minnows that regularly encounter pike were found to be more likely to respond to the presence of alarm substance than were minnows which were unfamiliar with pike predators. A lack of cover or refuges would represent an environment

with an increased risk of predation (Savino and Stein 1989) as a result of reduced opportunities of escape which would result in more responses to alarm substance.

Other factors will result in a reduced sensitivity to alarm substance. A lower risk of predation would result in fewer responses to alarm substance. A predator-free environment, knowledge of safe areas, or increased cover or refuges would lower the risk of predation. Fish in shoals have several benefits which reduce the risk of predation (Magurran *et al.* 1985). Larger shoals are more adept at detecting predators than are smaller shoals (Godin *et al.* 1988). If detected, shoals are less likely to be attacked (Keenleyside 1979), and if a shoal is attacked, individuals are less likely to be captured (Neill and Cullen 1974). Fish in larger shoals are less sensitive to alarm substance than fish in smaller shoals or single fish (Göz 1941; Magurran and Pitcher 1987). Acceptance of a greater level of risk due to reproductive behavior such as guarding a territory or offspring (Magnhagen 1990), or attempting to mate (Magnhagen 1992), would make fish less sensitive to alarm substance. The costs of fleeing become greater as a result of the time and energy invested in the reproductive activity which would be lost if the animal did flee (Magnhagen 1991, 1992).

The presence of alarm substance conveys information that a predator may be in the area but it does not identify the identity or current location of the predator. Detecting alarm substance allows the individuals to perform antipredator behavior. The response to alarm substance in turbid water should be one which allows the prey to respond to all types of predators, either bird, invertebrate, mammal, or fish. If a given population of minnows is predominately preyed upon by one type of predator, then the response to alarm substance should be the behavior which best counteracts the hunt and capture strategy of that predator. The type of response demonstrated should also reflect the characteristics of the environment. If certain areas are not accessible to

predators as a result of habitat structure, prey should dash and seek refuge in those areas when they are threatened with alarm substance. The characteristics of the environment must be considered along with the hunting strategies of the predator. An increased amount of cover would allow individuals an opportunity to hide rather than dash or freeze in the open but ambush predators would prefer hunting from areas with cover (Savino and Stein 1989).

Aquatic predators should also demonstrate an ability to compensate for a reduction in visual information. When predators are faced with a reduction in visual information, they could increase the sensitivity of their alternative sensory mechanisms, such as mechanical or chemical senses, to detect prey. It has been demonstrated that minnow predators are attracted to the presence of alarm substance (Mathis *et al.* 1995; Chivers *et al.* 1996). I do not believe that predators should alter their sensitivity to the presence of alarm substance when faced with changes in visibility. When alarm substance is present, it is a definite indication that injured prey is near. When a predator is hunting, it should use a chemical cue regardless of the visible conditions. Other than alarm substance, little is known about the chemicals released by minnows. If minnows did release other chemicals, there is no reason for the predators to ignore them in clear water. The costs to the predator associated with responding to a false chemical cue are only energetic costs.

When in turbid water, predators should maximize their ability to detect or contact prey but at the same time, avoid their own predators. Increasing movement will result in a higher probability of encountering prey because the rate of contact is a function of speed but it also increases the probability of encountering a predator (Werner and Anholt 1993). Experiments have demonstrated that prey may be less effective at escaping predators when the water is turbid. Abrahams and Kattenfeld (1997) found that perch predators did not display any size preference

in turbid water but they caught a similar number of minnows in clear and turbid water. This indicates that encountering prey in turbid water results in a much higher probability of capture as a result of ineffective antipredator behavior. Therefore, I would predict that predators can compensate for a reduction in visibility by increasing their movement. The encounter rate will increase and the probability of obtaining a meal from an encounter increases also but this increased movement must be balanced with the probability of encountering a predator.

My experiments have demonstrated that fish are capable of adjusting their sensitivity to chemical cues based on the level of risk, the sensitivity to risk, and the availability of visual information. There is information that this compensation can cause morphological changes over time. Huber and Rylander (1992) and Van Staaden *et al.* (1995) found that species of fish inhabiting turbid water had better developed olfactory apparatus when compared with species from clear water. The increased development of these structures suggests a greater reliance on the use of chemicals to obtain information from a more turbid environment (Van Staaden *et al.* 1995). Therefore it appears minnows in turbid water should have an increased ability to use alarm substance to detect the presence of a predator. This indicates that minnows in turbid water may have the ability to modify their behavior in an effort to decrease their mortality rates.

References

Åbjörnsson, K., B.M.A. Wagner, A. Axelsson, R. Bjerselius, and K. H. Olsén. 1997. Responses of *Acilius sulcatus* (Coleoptera: Dytiscidae) to chemical cues from perch (*Perca fluviatilis*). Oecologia, **111**:166-171.

Abrahams, M.V. 1994. Risk of predation and its influence on the relative competitive abilities of two species of freshwater fishes. Can. J. Fish. Aquat. Sci. **51**:1629-1633.

Abrahams, M.V. 1995. The interaction between anti-predator behavior and anti-predator morphology: experiments with fathead minnows and brook sticklebacks. Can. J. Zool. **73**:2209-2215.

Abrahams, M.V., and L.D. Townsend. 1993. Bioluminescence in dinoflagellates: a test of the burglar alarm hypothesis. Ecology, **74**:258-260.

Abrahams, M.V., and M.G. Kattenfeld. 1997. The role of turbidity as a constraint on predator-prey interactions in aquatic environments. Behav. Ecol. Sociobiol. **40**:169-174.

Adams, D.C., and C.D. Anthony. 1996. Using randomization techniques to analyze behavioral data. Anim. Behav. **51**:733-738.

Aksnes, D.L., and J. Giske. 1993. A theoretical model of aquatic visual feeding. Ecol. Modelling. **67**:233-250.

Alcock, J. 1993. Animal behavior: an evolutionary approach. Sinauer Associates, Inc.:Sunderland.

Anderson, O. 1984. Optimal foraging by largemouth bass in structured environments. Ecology, **65**:851-861.

Andraso, G.M., and J.N. Barron. 1995. Evidence for a trade-off between defensive morphology and startle-response performance in the brook stickleback (*Culaea inconstans*). Can. J. Zool. **73**:1147-1153.

Biase, D.V., and M. Zuckerman. 1964. Perceptual isolation as a stress situation. Arch. Gen. Psychiat. **11**:255-276.

Blake, M., and P.J.B. Hart. 1993. The behavioral responses of juvenile signal crayfish, *Pacifastacus leniusculus* to stimuli from perch and eels. Freshwater Biology, **29**:89-97.

Brown, G.E., and R.J.F. Smith. 1994. Fathead minnows use chemical cues to discriminate natural shoal-mates from unfamiliar conspecifics. J. Chem. Ecol. **20**:3051-3061.

Brown, G.E., and R.J.F. Smith. 1996. Foraging trade-offs in fathead minnows (*Pimephales promelas*, Osteichthyes, Cyprinidae): acquired predator recognition in the absence of an alarm response. Ethology, **102**:776-785.

Brown, G.E., D.P. Chivers, and R.J.F. Smith. 1995. Fathead minnows avoid conspecific and heterospecific alarm pheromones in the faeces of northern pike. J. Fish Biol. **47**:387-393.

Bruski, C.A., and D.W. Dunham. 1987. The importance of vision in agonistic communication in crayfish *Orconectes rusticus*. I: an analysis of bout dynamics. Behaviour, **103**:83-107.

Bumann, D., J. Krause, and D. Rubenstein. 1997. Mortality risk of spatial positions in animal groups: the danger of being in front. Behaviour, **134**:1063-1076.

Burghardt, G. 1964. Effects of prey size and movement on the feeding behavior of the lizards *Anolis carolinensis* and *Eumeces fasciatus*. Copeia, **1964**:576-578.

Canfield, J.G., and R.C. Eaton. 1990. Swimbladder acoustic pressure transduction initiates Mauthner-mediated escape. Nature, Lond. **347**:760-762.

Chivers, D.P., and R.J.F. Smith. 1993. The role of olfaction in chemosensory based predator recognition in the fathead minnow, *Pimephales promelas*. J. Chem. Ecol. **19**:623-633.

Chivers, D.P., and R.J.F. Smith. 1994a. Fathead minnows, *Pimephales promelas*, acquire predator recognition when alarm substance is associated with the sight of unfamiliar fish. Anim. Behav. **48**:597-605.

Chivers, D.P., and R.J.F. Smith. 1994b. The role of experience and chemical alarm signaling in predator recognition by fathead minnows, *Pimephales promelas*. J. Fish Biol. **44**:273-285.

Chivers, D.P., and R.J.F. Smith. 1995. Free-living fathead minnows rapidly learn to recognize pike as predators. J. Fish Biol. **46**:949-954.

Chivers, D.P., G.E. Brown, and R.J.F. Smith. 1995. Familiarity and shoal cohesion in fathead minnows (*Pimephales promelas*): implications for anti-predator behavior. Can. J. Zoo. **73**:955-960.

Chivers, D.P., G.E. Brown, and R.J.F. Smith. 1996. The evolution of chemical alarm signals: attracting predators benefits alarm signal senders. Am. Nat. **148**:649-659.

Confer, J.L., G.L. Howick, M.H. Corzette, S.L. Kramer, S. Fitzgibbon, and R. Landesberg. 1978. Visual predation by planktivores. Oikos, **31**:27-37.

Coull, B.C., and J.B.J. Wells. 1983. Refuges from fish predation: experiments with phytal meiofauna from New Zealand rocky intertidal. Ecology, **64**:1599-1609.

Davis, B.J., and R.J. Miller. 1967. Brain patterns in minnows of the genus *Hybopsis* in relation to feeding habits and habitat. Copeia, **1967**:1-39.

Dial, B.E., and K. Schwenk. 1996. Olfaction and predator detection in *Coleonyx brevis* (Squamata: Eublepharidae), with comments on the functional significance of buccal pulsing in geckos. J. Exp. Zoo. **276**:415-424.

Dill, L.M., and J.F. Gillett. 1991. The economic logic of barnacle, *Balanus glandula* (Darwin) hiding behavior. J. Exp. Mar. Biol. **153**:115-127.

Duda, P., and J.P. Zubek. 1965. Auditory sensitivity after prolonged visual deprivation. Psychon. Sci. **3**:359-360.

Dusenbery, D.B. 1992. Sensory ecology: how animals acquire and respond to information. W.H. Freeman and Company: New York.

Endler, J.A. 1986. Defense against predators. In: Predator-prey relationships. Perspectives and approaches (eds. M.E. Feder and G.V. Lauder). Univ. Of Chicago Press: Chicago. p.109-134.

Ellis, M.E. 1982. Evolution of aversive information processing: A temporal trade off hypothesis. Brain Behav. Evol. **21**:151-160.

Fuiman, L.A., and A.E. Magurran. 1994. Development of predator defenses in fishes. Rev. Fish Biol. Fish. **4**:145-183.

Gelowitz, C.M., Mathis, A. & Smith, R.J.F. 1993. Chemosensory recognition of northern pike (*Esox lucius*) by brook stickleback (*Culaea inconstans*): population differences and the influence of predator diet. Behaviour, **127**:105-118.

Gerkema, M.P., and S. Verhulst. 1990. Warning against an unseen predator: functional aspect of synchronous feeding in the common vole, *Microtus arvalis*. Anim. Behav. **40**:1169-1178.

Glass, N.R. 1971. Computer analysis of predation energetics in the largemouth bass. In: Systems analysis and simulation ecology. Vol.1 (ed. B.C. Patten). New York: Academic Press. pp. 325-363.

Godin, J-G. J., and S.L. Crossman. 1994. Hunger-dependent predator inspection and foraging in the threespine stickleback (*Gasterosteus aculeatus*) under predation risk. Behav. Ecol. Sociobiol. **34**:359-366.

Godin, J-G. J., L.J. Classon, and M.V. Abrahams. 1988. Group vigilance and shoal size in a small characin fish. Behaviour, **104**:29-40.

Gotceitas, V., and P. Colgan. 1987. Selection between densities of artificial vegetation by young bluegills avoiding predation. Trans. Am. Fish. Soc. **116**:40-49.

Göz, H. 1941. Über den Art- und Individualgeruch bei Fischen. Z. vergl. Physiol. **29**:1-45.

Gregory, R.S. 1993. Effect of turbidity on the predator avoidance behavior of juvenile chinook salmon (*Oncorhynchus tshawytscha*). Can. J. Fish. Aquat. Sci. **50**:241-246.

Gregory, R.S., and T.G. Northcote. 1993. Surface, planktonic and benthic foraging by juvenile chinook salmon (*Oncorhynchus tshawytscha*) in turbid laboratory conditions. Can. J. Fish. Aquat. Sci. **50**:233-240.

Heale, V.R., and C.H. Vanderwolf. 1994. Toluene and weasel (2-propyltheitane) odors suppress feeding in the rat. J. Chem. Ecol. **20**:2953-2958.

Henderson, P.A., P.W. Irving, and A.E. Magurran. 1997. Fish pheromones and evolutionary enigmas: a reply to Smith. Proc. R. Soc. Lond. B. **264**: 451-453.

Högstedt, G. 1983. Adaptation unto death: function of fear screams. Am. Nat. **121**:562-570.

Holomuzki, J.R., and T.M. Short. 1988. Habitat use and fish avoidance by the stream dwelling isopod *Lirceus fontinalis*. Oikos, **52**:79-86.

Huber, R., and M.K. Rylander. 1991. Quantitative histological studies of the optic tectum in six species of *Notropis* and *Cyprinella* (Cyprinidae, Teleostei). J. Hirnforsch. **32**:309-316.

Huber, R., and M.K. Rylander. 1992. Brain morphology and turbidity preference in *Notropis* and related genera (Cyprinidae, Teleostei). Env. Biol. Fish. **33**:153-165.

Irving, P.W., and A.E. Magurran. 1997. Context-dependent fright reactions in captive European minnows: the importance of naturalness in laboratory experiments. Anim. Behav. **53**:1193-1201.

Jachner, A. 1995. Changes in feeding behavior of bleak (*Alburnus alburnus L.*) in response to visual and chemical stimuli from predators. Arch. Hydrobiol. **133**:305-314.

Kattenfeld, M.G. 1995. The effect of turbidity and predation risk on the habitat choice of Fathead Minnows (*Pimephales promelas*). M.Sc. Thesis, University of Manitoba. pp.91.

Keenleyside, M.H.A. 1979. Diversity and adaptation in fish behavior. New York:Springer -Verlag.

Kishida, R. 1978. Comparative study on the optic tectum. Lamination and cytoarchitecture. J. Hirnforsch. **20**:57-67.

Koops, M.A. 1998. Misinformation and assessment uncertainty in the ecology of information use. Ph.D. Thesis, University of Manitoba. pp. 215.

Kotrschal, K., and H. Junger. 1988. Patterns of brain morphology in mid-European Cyprinidae (Pisces, Teleostei): A quantitative histological study. J. Hirnforsch. **29**:341-352.

Krause, J. 1993a. The effect of "Schreckstoff" on the shoaling behavior of the minnow: a test of Hamilton's selfish herd theory. Anim. Behav. **45**:1019-1024.

Krause, J. 1993b. The relationship between foraging and shoal position in a mixed shoal of roach (*Rutilus rutilus*) and chub (*Leuciscus cephalus*): a field study. Oecologia, **93**:356-359.

Lawler, S.P. 1989 Behavioral responses to predators and predation risk in four species of larval anurans. Anim. Behav. **38**:1039-1047.

Lawrence, B.J., and R.J.F. Smith. 1989. Behavioral response of solitary fathead minnows, *Pimephales promelas*, to alarm substance. J. Chem. Ecol. **15**:209-219.

Levesley, P.B., and A.E. Magurran. 1988. Population differences in the reaction of minnows to alarm substance. J. Fish Biol. **32**:699-706.

Lima, S.L., and L.M. Dill. 1990. Behavioral decisions made under the risk of predation: a review and prospectus. Can. J. Zool. **68**:619-640.

Magnhagen, C. 1988a. Predation risk and foraging in juvenile pink (*Oncorhynchus gorbuscha*) and chum salmon (*O. Keta*). Can. J. Fish. Aquat. Sci. **45**:592-596.

Magnhagen, C. 1988b. Changes in foraging as a response to predation risk in two gobiid fish species, *Pomatoschistus minutus* and *Gobius niger*. Mar. Ecol. Prog. Ser. **49**:21-26.

Magnhagen, C. 1990. Reproduction under the predation risk in the sand goby, *Pomatoschistus minutus*, and the black goby, *Gobius niger*: the effect of age and longevity. Behav. Ecol. Sociobiol. **26**:331-335.

Magnhagen, C. 1991. Predation risk as a cost of reproduction. TREE. **6**:183-186.

Magnhagen, C. 1992. Parental care and predation risk in fish. Ann. Zool. Fennici. **29**:227-232.

Magurran, A.E. 1990a. The adaptive significance of schooling as an anti-predator defense in fish. Annls. Zool. Fenn. **27**:51-66.

Magurran, A.E. 1990b. The inheritance and development of minnow anti-predator behavior. Anim. Behav. **39**:834-842.

Magurran, A.E., W.J. Oulton, and T.J. Pitcher. 1985. Vigilant behavior and shoal size in minnows. Z. Tierpsychol. **67**:167-178.

Magurran, A.E., and S.L. Girling. 1986. Predator model recognition and response habituation in shoaling minnows. Anim. Behav. **34**:510-518.

Magurran, A.E., and A. Higham. 1988. Information transfer across fish shoals under predator threat. Ethology, **78**:153-158.

Magurran, A.E., and T.J. Pitcher. 1987. Provenance, shoal size and the sociobiology of predator-evasion behavior in minnow shoals. Proc. R. Soc. Lond. B. **229**: 439-465.

Magurran, A.E., P.W. Irving, and P.A. Henderson. 1996. Is there a fish alarm pheromone? A wild study and critique. Proc. R. Soc. Lond. B. **263**:1551-1556.

Mangel, M., and C.W. Clark. 1986. Towards a unified foraging theory. Ecology, **67**:1127-1138.

Manly, B.F.J. 1991. Randomization and Monte Carlo Methods in Biology. New York: Chapman & Hall.

Martel, G., and L.M. Dill. 1995. Influence of movement of coho salmon (*Oncorhynchus kisutch*) parr on their detection by common mergansers (*Mergus merganser*). Ethology, **99**:139-149.

Mathis, A., and R.J.F. Smith. 1992a. Avoidance of areas marked with a chemical alarm substance by fathead minnows (*Pimephales promelas*) in a natural habitat. Can. J. Zoo. **70**:1473-1476.

Mathis, A., and R.J.F. Smith. 1992b. Intraspecific and cross-superorder responses to chemical alarm signals by brook stickleback. Ecology, **74**:2395-2404.

Mathis, A., and R.J.F. Smith. 1993a. Chemical labeling of northern pike (*Esox lucius*) by the alarm pheromone of fathead minnows (*Pimephales promelas*). J. Chem. Ecol. **19**:1967-1979.

Mathis, A., and R.J.F. Smith. 1993b. Fathead minnows, *Pimephales promelas*, learn to recognize northern pike, *Esox lucius*, as predators on the basis of chemical stimuli from minnows in the pike's diet. Anim. Behav. **46**:645-656.

Mathis, A., D.P. Chivers, and R.J.F. Smith. 1993. Population differences in responses of fathead minnows (*Pimephales promelas*) to visual and chemical stimuli from predators. Ethology, **93**:31-40.

Mathis, A., D.P. Chivers, and R.J.F. Smith. 1995. Chemical alarm signals: predator deterrents or predator attractants? Am. Nat. **145**:994-1005.

McLean, E.B., and J-G.J. Godin. 1989. Distance to cover and fleeing from predators in fish with different amounts of defensive armor. Oikos, **55**:281-290.

McNamara, J.M., and A.I. Houston. 1986. The common currency for behavioral decisions. Am. Nat. **127**:358-378.

Miner, J.G., and R.A. Stein. 1993. Interactive influence of turbidity and light on larval bluegill (*Lepomis macrochirus*) foraging. Can. J. Fish. Aquat. Sci. **50**:781-788.

Miner, J.G., and R.A. Stein. 1996. Detection of predators and habitat choice by small bluegills: effects of turbidity and alternate prey. Trans. Am. Fish. Soc. **125**:97-103.

Naish, K.-A., G.R. Carvalho, and T.J. Pitcher. 1993. The genetic structure and microdistribution of shoals of *Phoxinus phoxinus*, the European minnow. J. Fish Biol. (Suppl. A). **43**:75-89.

Neill, S.R.St.J., and J.M. Cullen. 1974. Experiments on whether schooling by their prey affects hunting behavior of cephalopods and fish predators. J. Zool. **172**:549-569.

Noreen, E.W. 1989. Computer intensive methods for testing hypotheses: An introduction. New York: Wiley. 229 p.

Ode, P.R., and S.A. Wissinger. 1993. Interaction between chemical and tactile cues in mayfly detection of stoneflies. Freshwater Biology, **30**:351-357.

Partridge, B.L., and T.J. Pitcher. 1980. The sensory basis of fish schools: relative roles of lateral line and vision. J. Comp. Physiol. **135**:315-325.

Peckarsky, B.L. 1980. Predator-prey interactions between stoneflies and mayflies: behavioral observations. Ecology, **61**:1283-1290.

Pfeiffer, W. 1962. The fright reaction of fish. Biol. Rev. **37**:495-511.

Pfeiffer, W. 1963a. Alarm substances. Experientia, **19**:113-123.

Pfeiffer, W. 1963b. The fright reaction in North American fish. Can. J. Zoo. **41**:69-77.

Pfeiffer, W. 1978. Heterocyclic compounds as releasers of the fright reaction in the giant danio (*Danio malabricus*) (Jerdon) (Cyprinidae, Ostariophysi, Pisces). J. Chem. Ecol. **4**:665-673.

Pfeiffer, W. and J. Lemke. 1973. Untersuchungen zur Isolierung und Identifizierung des Schreckstoffes aus der Haut der Elzrite, *Phoxinus phoxinus* (L.) (Cyprinidae, Ostariophysi, Pisces). J. Comp. Physiol. **82**:407-410.

Pfeiffer, W. 1982. Chemical signals in communication. In: Chemoreception in fishes (ed. T.J. Hara). Amsterdam: Elsevier.

Pfeiffer, W. and G. Riegelbauer. 1978. The effect of the alarm substance on the central nervous excitation of the black tetra *Gymnocorymbus ternetzi* (Characidae, Ostariophysi, Pisces) indicated by dorsal light response. J. Comp. Physiol. **123**:281-288.

Pfeiffer, W., G. Riegelbauer, G. Meier, and B. Scheibler. 1985. Effect of hypoxanthine-3-N-oxide and hypoxanthine-1-N-oxide on central nervous excitation of the black tetra, *Gymnocorymbus ternetzi* (Characidae, Ostariophysi, Pisces) indicated by dorsal light response. J. Chem. Ecol. **11**:507-523.

Pijanowska, J. 1997. Alarm signals in *Daphnia*? Oecologia, **112**:12-16.

Pitcher, T.J., D.A. Green, and A.E. Magurran. 1986. Dicing with death: predator inspection behavior in minnow shoals. J. Fish Biol. **28**:439-448.

Rangeley, R.W., and D.L. Kramer. 1998. Density-dependent anti-predator tactics and habitat selection in juvenile pollock. Ecology, **79**:943-952.

Reissen, H.P. 1992. Cost-benefit model for the induction of an anti-predator defense. Am. Nat. **140**:349-362.

Savino, J.F. & Stein, R.A. 1982. Predator-prey interaction between largemouth bass and bluegills as influenced by simulated, submerged vegetation. Trans. Am. Fish. Soc. **111**:255-266.

Savino, J.F. & Stein, R.A. 1989. Behavioural interactions between fish predators and their prey: effects of plant density. Anim. Behav. **37**:311-321.

Schellart, N.A.M. 1992. Interrelationships between the auditory, the visual and the lateral line systems of teleosts; a mini-review of modeling sensory capabilities. Neth. J. Zoo. **42**:459-477.

Schutz, F. 1956. Vergleichende Untersuchungen über die Schreckreacktion bei Fischen und deren Verbreitung. Z. Vergl. Physiol. **38**:84-135.

Schwenk, K. 1993. Are geckos olfactory specialists? J. Zoo. Lond. **229**:289-302.

Scott, W.B., and E.J. Crossman. 1973. Freshwater fishes of Canada. Bull. Fish. Res. Bd. Can. **184**:1-966.

Seeley, T.D. 1989. The honey bee colony as a superorganism. Amer. Sci. **77**:546-553.

Sih, A., and R.D. Moore. 1989. Interacting effects of predator and prey behavior in determining diets. In: Behavioral mechanisms of food selection (ed. R.N. Hughes). Springer, Heidelberg.

Smith, R.J.F. 1976. Male fathead minnows (*Pimephales promelas* Rafinesque) retain their fright reaction to alarm substance during the breeding season. Can. J. Zoo. **54**:2230-2231.

Smith, R.J.F. 1981. Effect of food deprivation on the reaction of Iowa darters (*Etheostoma exile*) to skin extract. Can. J. Zoo. **59**:558-560.

Smith, R.J.F. 1982. The adaptive significance of the alarm substance-fright reaction systems. In: Chemoreception in fishes (ed. T.J. Hara). Elsevier: Amsterdam.

Smith, R.J.F. 1992. Alarm signals in fishes. Rev. Fish Biol. Fish. **2**:33-63

Smith, R.J.F. 1997. Does one result trump all others? A response to Magurran, Irving and Henderson. Proc. R. Soc. Lond. B. **264**:445-450.

Suboski, M.D., S. Bain, A.E. Carty, L.M. McQuoid, M.I. Seelen, and M. Seifert. 1990. Alarm reaction in acquisition and social transmission of simulated-predator recognition by zebra danio fish (*Brachydanio rerio*). J. Comp. Psychol. **104**:101-112.

Thomas, F. and R. Poulin. 1997. Using randomization techniques to analyze fluctuating asymmetry data. Anim. Behav. **54**:1027-1029.

Valone, T.J. 1989. Group foraging, public information, and patch estimation. Oikos, **56**:357-363.

Van Staaden, M.J., R. Huber, L.S. Kaufman, and K.F. Liem. 1995. Brain evolution in cichlids of the Great African Lakes: brain and body size, general patterns, and evolutionary trends. Zoology, **98**:165-178.

Vandenbyllaardt, L., F.J. Ward, C.R. Braekevelt, and D.B. McIntyre. 1991. Relationships between turbidity, piscivory and development of the retina in juvenile walleyes. Trans. Am. Fish. Soc. **120**:382-390.

VanHavre, N., and G.J. FitzGerald. 1988. Shoaling and kin recognition in the threespine stickleback, (*Gasterosteus aculeatus* L.). Biol. Behav. **13**:190-201.

Verheijen, F.J. 1956. Transmission of a flight reaction amongst a school of fish and the underlying sensory mechanisms. Experientia, **12**:202-204.

Vernon, J., and T. McGill. 1961. Sensory deprivation and pain thresholds. Psychosom. Sci. **133**:330-331.

Vinyard, G.L., and W.J. O'Brien. 1976. Effects of light and turbidity on the reactive distance of bluegill (*Lepomis macrochirus*). J. Fish. Res. Board. Can. **33**:2845-2849.

von Frisch, K. 1938. Zur psychologie des Fisch-Schwarmes. Naturwissenschaften. **26**:601-606.

Weldon, P.J. 1990. Responses by vertebrates to chemicals from predators. In: Chemical signals in vertebrates 5 (eds. D.W. MacDonald, D. Müller-Schwartze & S.E. Natynczuk). Oxford University Press: New York. pp.500-521.

Werner, E.E., and B.R. Anholt. 1993. Ecological consequences of the trade-off between growth and mortality rates mediated by foraging activity. Am. Nat. **142**:242-272.

Williams, G.C. 1992. Natural selection: domains, levels and challenges. Oxford University Press.

Wodinsky, J. 1971. Movement as a necessary stimulus of *Octopus* predation. Nature, **229**:493-494.

Wright, D.I., and W.J. O'Brien. 1984. The development and field test of a tactical model of the planktivorous feeding of white crappie (*Pomoxis annularis*). Ecol. Monogr. **54**:65-98.

Ydenberg, R.C., and L.M. Dill. 1986. The economics of fleeing from predators. Adv. Study Behav. **16**:229-249.

Zubek, J.P. 1964. Effects of prolonged sensory and perceptual deprivation. Brit. Med. Bull. **20**:38-42.

Zubek, J.P., J. Flye, and M. Aftanas. 1964. Cutaneous sensitivity after prolonged visual deprivation. Science, **144**:1591-1593.

Zubek, J.P., J. Flye, and D. Williams. 1964. Changes in cutaneous sensitivity after prolonged exposure to unpatterned light. Psychon. Sci. **1**:283-284.

Zuckerman, M., S. Levine, and D.V. Biase. 1964. Stress response in total and partial perceptual isolation. Psychosom. Med. **26**:250-260.

Appendix 1. The measurements taken from the donor fish used to make to alarm substance and muscle control in A) Experiment 1 and B) Experiment 2.

A)

Fish	Total length (mm)	Wet weight (g)	Skin area (cm^2)	Skin weight (g)	Caudal weight (g)
1	73.73	4.47	11.38	0.41	0.42
2	54.33	1.16	6.11	0.17	0.17
3	70.31	3.24	6.30	0.12	0.22
4	69.81	3.39	12.31	0.31	0.20
5	60.40	2.06	6.56	0.33	0.25
6	77.81	4.30	10.22	0.50	0.60
7	65.81	2.44	8.24	0.27	0.23
8	57.45	1.76	4.17	0.17	0.24
9	55.07	1.44	6.12	0.16	0.19
10	59.90	1.75	7.38	0.20	0.16
11	71.15	3.51	9.67	0.45	0.51
12	56.51	1.62	4.20	0.10	N/A
Mean	64.36	2.60	7.72	0.27	0.29
Std. Error	2.33	0.33	0.77	0.04	0.05
Total	772.28	31.14	92.66	3.19	3.19

B)

Fish	Total length (mm)	Wet weight (g)	Skin area	Skin weight (g)	Caudal weight (g)
1	69.20	3.13	9.29	0.37	0.35
2	68.40	3.16	8.98	0.47	0.48
3	60.08	1.81	4.83	0.24	0.20
4	60.66	2.11	6.72	0.30	0.33
5	64.57	2.65	8.98	0.40	0.40
6	57.68	1.61	6.62	0.20	0.22
7	65.81	2.44	8.24	0.27	0.23
Mean	63.43	2.41	7.57	0.33	0.33
Std. Error	1.93	0.27	0.73	0.04	0.05
Total	380.59	14.47	45.42	1.98	1.98

Appendix 2. The Visual Basic program used for the approximate randomization. This version involved the presence or absence of a predator for Experiment 1. To analyze the other treatments and the other experiment, the program was slightly modified. Program comment statements are capitalized.

```
Sub predprog()
Worksheets(2).Select
Dim y(80), x(80), v(97)
Range("b5:b8").ClearContents
'SET THE SAMPLE SIZE
m = Cells(2, 2)
'DEFINE THE NUMBER OF REPLICATIONS
NS = Cells(4, 2)
'NGE = THE NUMBER GREATER THAN OR EQUAL TO; SET THE VALUE TO 0
nge = 0
'ENTER THE DATA
For i = 1 To m
   x(i) = Cells(11 + i, 2)
   y(i) = Cells(11 + i, 3)
Next i
'GO TO THE FUNCTION THAT CALCULATES THE DIFFERENCE BETWEEN THE
MEANS OF THE TREATMENTS
ActualStat = statistic(m, x(), y())
Cells(3, 2) = ActualStat
'SHUFFLE AND RECALCULATE THE TEST STATISTIC
For shuffle = 1 To NS
   Call simpleshuffle(y(), m)
   Pseudostat = statistic(m, x(), y())
   If Pseudostat >= ActualStat Then nge = nge + 1
Next shuffle
'CALCULATION OF THE P-VALUE
Cells(5, 2) = (nge + 1) / (NS + 1)
End Sub

'FUNCTION USED TO CALCULATE THE DIFFERENCE BETWEEN THE MEANS
Function statistic(m, x(), y())
Dim table(2, 2)
'SET TABLE VALUES TO 0
For i = 0 To 1
   For j = 0 To 1
      table(i, j) = 0
   Next j
```

```
Next i
'ENTER THE DATA INTO THE TABLE
For replicate = 1 To 80
   i = x(replicate)
   j = y(replicate)
   table(i, j) = table(i, j) + 1
Next replicate
'OBSERVED - EXPECTED THEN SUM THE DIFFERENCES
predator = (table(1, 1) - 25.5) + (14.5 - table(1, 0))
nopredator = (25.5 - table(0, 1)) + (table(0, 0) - 14.5)
statistic = predator + nopredator
End Function

'SUBROUTINE USED TO RANDOMLY SHUFFLE THE DATA
Sub simpleshuffle(y(), m)
For j = 1 To m - 1
   ' RANDOM NUMBER GENERATOR
   u = Rnd
   k = j + Int(u * (m - j + 1))
   temp = y(k)
   y(k) = y(j)
   y(j) = temp
Next j
End Sub
```