ANTI-PREDATOR BEHAVIOUR OF JUVENILE ATLANTIC COD: THE ROLE OF WILD EXPERIENCE AND EFFECT OF MULTIPLE PREDATORS

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by

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ABSTRACT

Community structure is largely influenced by predator-prey interactions; however, the complex nature of anti-predator behaviours, especially when more than one predator is present, remains unclear. The work outlined in this thesis investigated the effect of experience with multiple predators on the anti-predator behaviour of juvenile Atlantic cod (*Gadus morhua*). Hatchery-reared ("naïve") individuals lacking the experience of their wild counterparts may be unable to recognize or evade predators effectively. The anti-predator behaviour of naïve juvenile cod and two wild cod species, Atlantic cod and rock cod (*Gadus ogac*) in the presence of either one or two shorthorn sculpin, was examined. Both wild species demonstrated schooling behaviour in the presence of a predator, and no predation was observed over the course of the trials. However, effective schooling behaviour, as determined by relative mortality, was not observed with the naïve Atlantic cod as predation was observed in 50% of the trials.

The effect of multiple predators on schooling behaviour and mortality was also investigated in naïve juvenile Atlantic cod exposed to both adult cod and sculpin in a substitutive design. Non-additive effects on the relative mortality of juvenile Atlantic cod under multiple predator threat were observed, while the effects on schooling behaviour were less clear. This study was the first to investigate multiple predator effects in Atlantic cod, and one of the first marine studies in the multiple predator effects literature. The results demonstrate the plasticity of prey responses and the importance of experience in the development of efficient anti-predator behaviours.

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CHAPTER 1

General Introduction

1.1 Predator-prey interactions

Predator-prey interactions shape the dynamics of natural communities. The direction of this interaction can be shaped by how prey respond to a predatory threat (Kerfoot and Sih, 1987; Soluk and Collins, 1988; Power, 1990; Crowder *et al.*, 1997; Sih *et al.*, 1998). The role of prey behavioural responses in predator-prey interactions was emphasized by Sih (1979), who suggested that the importance of predator functional responses had been overstated. The diversity of prey responses to predatory threat is impressive, as is the plasticity of those responses (Relyea, 2003). For instance, bluegill sunfish (*Lepomis macrochirus*) shoaling decreases when vegetative cover is available, and schools of fish are observed to become more concentrated in the presence of a predator (Savino and Stein, 1989; Pitcher and Parrish, 1993). Schooling is a well-established anti-predator behaviour, reducing the per capita predation risk of prey with prey group size (Sih, 1987; Pitcher and Parrish, 1993). The flexibility of these behaviours reflects the sensitivity of prey to the level of predation risk and their active choice of avoidance behaviour.

1.2 The role of experience in avoiding predators

Predator avoidance behaviours have both an innate and learned component. This is well exemplified by observing the behavioural capacities of fish that are naïve to predators. There exist some mixed results on both the anti-predator behaviour and foraging ability of naïve fish. In general, naïve fish initially exhibit a lack of certain abilities, but these abilities can be quickly learned (Benzie, 1965; Colgan *et al.*, 1986; Järvi and Uglem, 1993; Steingrund and Fernö, 1997). Milinski (1979) was able to show that as predators, sticklebacks with previous experience attacking high density swarms of prey were more effective in subsequent attempts than sticklebacks that had experience with only smaller swarms. This demonstrates that many of the differences between experienced and inexperienced fish lie not in the presence or absence of certain behaviours, but in the extent of their development.

1.3 Multiple predator effects

In recent years, the multiple predator environment has become of interest to behavioural ecologists in determining how prey respond to the complex predation risks. Studies involving multiple predators have found that predator-prey relationships are less clear than once imagined. While most studies on predator-prey interactions focus on lethal effects, the indirect effects of predator-prey interactions have become the focus of some interest with respect to the multiple predator environment. Prey mortality in the presence of multiple predators is often compared to a theoretical value for prey mortality attained by adding mortality in the presence of either predator alone. However, interactions among predator and prey are often qualitatively different when more than one predator is present. Emergent multiple predator effects are said to be present when the observed effect on prey cannot be predicted merely by summing the effects of the predators individually; that is, the presence of more than one predator results in either risk enhancement or risk reduction for the prey (Sih *et al.*, 1998).

Research is now finding that interactions between predator and prey are qualitatively different when more than one predator is present. For example, *Daphnia sp.*, an aquatic invertebrate, migrate towards the water surface at night, which is a seemingly inefficient anti-predator defense, as this makes them more vulnerable to predation by fly larvae. However, this migratory behaviour actually mitigates potential predation since bluegill sunfish prey upon fly larvae that hover near the water surface waiting for *Daphnia* (González and Tessier, 1997).

Emergent multiple predator effects (MPEs) are the result of indirect effects among predator and prey species. Indirect effects have been given many definitions, including all those effects that are not direct (Wootton, 1994), but in this case it refers to an effect that modifies the direct effect of one species on another (Kerfoot and Sih, 1987). Essentially, indirect effects refer to competition, interference, or facilitation between predators. In recent years these effects have been described using many different terms; however, the most concise terminology has been set out by Miller and Kerfoot (1987). Indirect effects are of three qualitatively different categories: trophic linkages, whereby one species affects another by altering the abundance of a third species; behavioural effects, whereby one species affects another by altering the behaviour of a third species; and chemical response effects, which is a behavioural effect that has been chemically mediated (Miller and Kerfoot, 1987). 'MPE', then, is a broad term for predator-prey interactions, but in this thesis I am concerned with the behaviour of a single prey species in the presence of multiple predators of different species.

1.4 Objectives

This thesis examines the behavioural response and relative risk of predation of juvenile cod when exposed to different types and densities of predators, both from the applied perspective of aquaculture and the theoretical perspective of emergent effects. In

Chapter 2, I set out to determine whether or not differences exist in schooling behaviour and mortality of hatchery-reared Atlantic cod (*Gadus morhua*), a wild conspecific, and Rock cod (*Gadus ogac*), when exposed to a predator based on their previous experience. Chapter 3 examines the theoretical question of multiple predator effects by looking at the schooling behaviour and relative mortality of hatchery-reared Atlantic cod in a multiple predator environment. Some data from Chapter 2 is used for comparison in Chapter 3; the purpose of this was to limit the number of fish used in the experiment. The impacts of previous experience on anti-predator behaviour and fitness are examined.

CHAPTER 2

Intra- and interspecific differences in predation risk: A comparison of wild and naive fish

2.1 Introduction

Predator-prey interactions shape aquatic communities in complex ways (Sih *et al.*, 1998). These interactions can have both direct and indirect effects on individuals and communities. Predators can directly affect prey mortality, and indirectly affect different behaviours, including feeding, schooling, refuge use, and habitat choice (Sih, 1987). The environment in which these relationships develop has fitness and behavioural consequences, influenced by life history and experience of both predator and prey.

Differences between the anti-predator behaviour of different species are due to unique predation pressures. Two congenerics, juvenile Atlantic (*Gadus morhua*) and rock cod (*Gadus ogac*), have few morphological differences while at a young age, but very different early life histories (Methven and McGowan, 1998). While *G.morhua* spawns offshore and has pelagic eggs, *G.ogac* spawns inshore and has demersal eggs (Scott and Scott, 1988). Laurel *et al.* (2004a) suggests this difference may explain differences in habitat use and aggregative behaviour between the two species. These differences in behaviour and habitat use reflect the variation in predation pressure experienced by the species, and the magnitude of predatory threat in the wild from such predators as larger cod, sculpin and seals (Scott and Scott, 1988). Atlantic cod are also raised in hatcheries, where they never experience the threat of predation in a wild environment. These naïve cod do not have the experience necessary to develop behavioural mechanisms that enable wild fish to recognize and evade predators effectively. Although anti-predator behaviours are based on unlearned predispositions, for example, the fast-start startle response (Eaton *et al.*, 1977), they are also shaped through experience (Magurran, 1990; Olla *et al.*, 1998). The exact nature of hatchery-reared behavioural deficits is unknown, but likely involves the inability to recognize predators, to discern their level of motivation, and poor anti-predator behaviours, including schooling (Olla *et al.*, 1998). In fact, Thompson and Tufts (1967) found that a predator may even switch from wild to reared prey when they are available, presumably due to the ease of their capture. Attempts to release hatchery-reared fish into the wild have largely been met with failure, due to high predation rates and their inability to forage on live prey (Svåsand and Kristiansen, 1990). Skreslet *et al.* (1999) concluded from a survival study on stocked wild Atlantic cod that release programs provided no potential for commercial fishing due to predation and cannibalism. Fortunately, some research shows that these anti-predator behavioural deficits can be corrected with predator "training"; that is, acquainting prey with a predator in either a contact or non-contact situation (Benzie, 1965; Järvi and Uglem, 1993; for review see Maynard *et al.*, 1995). Järvi and Uglem (1993) were able to improve the anti-predator behaviour of reared Atlantic salmon smolts with one-predator non-contact training, and even more so with contact training. Olla *et al.* (1992) found that coho salmon smolts that had previously survived predation avoided it in greater numbers upon reintroduction to a predator, and were also able to avoid capture longer. Even training with a predator model (Kanayama and Tuge, 1968) or with the visual, chemical and tactile stimuli associated with predation is sufficient to see some improvement in mortality rates (Olla *et al.*, 1998).

Although the behavioural differences of hatchery-reared and wild fish have been studied in the past, much of this research concerns salmon. Many anti-predator behaviour studies involve only one predator and one or two prey at a time, which is an unlikely situation in the wild. Additionally, these comparison studies often expose prey to predators in a non-contact situation (behind a plexiglass divider or net) making estimations of relative mortality impossible. The two studies that do specifically address differences between wild and hatchery cod both only used one predator (Nordeide and Svåsand, 1990; Nødtvedt *et al.*, 1999), while Schoener (1989), in a review of food web literature, found a median of 2-3 predator taxa feeding on a given prey taxon. There has been some research on the behavioural differences of hatchery-reared and wild cod, but these studies have found conflicting results. Nødtvedt *et al.* (1999) found that hatchery-reared juvenile cod maintained closer distances to an adult cod than did wild cod, in contrast to the findings of Nordeide and Svåsand (1990) that hatchery-reared cod were more vigilant and stayed further away from an adult cod.

Recent interest in aquaculture has increased due to the declining condition of fish stocks and fisheries worldwide (Brown *et al.*, 2003). While some of these efforts focus on full-cycle ("egg to plate") aquaculture, others programs are being developed for release, i.e. a "put and take" fishery (Svåsand *et al.*, 1998). The mass production of cod has been possible since the early 1980s, and enhancement initiatives have been attempted in Norway, Iceland, the United States and Canada (Svåsand and Kristiansen, 1990; Brown and Laland, 2001). The rates of juveniles surviving to adulthood in these initiatives have been notoriously low, some as little as 5% (for review see Brown and Laland, 2001). One study that investigated the mortality of released reared and wild cod found a similar mortality pattern, but also noted that reared cod mortality was often due to larger cod and short-horn sculpin (Larsen and Pedersen, 2002). However, Atlantic salmon survival from egg to smolt in the hatchery can reach 50%, while it is less than 1% in the wild (Piggins and Mills, 1985). Skreslet *et al.* (1999) found higher rates of angling returns for tagged wild cod versus reared cod, suggesting that more reared cod suffered natural mortality due to predation, and similar results have been found for Atlantic salmon (in Svåsand *et al.*, 1998). Mortality for hatchery-reared fish is highest immediately after release (Howell, 1994; Olla *et al.*, 1998). For these initiatives, it is of the utmost importance that reared individuals are equipped for surviving in a wild environment (Olla *et al.*, 1998).

This study will investigate the schooling behaviour and mortality rates of wild Atlantic cod, wild rock cod and hatchery-reared, naïve Atlantic cod under multiple predator threat. I predict that the schooling behaviour of naïve cod will be lacking in comparison to wild individuals of either species, and that this will be reflected in their relative mortality rates.

2.2 Materials and Methods

2.2.1 Experimental animals

Age 0 hatchery-reared juvenile Atlantic cod (*Gadus morhua*, 12.9±1.0 cm, s.d.) were obtained from the Aquaculture Research and Development Facility at the Ocean Sciences Centre in Logy Bay, Newfoundland (see Brown *et al.*, 2003 for hatchery protocols) in January, 2004. All wild prey were collected from Mosquito Cove, Trinity Bay in Newfoundland by beach seine. Wild age 0+ Atlantic cod (*Gadus morhua*, 10.7±1.7 cm, s.d.) were collected in May 2003 and age 0 rock cod (*Gadus ogac* 11.9±0.9 cm, s.d.) were collected in December of 2003. In the laboratory, fish were maintained in a holding tank (2 m x 1.5 m x 0.4 m) lined with a sand substrate and fitted with a flow-through system at ambient seawater temperature (8.9 ±0.3°C Standard Error of the Mean).

Wild and hatchery-reared fish were held separately. Wild cod (prey) were fed chopped herring approximately 3-5 times per week, depending on water temperature, while hatchery-reared prey were fed pellets daily, as per hatchery practice. These different feeding regimes were maintained in an attempt to preserve the "wild" and "naïve" dispositions of the prey.

When small and large Atlantic cod occur together naturally, intercohort cannibalism is often observed in the hatchery environment (Howell, 1984). Therefore, age 2+ Atlantic cod (39.5±5.9 cm, s.d.) were used as predators in this study, along with Shorthorn sculpin (*Myoxocephalus scorpius* 28.3±2.7 cm, s.d.). Blom and Folkvold (1997) found that a size factor ratio of 1.6-3.2 was sufficient to observe intercohort cannibalism in cod, and most of the predators were in this range. Both species of predator were collected from the wild to ensure they had previous experience as predators. Older Atlantic cod were collected off the eastern shore of Newfoundland by angling. Shorthorn sculpin were collected by SCUBA. Adult cod and sculpin were held in separate tanks with a flow-through system at ambient temperature (6.7±0.2°C SEM and 8.8±0.4°C SEM, respectively). Predators were fed minimally (once or twice per week) with chopped herring over the course of the experiments to maintain predator motivation. All prey and predators were subject to ambient daylight light levels and photoperiod.

2.2.2 Experimental set-up

The experimental set-up consisted of a tank on a flow-through system (10.5±0.2°C SEM) divided into three sections: the experimental arena (2 m x 1.5 m x 0.4 m) and two smaller compartments (0.6 m x 0.9 m and 0.6 m x 1.1 m) that open to the experimental arena with a removable opaque divider. The experimental arena was fitted with artificial eelgrass, constructed by attaching lengths of polypropylene rope (W: 0.8 cm H: 20 cm) to galvanized wire fencing at a density of 400 stems/m^2. Sand (< 1 mm) was washed and placed in the tank to cover the bottom 10cm (Gotceitas *et al.*, 1997; Laurel *et al.*, 2004a). This set-up has been used successfully in previous studies (Gotceitas *et al.*, 1995; Gotceitas *et al.*, 1997; Laurel *et al.*, 2004a).

2.2.3 Schooling behaviour trials

To determine the effects of predators on schooling behaviour, naïve and wild prey were observed in the presence of different densities of sculpin. All trials were conducted at the Ocean Sciences Centre in Logy Bay, Newfoundland. Each prey group (wild Atlantic cod, rock cod, or naïve cod), composed of five individuals, was tested in three treatments: no predator (control), in the presence of one adult sculpin, and in the presence of two adult sculpin. No individual juvenile cod was exposed to more than one trial with a predator, although some juveniles participated in both a control and one experimental trial. Trials were conducted during daylight hours between July 8 and August 7, 2003 (wild prey) and January 20-February 18, 2004 (rock cod and hatchery cod). The trials were conducted in a rotation that precluded the use of the same predator more than once in a day. A total of eight trials were run for each treatment (n=8).

The trials were digitally video recorded by a camera fitted with a wide-angle lens mounted 1.9 m directly above the experimental arena, so that the view of the camera was directly perpendicular to the tank (Laurel *et al.*, 2004a, 2005). The tank was illuminated by two 500 Watt halogen lights placed on either side of the tank. Five juvenile cod were selected without known bias and were transferred to the experimental arena by dip netting and remained undisturbed for a 15-minute acclimation period. This period allowed the fish a chance to acclimate to the water temperature and settle down after the tank transfer. The predator(s) were also acclimated for the same duration in the smaller predator compartment, with the opaque partition in place. Once the partition was removed and the predator entered the experimental arena, the trial began. Trial duration was one hour.

2.2.4 Video analysis and computations

For the video analysis, an hour-long trial was sampled once every 6 minutes (n = 10 observation periods/trial), modified from Laurel *et al.* (2004a). The location of each juvenile in the tank and water column (x, y coordinates), angle of orientation (in degrees, relative to the centre of the tank) and distance to predator was determined using Matrox Inspector 3.0 image analysis software. Distances between fish were measured from head to head. All observation periods where 2 or fewer juveniles could be located were excluded from the analysis. This occurred when prey swam in the shadowy areas around the edges of the tank.

Measures of aggregation and orientation were modified from Laurel *et al.* (2004a). The mean inter-prey distance was calculated by taking the mean of all possible distances from each juvenile to any other juvenile in an observation period and averaging them over the course of a trial. Low values of the mean inter-prey distance indicate that prey were tightly aggregated, while high values indicate little aggregation. The extent to which juveniles were commonly orienting in the same direction was measured by calculating the mean inter-prey angle difference, which is the mean of the acute angle differences from one reference fish in an observation period averaged over the course of a trial. The value for absolute angle difference were adjusted by subtracting from 360 if they were greater than 180 degrees. Low values of the mean inter-prey angle difference indicated that prey were more commonly oriented while high values reflected divergent orientations. Distance to the predator is reported as a mean distance to a predator for the two predator trials and all distance to predators are averaged over the course of a trial.

2.2.5 Cod predation trials

Mortality trials were run concurrently with the behavioural trials, where sculpin was the predator. Trials with cod as a predator were also conducted, with the result that each juvenile group (wild Atlantic, rock, and naïve cod) was exposed to six treatments: the three described in the schooling experiment, plus the presence of one adult Atlantic cod, two adult Atlantic cod, and a mixed trial of one cod and one sculpin. Mortality was measured by counting the number of prey consumed by the predator over the course of the one hour trial. Due to ethical concerns, an "LD50-like measure" was used (time to mortality of 50% of juveniles) to ensure that no more that 50% of the prey were consumed. There was no need to terminate experiments early (the LD50) since only two trials saw this criterion exceeded.

2.2.6 Experience trials

Hatchery-reared Atlantic cod that had now been exposed to a predator once and survived were retested under the same experimental conditions. The time elapsed from the previous exposure ranged from a potential 1-14 days. A set of trials (n=4) was conducted for each of two experimental treatments (no predator, one sculpin). Trial duration was 15 minutes. Each trial was sampled once every 3 minutes (n = 5 observation periods/trial). Video analysis, computations and comparisons were conducted as mentioned above.

2.2.7 Statistics

All comparisons were made using the General Linear Model ANOVA, using Minitab v.14.0. The residuals were examined in all cases to determine whether or not the assumptions (normality, homogeneity of variance and independence of residuals) of the model were met. In any cases where the assumptions were not met, and the obtained p-value was close to the criterion alpha of 5% (within a factor of 5), the decision was made *a priori* to obtain a new p-value by randomization of the response variable. Randomization is a technique testing the chance of a type I error (rejection of a true null hypothesis) by repeated recalculation of a statistic with the response variable randomized between all treatment levels, which is then compared with the original obtained statistic. The p-value is the likelihood of obtaining the observed F due to chance based on the new distribution (more details see Sokal and Rolfe, 1995). As the new p-value in the case of randomization is based upon a distribution constructed from the raw data, it is free of assumptions regarding the distribution. A minimum of 1000 computations was used to create the new distribution. In the case that the assumptions were not met and the obtained p-value was far from alpha (greater than a factor of 5), no randomization was conducted since a randomized p-value in this case would not alter the decision regarding statistical significance. This is due to the fact that randomization rarely changes an obtained p-value by more than a factor of five in either direction.

2.3 Results

2.3.1 Schooling behaviour

The presence of schooling was assessed by examining two aspects of this behaviour: aggregation (inter-prey distance, IPD) and orientation (inter-angle differences, IAD). The normality assumption was violated in testing for differences in the IPD, therefore randomizations were carried out. In terms of the IPD, no interaction was found between prey type and treatment ($F_{4,66} = 1.53$, $p = 0.192$ by randomization (1000 iterations), Figure 2.1). There was a main effect of prey type ($F_{2,66} = 68.85$, $p = 0.001$ by randomization (1000 iterations), Figure 2.1), and the confidence intervals show the higher IPD is for naïve fish compared to either wild species ($CI_{naïve\_cod}$ {84.377 cm < IPD < 91.779 cm} = 95%, $CI_{atlantic\_cod}$ {50.470 cm < IPD < 56.344 cm} = 95%, CI_{rock_cod} {51.840 cm < IPD < 58.711 cm} = 95%). There was no effect of predator type ($F_{2,66} = 0.45$, $p = 0.641$). For the IAD, all the assumptions of the GLM were met. A similar trend in the differences for IPD was found for differences in orientation (Figure 2.2). The interaction was not significant ($F_{4,63} = 1.90$, $p = 0.121$), but a significant main effect of prey type on mean IAD ($F_{2,63} = 23.01$, $p = 0.001$) was found, while there was no significant effect of the number of predators present ($F_{2,63} = 1.46$, $p = 0.241$; Figure 2.3).

2.3.2 Distance to predator

The p-values obtained through the GLM are unreliable as the normality assumption was violated, therefore p-values were obtained by randomization (1067 iterations). The interaction of prey type and number of predators on the distance to predator was not significant ($F_{2,42} = 1.56$, $p = 0.23$ by randomization; Table 2.1). There was, however, an effect of number of predators ($F_{1,42} = 11.35$, $p = 0.0046$ by randomization; Figure 2.4). Wild Atlantic cod had the smallest difference in distance from one predator to two predators. Naïve cod had a larger difference, although they generally were closer to their predators overall and rock cod showed the greatest difference in distance when two predators were present. Overall, wild Atlantic cod kept the greatest distance from their predators, regardless of how many were present (mean

112.46cm±1.69 SEM). Wild rock cod and naive cod kept similar distances from predators (mean 105.43cm±1.86 SEM and 101.70cm±2.36 SEM, respectively), although naive cod remained slightly closer overall to the predator. In general, when two predators were present, prey were less able to keep their distance (118.99cm±1.76 SEM for one predator, 99.73cm±1.38 SEM for two predators).

Additionally, naïve cod were observed to behave unusually in the experimental tank. They more frequently engaged in freezing behaviour (no movement) in the presence of a predator, and were observed swimming into tank corners. Freezing behaviour was noted four times in the naïve prey trials and was not observed in the wild trials. Naïve prey were also more often sighted resting at the water surface and on the sand substrate than wild prey, were observed to have difficulty swimming through the eelgrass and also became lodged in the mesh-wire base of eelgrass mats. This was also observed with wild prey, although less frequently (two wild fish versus 17 naïve fish). In these entanglement cases, the fish were omitted from the analysis.

2.3.3 The role of experience

"Experienced" naïve cod, i.e. those that had been exposed to a predator once, were re-examined under the same treatments in a small study (Table 2.2). The normality assumption of the GLM was not met for the mean inter-prey distance and mean inter-angle difference, but due to the obtained p-values being so far from the criterion alpha, randomization would not alter the outcome; therefore a decision was made not to undertake randomization. Differences in behaviour were observed from the purely naïve cod. In general, there was an effect of experience on orientation, as experienced fish were more commonly oriented ($F_{1,20} = 9.62$, $p = 0.006$). There was no interaction of experience and number of predators ($F_{1,20} = 2.97$, $p = 0.100$), and no difference in the behaviour when a predator was present (treatment, $F_{1,20} = 0.03$, $p = 0.871$) (Figure 2.5a). No significant interaction of experience and number of predators ($F_{1,20} = 0.10$, $p = 0.754$) was found on the IPD, and no main effects ($F_{1,20} = 0.09$, $p = 0.766$ for experience; $F_{1,20} = 0.04$, $p = 0.847$ for number of predators) (Figure 2.5). For distance to the predator, the normality assumption was violated. Experienced naïve prey did differ in the distance kept from the predator than naïve prey (67.42±3.53cm SEM for naïve prey and 89.74±4.39cm SEM for experienced prey), although this was not statistically significant ($F_{1,8} = 1.74$, $p = 0.224$). Given the smaller sample size for this experiment, it is possible that I did not have the power to detect real, but small, differences.

2.3.4 Cod predation trials

Predation was only observed in the naïve prey trials, despite the fact that the first half of the wild Atlantic cod trials were extended for an additional 23 hours after the initial 1-hour videotaping. When no predation was observed in this 24-hour period, the extended trials were discontinued.

Predation was observed in 50% of the naïve prey trials (16 in total with a predator present). All mortality was due to predation by sculpins, despite the fact that the cod predators were much larger. Even in the mixed predator trial, sculpin were responsible for all mortalities (Figure 2.6).

2.4 Discussion

Differences in schooling behaviour were found between wild and naïve cod. Naïve cod were much more loosely aggregated than either wild species, and were significantly less oriented in a common direction. Ryer and Olla (unpublished, in Olla *et* *al.*, 1998) found that the nearest-neighbour distance in a marine pelagic fish increased after a predator fright, and naïve salmon smolts were found to react more rapidly to the presence of a predator (Järvi and Uglem, 1993). However, both the addition of a predator and an increase in the number of predators resulted in increased aggregation (the mean inter-prey distance) for rock cod, but this was not true for naïve cod. Predator presence decreased aggregation in wild Atlantic cod from the no predator condition, but increased when the second predator was added. The orientation (mean inter-prey angle difference) results were much less clear. In general, both wild species became less commonly oriented with predator presence, and the naïve cod showed an initial increase in common orientation, but this decreased with the addition of a second predator.

In terms of the distance maintained from the predator, a significant effect of prey type was found. Rock cod demonstrated heightened vigilance in response to the addition of, and increase in numbers of, predators. In contrast, both wild and naïve Atlantic cod each had more subtle increases in distance. However, naïve cod still approached closer to the predator than either wild forms, and wild Atlantic cod maintained the greatest distance from the predators overall. This differs from Nordeide and Svåsand (1990) who found that reared (naïve) cod kept greater distances from an adult cod predator and were slower to approach the predator than wild cod when exposed in a non-contact situation. However, Nødtvedt *et al.* (1999) found that hatchery-reared (naïve) cod maintained a shorter distance to the predator, although this distance increased with increasing length of exposure to a predator. Cod used as naïve prey in both aforementioned studies were raised in the same outdoor pond using the same rearing protocol, so differences in behaviour must be attributable to the experimental environment. Nødtvedt *et al.* (1999)

exposed prey in a contact situation while in Nordeide and Svåsand (1990) the predators were separated from prey by a transparent plexiglass divider. Nødtvedt *et al.* (1999) experiment had artificial habitat and that may have provided the cover necessary for prey to safely approach the predator, unlike the unvegetated tank of Nordeide and Svåsand (1990). Prey may have felt more vulnerable with no refuge despite that fact that there was no direct risk of predation. Additionally, the size of predator used in my study is more similar to the size of predators used by Nødtvedt *et al.* (1999), as opposed to the larger predators used by Nordeide and Svåsand (1990), which may account for the similarities in results with Nødtvedt *et al.* (1999).

It appears that familiarity, or experience, plays a role in the behaviour of naïve cod toward a predator. Re-exposing previously-naïve cod (referred to as 'experienced') in the same experimental design showed that even minimal experience with a predator increases schooling behaviour, but the power of these tests was low due to the small sample size and future research should seek to replicate these findings. The mean inter-prey angle difference decreased, indicating that prey were more commonly orienting in the same direction. However, no increase was seen in the mean inter-prey distance. Unlike Nødtvedt *et al.* (1999), 'experienced' prey in this experiment stayed further away from the predator than they did when naïve, although it was not statistically significant. Nødtvedt *et al.* (1999) suggested that a 3-staged anti-predator behaviour learning process takes place whereby naïve cod initially demonstrate little vigilance in the presence of a predator, then too much, and finally have an appropriate, reasonable reaction. It is possible that after only one hour with a predator, the 'experienced' cod have benefited from predator experience. Olla and Davis (1989) found that one round of training was insufficient, but their training period was only 15 minutes. However, in the current study, no predation was observed during the course of the 'experienced' trials, indicating that a one-hour exposure to a predator was sufficient to develop at least some vigilance. The anti-predator behaviours of Atlantic salmon smolts were improved by training both in a contact and non-contact situation (Järvi and Uglem, 1993), and the same was found for hatchery-naive coho and sockeye salmon (Olla and Davis, 1989; Ginetz and Larkin, 1976). However, improvements in anti-predator behaviours have only been evaluated in the laboratory, while a study attempting to reduce hatchery-reared brook trout predation in the wild was largely unsuccessful (Fraser, 1974).

The artificial eelgrass posed problems for the naïve prey that were unaccustomed to navigating a vegetated terrain. The "vegetated" habitat appeared to confuse some who got stuck both in the artificial grass and under the wire mesh to which the artificial grass was attached. For this reason, naïve prey may be avoiding the grass while wild prey are using it as a refuge. Braithwaite and Salvanes (2005) found that experience with a cobble and kelp substrate promoted improved anti-predator behaviours from hatchery-reared cod and it is possible that prey in the experience trials benefited from the time with the cobble/eelgrass substrate. The 'experienced' prey gained their experience in naïve trials, where some of their counterparts were eaten by sculpins, therefore these prey have also witnessed and survived predation. There is evidence that simply being with other prey that have survived predation, such as these experienced prey, may improve the anti-predator behaviours of naïve fish (Patten, 1977; Olla *et al.*, 1998; Kelley *et al.*, 2003). It is possible for fish to retain this knowledge to escape direct contact with a predator at a later time (Brown and Laland, 2001).

Differences were found between wild Atlantic cod and wild rock cod, which may be related to their different life histories. Trials were first attempted with a mix of Atlantic cod and rock cod, but the species were observed to school separately, resulting in the separation of the wild species for the experiment. In general, rock cod responded more to the presence of a predator. An increase in aggregation was observed, and their degree of orientation fell between that of the wild Atlantic cod and their naïve counterparts. Rock cod have demersal eggs, and hatch in the environment they will inhabit. However, Mikhail and Welch (1989) describe rock cod as a non-schooling species in the wild, which is clearly the opposite of what was found in this laboratory study, and other field observations (Laurel *et al.*, 2003). Conversely, Atlantic cod have pelagic eggs and settle into inshore areas as juveniles. However, given the differences in the aggregation response to a predator and orientation means, I conclude that there are schooling differences between wild Atlantic cod and rock cod when a predator is present, although the cause of these differences is not obvious in my study.

Predation was observed only in the naïve prey trials, and was seen in 50% of these trials. This increase in mortality by predation is not surprising given that schooling behaviour has long been considered to provide protection from predation by reducing the per capita predation risk of prey with prey group size (Godin *et al.*, 1988; Pitcher and Parrish, 1993; Krause and Godin, 1995; Krause *et al.*, 1998). Non-schooling guppies have been found to have higher mortality rates (Seghers, 1974), and cod have been found to need less time catching prey alone than from a school (Radakov, 1958), an observation made in other fish species (Milinski, 1977).

All observed predation in my study was attributable to the sculpin predators; none was observed by older Atlantic cod. Despite the fact that juvenile cod have experience with cannibalism from larger juveniles in hatcheries (Howell, 1984), it is possible that this experience is insufficient for the development of generalized anti-predator behaviours. Nødtvedt *et al.* (1999) argue that experience in the hatchery causes habituation, resulting in little response to the presence of other cod of any size, and that this may apply to other predatory species. However, we should expect that cod would quickly learn that larger fish should be avoided in the hatchery due to cannibalism events. Even if habituation to conspecifics was occurring in the hatchery, demonstrating little vigilance in the presence of a "novel" predator would more likely be due to lack of experience than to habituation. In this study, the increase in mortality may be due in part to exposure to a sculpin. This is especially true since cod and sculpin use different predatory tactics, and the naïve prey would never have encountered an ambush predator such as sculpin. However, Nordeide and Salvanes (1991) found that newly released reared cod were subject to predation mainly by large cod, pollack and ling, but suggest that this is due to the novelty of an unfamiliar species and the size of the predators. In my study, larger cod were not often observed in the artificial eelgrass, likely due to their size. Sogard and Olla (1993) also found that adult pollock would not enter artificial eelgrass. It could be that this eelgrass avoidance by larger cod offered the prey an opportunity to avoid predation by cod that was unavailable when sculpin were present. Olla *et al.* (1998) state that predator recognition has a strong innate component that is somehow repressed by the rearing environment, and new research into the roles of different sensory cues has found that visual predator recognition skills are largely based on unlearned predispositions (Kelley and Magurran, 2003). However, as demonstrated by the improvement of anti-predator behaviours with predator exposure in the current study (and others), including reduced mortality, suggest that predator recognition is more of a learned than innate behaviour. It is possible, as suggested by Krause *et al.* (1998), that shoaling is an ineffective strategy for averting surprise (ambush) attacks, which may account for the predation credited to sculpin in this study.

In addition to reduced schooling, naïve prey frequently skimmed the water surface. While the motivation for this behaviour was not apparent, the resulting increase in activity may have contributed to the increased frequency of predation, as prey locomotion is positively correlated with possibility of their detection by predators (Lima and Dill, 1990). In fact, although Godin *et al.* (1988) found the shoal size that provided the most anti-predator benefit was 1-10 individuals, Krause and Godin (1995) found that it was not necessarily shoal size that determined what a predator would find attractive, but conspicuousness. Nødtvedt *et al.* (1999) found that predator-naïve cod had higher activity levels than wild cod in the presence of a free-swimming predator, increasing conspicuousness. However, when the predator was enclosed, the trend reversed and the wild cod were more active. Gotceitas *et al.* (1995) determined that juvenile wild cod are able to distinguish between a passive and an actively foraging predator, and it is possible that the wild fish were simply better able to assess the actual risk posed by the predator.

Understanding how hatchery-rearing influences the development of predator avoidance ability is crucial to "grow-and-release" aquaculture. The costs associated with predator training should be evaluated and weighed against the overall benefits, given that hatchery-rearing is an expensive undertaking (Wiley *et al.*, 1993). However, given the costs, outplanting success for release initiatives will be dependent upon, among other things, the behavioural capabilities of released fish. Therefore, it is important that hatchery-reared fish develop behavioural skills that reflect the life history of their species in the wild (Olla *et al.*, 1998). Given that the hatchery-reared cod in this study were the F_1 of wild brood stock, the differences in this study can be attributed to the hatchery environment, and not selective breeding, as has been suggested (Olla *et al.*, 1992). Reduced schooling of naïve cod and the increased susceptibility to predation indicate that current rearing protocols are insufficient for outplanting success. However, the benefits of schooling may also depend on the type of predator imposing the risk. Kelley *et al.* (2003) found that association with experienced conspecifics in the presence of a predator model improved anti-predator behaviours, including schooling, and the social transmission of predator recognition has been observed in a number of fish species (Brown and Laland, 2001). It may not be the sensory-deprived hatchery that causes behavioural deficits but the social deprivation, meaning that there may be benefit in both predator training and wild conspecific exposure to improve anti-predator behaviours.

Table 2.1 Results of GLM for the mean inter-prey distance, mean inter-angle difference and distance to predator for naïve versus wild prey. * Number in parentheses is the p-value obtained by randomization (1000 iterations for IPD and 1067 for distance to predator).

Response variable	Source	df	MS (adj)	F-value	p-value*
Mean inter-prey distance	Prey type	2	10388.5	68.85	0.000 (0.001)
	# of predators	2	67.6	0.45	0.641 (0.65)
	Prey type*# of predators	4	230.8	1.53	0.204 (0.192)
	Error	66	150.9		
Mean inter-angle difference	Prey type	2	2296.03	23.01	0.000
	# of predators	2	145.27	1.46	0.241
	Prey type*# of predators	4	189.87	1.90	0.121
	Error	63	99.77		
Distance to predator	Prey type	2	386.0	1.07	0.351 (0.35)
	# of predators	1	4074.0	11.35	0.002 (0.0046)
	Prey type*# of predators	2	560.5	1.56	0.222 (0.23)
	Error	42	359.1		

Table 2.2 Results of GLM for the mean inter-prey distance, mean inter-angle difference and distance to predator for naïve versus once-exposed prey.

Response variable	Source	df	MS (adj)	F-value	p-value
Mean inter-prey distance	Past experience	1	16.9	0.09	0.766
	# of predators	1	7.1	0.04	0.847
	Past experience*# of predators	1	18.8	0.10	0.754
	Error	20	185.8		
Mean inter-angle difference	Past experience	1	1419.0	9.62	0.006
	# of predators	1	4.0	0.03	0.871
	Past experience*# of predators	1	438.2	2.97	0.100
	Error	20	147.5		
Distance to predator	Past experience	1	938.5	1.74	0.224
	Error	8	539.7		

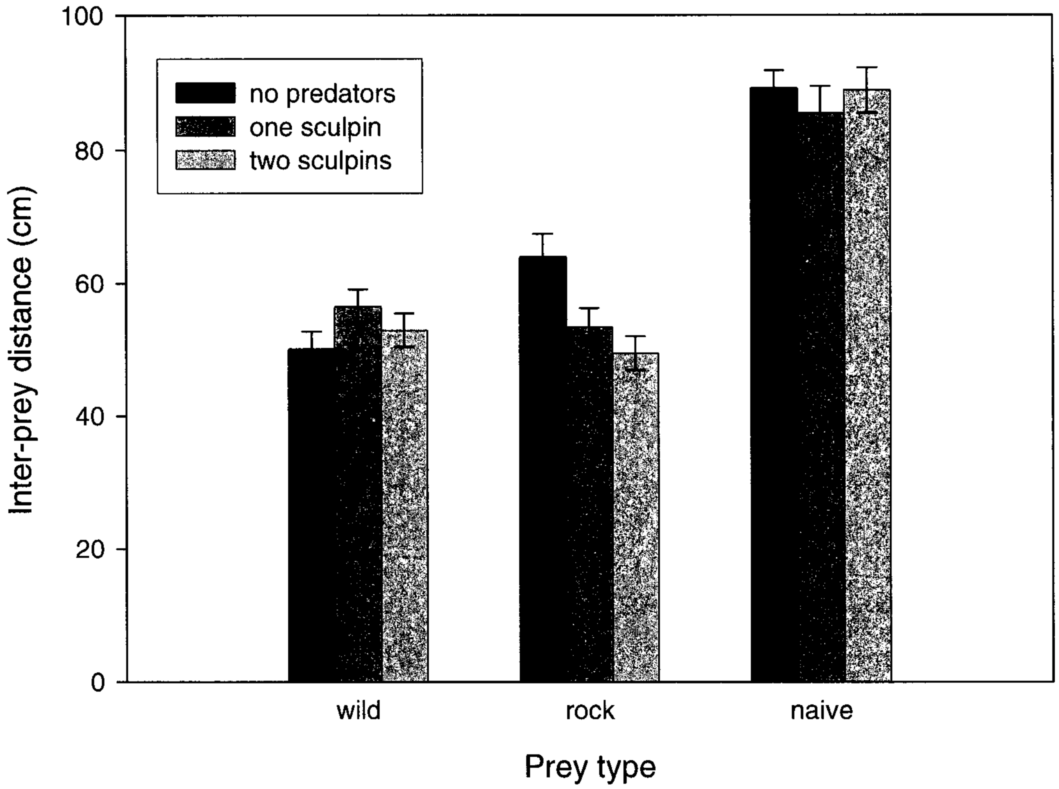

Figure 2.1 Differences in inter-prey distance (IPD) between wild Atlantic, rock and naïve cod under predation threat by zero, one or two sculpin predators. Columns represent group means ± SEM. See Table 2.1 for details.

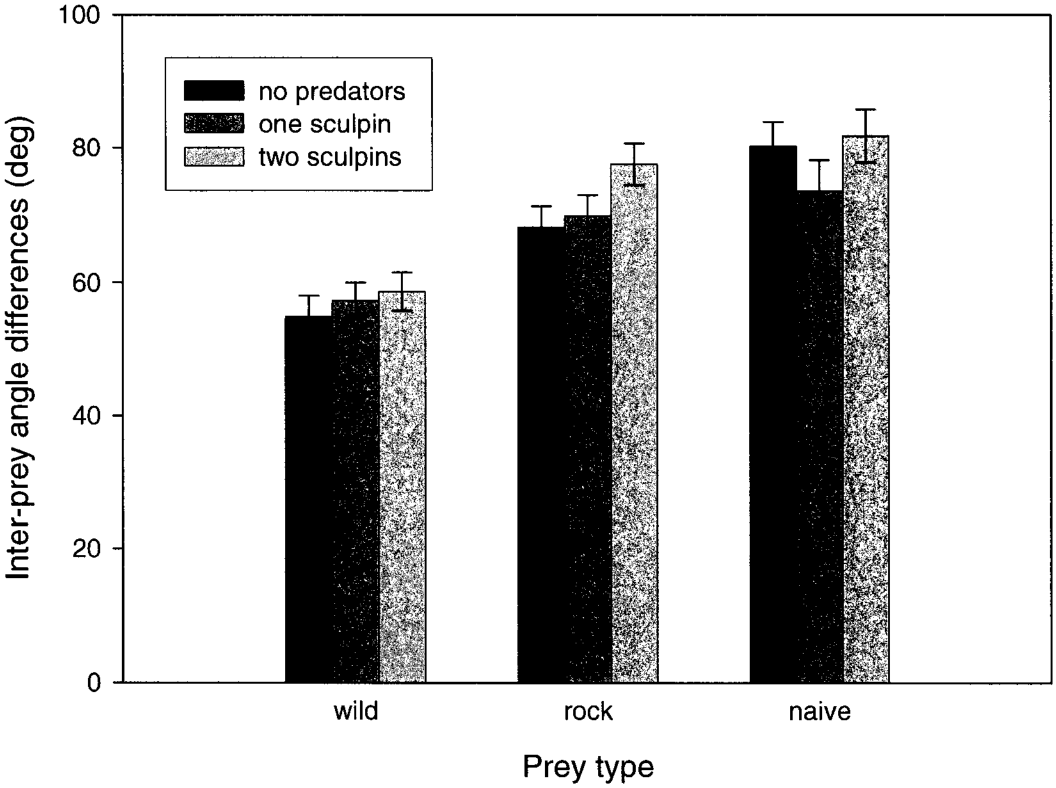

Figure 2.2 Differences in group orientation between wild Atlantic, rock and naïve cod under predation threat by zero, one, or two sculpin predators, as measured by the inter-prey angle difference (IAD). Columns represent group means ± SEM. See Table 2.1 for details.

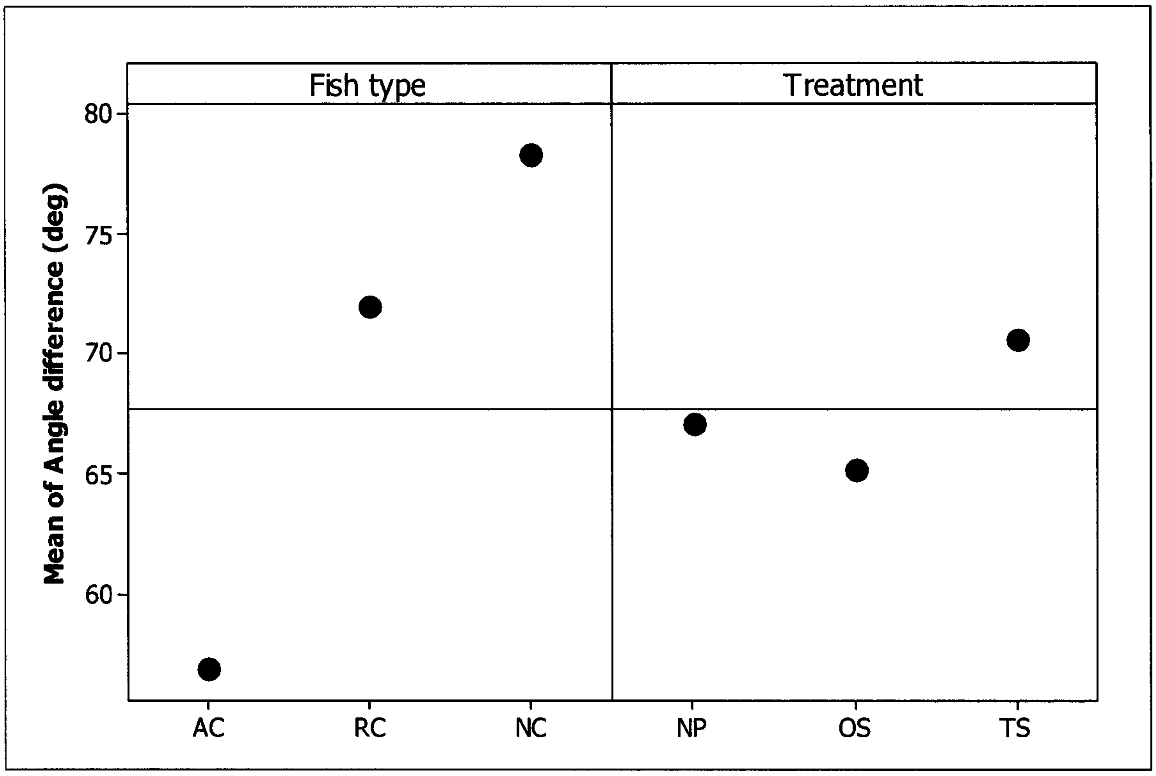

Figure 2.3 Main effects of prey type and predator density on the mean inter-prey angle difference. AC = Wild Atlantic cod, RC = Rock cod, NC = Naïve cod, NP = No predator, OS = One sculpin, TS = Two sculpin. Points represent group means and the line represents the overall mean, collapsed across fish type and predator density.

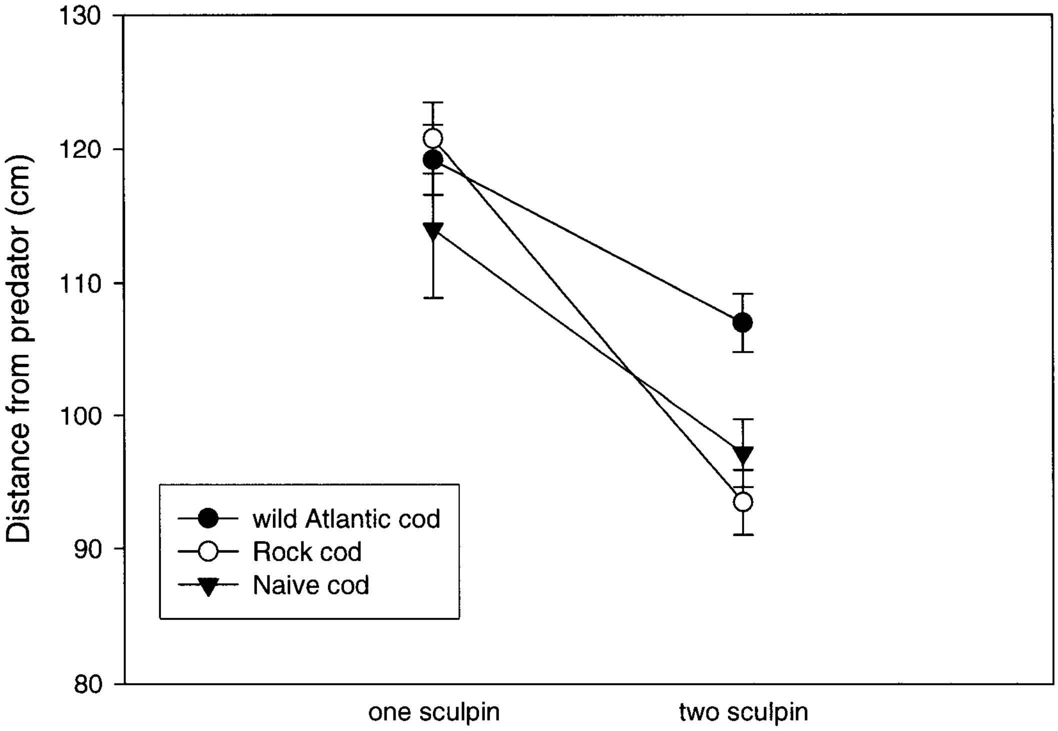

Figure 2.4 Distance to predator under different predator densities, by prey type. Points are group means ± SEM.

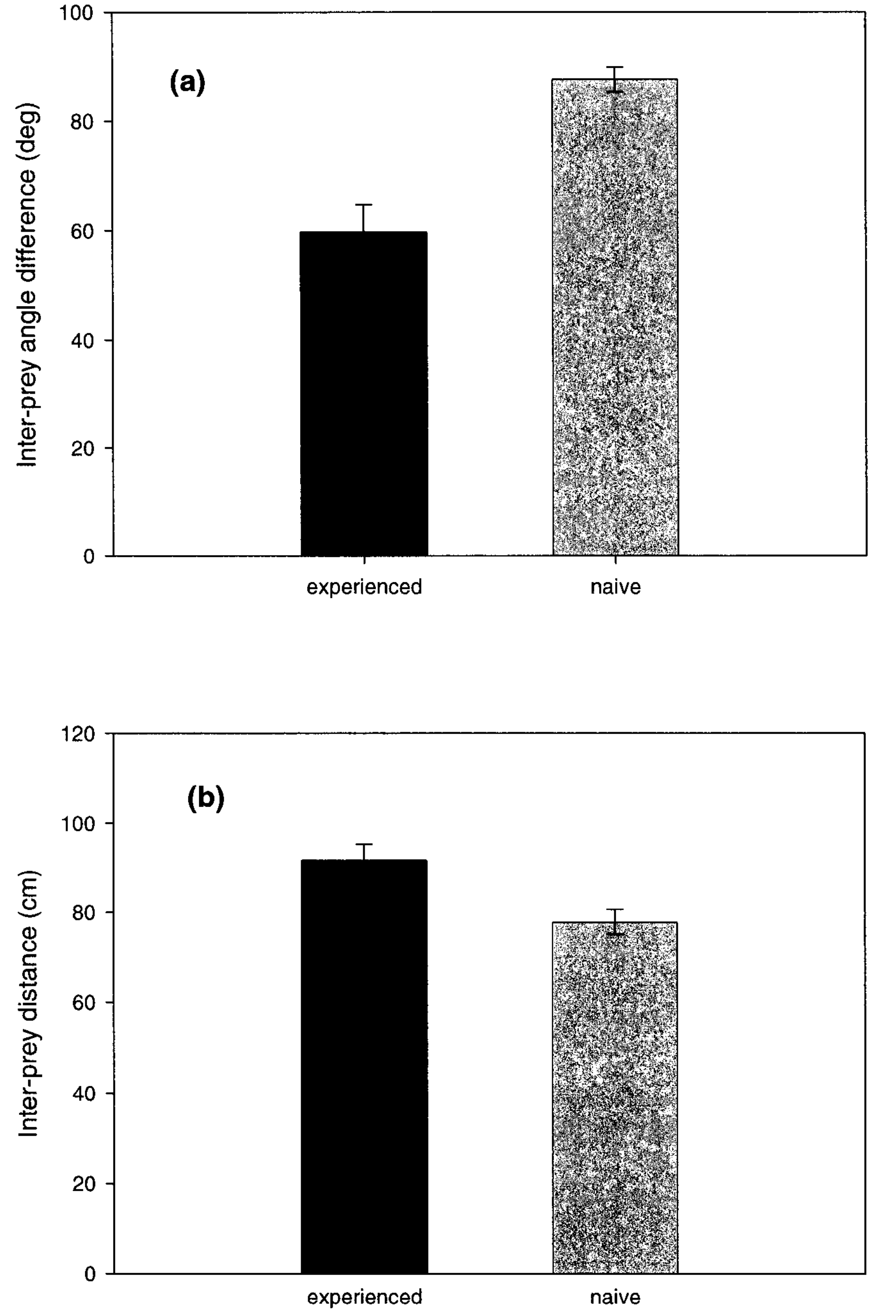

Figure 2.5 Change in the mean inter-prey angle difference (a) and inter-prey distance (b) after one predator exposure "event". Columns represent the mean of both control and predator trials, as no effects of predator presence were found, ± SEM. See Table 2.2 for details.

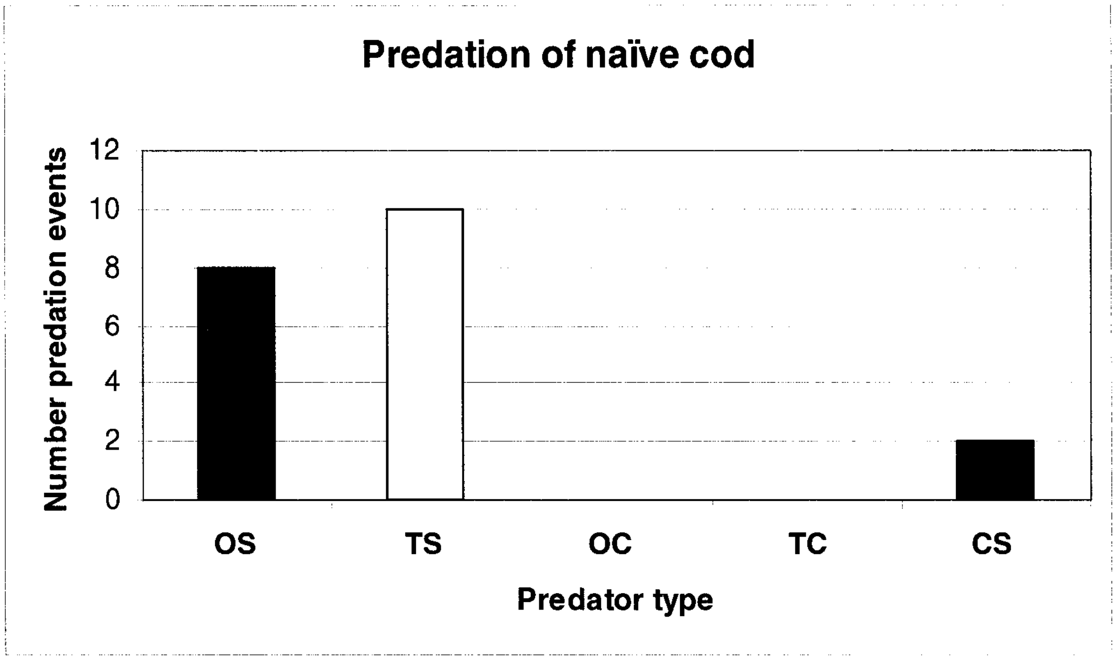

Figure 2.6 Number of predation events observed. Columns represent the sum of all predation events in a given treatment. OS = One sculpin, TS = Two sculpin, OC = One cod, TC = Two cod, CS = One cod and one sculpin.

CHAPTER 3

Multiple predator effects: Do non-additive effects exist on prey schooling behaviour and mortality rates?

3.1 Introduction

Predator-prey interactions have often been studied in aquatic systems in the presence of one species of predator despite the fact that most prey are subject to predation by more than one species (Sih *et al.*, 1998). Of those studies that do consider multiple predators, few consider more than one combination of predator (Relyea, 2003). Recently, researchers have begun to consider the fitness and behavioural consequences of living in a multiple predator environment and studies involving multiple predators have found that predator-prey relationships are less clear than once proposed.

Emergent, multiple predator effects (MPEs) are said to be present when the observed effect on prey cannot be predicted merely by summing the effects of the predators individually; that is, the presence of more than one predator results in either risk enhancement or risk reduction for the prey. Mutual interference among predators or predator facilitation results in emergent effects because the interaction of predators alters the number or prey consumed than if either had been present alone (Sih *et al.*, 1998). This interaction is dependent on predator foraging habitat and predation style. For instance, two ambush predators are least likely to create risk reduction because they will rarely encounter one another (Sih *et al.*, 1998). Other important factors influencing the outcome of MPE studies are predator density and habitat complexity. Prey mortality is typically the variable of interest in multiple predator studies, although some studies examine prey behaviour (Crowder *et al.*, 1997; González and Tessier, 1997; Peckarsky and McIntosh, 1998). Interest in how prey defenses affect MPEs has prompted the study of both lethal and sublethal effects of predator-prey interactions and the role of prey behaviour and the factors that influence it, such as habitat complexity (Sih *et al.*, 1998).

MPEs are influenced by many factors, including habitat complexity and prey defenses. Both bluegills and fathead minnows use structured habitats differently based on the type of predator present (Savino and Stein, 1982), and these predator-specific behavioural responses have also been observed in tadpoles (Relyea and Werner, 1999). Juvenile Atlantic cod specifically utilize areas of greater habitat complexity as a refuge from predators, and increased survival is observed in such habitat (Gotceitas *et al.*, 1997; Laurel *et al.*, 2004a). In the presence of a predator juvenile Atlantic cod will increase refuge use, differentially utilizing eelgrass compared to sand and gravel in the absence of a predator (Gotceitas *et al.*, 1997). Recent experiments show that juvenile cod will also aggregate more often over sand habitat than eelgrass habitat in the presence of a predator suggesting habitat-specific anti-predator behaviour (Laurel *et al.*, 2004a). It has also been shown that juvenile cod will occupy different areas of the water column in response to exposure to different predators (Laurel, 2003). These observations indicate that prey, including juvenile Atlantic cod, restrict their habitat use based on the habitat type and the degree of risk posed by the type of predator; that is, they are actively responding to the level of predation risk.

Most research concerning MPEs has focused on insects as prey and other insects or terrestrial organisms as predators. The results from these studies vary greatly, some finding support for emergent MPEs (Soluk and Collins, 1988; Martin *et al.*, 1989; Soluk, 1993; Crowder *et al.*, 1997) and others nonemergent effects (Van Buskirk, 1988; Sokol-

Hessner and Schmitz, 2002). The evidence to date indicates that general principles describing MPEs may be inadequate or limited to certain prey and predator taxa (Soluk 1993). MPE studies aid in determining the relative frequency of indirect effects (Wooton, 1993) when a third predator is involved. Currently, no research addresses the question of emergent MPEs in Atlantic cod.

This study will investigate juvenile Atlantic cod anti-predator behaviour and mortality under multiple predator threat in a substitutive design. Relyea's (2003) review paper of multiple predator studies did not find one study that controlled for predator density. A substitutive design allows for this as it is essentially a factorial design, with replacement. For this study I used the substitutive design to test the hypothesis that contrasting foraging strategies (pursuit versus ambush) of the predators and familiarity with one of the predators on the part of the prey will result in nonlinear, emergent effects on anti-predator behaviours and relative mortality rates.

3.2 Materials and Methods

3.2.1 Experimental animals

Hatchery-reared juvenile Atlantic cod (*Gadus morhua*, 12.9±1.0 cm, s.d.) were obtained from the Aquaculture Research and Development Facility at the Ocean Sciences Centre in Logy Bay, Newfoundland (see Brown *et al.*, 2003 for hatchery protocols) in January, 2004. Wild juveniles were preferred for this experiment, but low catch rates in Terra Nova National Park necessitated the use of hatchery-reared fish as prey. In the laboratory, fish were maintained in a holding tank (2 m x 1.5 m x 0.4 m) with a sand substrate and fitted with a flow-through system at ambient temperature (5.7±0.2°C

Standard Error of the Mean (SEM)). Fish were fed pellets daily to satiation, as per hatchery practice.

When small and large Atlantic cod occur together naturally, intercohort cannibalism is often observed (Howell, 1984). Blom and Folkvold (1997) found that a size factor ratio of 1.6-3.2 was sufficient to observe intercohort cannibalism in a hatchery environment; therefore, the adult cod (39.5±5.5 cm, s.d.) and shorthorn sculpin (*Myoxocephalus scorpius* 28.1±2.7 cm, s.d.) predators in this study were mostly in this range. All predators were collected from the wild to ensure they had previous wild experience. Larger Atlantic cod were collected off the eastern shore of Newfoundland by angling, and shorthorn sculpin were collected by divers. Adult cod and sculpin were held in separate tanks with a flow-through system at ambient temperature (5.7±0.2°C SEM, 8.3±0.4°C SEM, respectively). Predators were fed once or twice per week with chopped herring over the course of the experiments. Prey and predators were subject to ambient daylight light levels and photoperiod.

3.2.2 Experimental set-up

The experimental set-up consisted of a tank on a flow-through system (8.0±0.2°C SEM) divided into three sections: the experimental arena (2 x 1.5 x 0.4 m) and two smaller compartments (0.6 m x 0.9 m and 0.6 m x 1.1 m) that open to the experimental arena with a removable opaque divider. The experimental arena was fitted with artificial eelgrass, constructed by attaching lengths of polypropylene rope (W: 0.8 cm H: 20cm) to galvanized wire fencing at a density of 400 stems/m^2. Sand (< 1 mm) was washed and placed in the tank to cover the bottom 10cm (Gotceitas *et al.*, 1997; Laurel *et al.*, 2004a).

This set-up has been used successfully in previous studies (Gotceitas *et al.*, 1995; Gotceitas *et al.* 1997; Laurel *et al.*, 2004a).

3.2.3 Behavioural trials

To determine the effects of multiple predators on schooling behaviour, naïve prey were observed in the presence of different densities and combinations of older sculpin and cod. Prey were tested in six treatments: no predators (control), in the presence of one type of predator (Atlantic cod or shorthorn sculpin), in the presence of another predator (sculpin), in the presence of both predators (cod and sculpin), and in the presence of two of the same predator. This last treatment accounted for predator density. The control, one and two sculpin trials were initially conducted in Chapter 2 experiments, and these data are being used here for comparison. This was done to limit the number of fish used as experimental animals, as required by animal care regulations. No juvenile cod was exposed to more than one trial with a predator, although some juveniles participated in both a control and one experimental trial. Trials were conducted during daylight hours between February 2-18, 2004. The trials were conducted in a rotation that precluded the use of the same predator more than once in a day. A total of eight trials were run for each treatment (n=8).

The trials were digitally video recorded by a camera fitted with a wide-angle lens mounted 1.9 m directly above the experimental arena, so that the view of the camera was directly perpendicular to the tank (see also Laurel *et al.*, 2004a, 2005). The tank was illuminated by two 500 Watt halogen lights placed on either side of the tank. Five naïve juvenile cod were transferred to the experimental arena by dip netting and remained undisturbed for a 15 minute acclimation period. The predator(s) were also acclimated for the same duration in the smaller predator compartment, with the opaque partition in place. This period allowed the fish a chance to acclimate to the water temperature and settle down after the tank transfer. Once the partition was removed and the predator entered the experimental arena, the trial began. Trial duration was one hour.

3.2.4 Video analysis and computations

For the video analysis, the hour-long trial was sampled once every 6 minutes (n = 10 observation periods/trial), modified from Laurel *et al.* (2004a). The location of each juvenile in the tank and water column (x, y coordinates), angle of orientation (in degrees, relative to the centre of the tank) and distance to predator was determined using Matrox Inspector 3.0 image analysis software. Distances between fish were measured from head to head. All observation periods where 2 or fewer juveniles could be located were excluded from the analysis. This occurred when prey swam in the shadowy areas around the edges of the tank.

Measures of aggregation and orientation were modified from Laurel *et al.* (2004a). The mean inter-prey distance was calculated by taking the mean of all possible distances from each juvenile to any other juvenile in an observation period and averaging them over the course of a trial. Low values of the mean inter-prey distance indicate that prey were tightly aggregated, while high values indicate little aggregation. The extent to which juveniles were commonly orienting in the same direction was measured by calculating the mean inter-prey angle difference, which is the mean of the acute angle differences from one reference fish in an observation period averaged over the course of a trial. The values for absolute angle difference were adjusted by subtracting from 360 if they were greater than 180 degrees. Low values of the mean inter-prey angle difference indicated that prey were more commonly oriented while high values reflected divergent orientations. Distance to the predator is reported as a mean distance to a predator for the two predator trials all distance to predators are averaged over the course of a trial.

3.2.5 Cod predation trials

Predation was observed throughout the behaviour trials. Mortality was measured by counting the number of prey consumed by the predator over the course of the one hour trial in each of the six experimental treatments. Due to ethical concerns, an "LD50-like measure" was used (time to mortality of 50% of juveniles) to ensure that no more that 50% of the prey were consumed. There was no need to terminate experiments early (the LD50) since only two trials saw this criterion exceeded.

3.2.6 Statistics

All comparisons were made using the General Linear Model ANOVA, using Minitab v.14.0. The residuals were examined in all cases to determine whether the assumptions of the model were met. In any cases where the assumptions were not met, and the obtained p-value was close to the criterion alpha of 5% (within a factor of 5), the decision was made *a priori* to obtain a new p-value by randomization of the response variable. Randomization is a technique testing the chance of a type I error (rejection of a true null hypothesis) by repeated recalculation of a statistic with the response variable randomized between all treatment levels, which is then compared with the original obtained statistic. The p-value is the likelihood of obtaining the observed F due to chance based on the new distribution (more details see Sokal and Rohlf, 1995). As the new p-value in the case of randomization is based upon a distribution constructed from the raw data, it is free of assumptions regarding the distribution. A minimum of 1000 computations was used to create the new distribution. In the case that the assumptions were not met and the obtained p-value was far from alpha (greater than a factor of 5), no randomization was conducted since a randomized p-value in this case would not alter the decision regarding statistical significance. This is due to the fact that randomization rarely changes an obtained p-value by more than a factor of five in either direction.

3.3 Results

3.3.1 Schooling behaviour

The effect of predator type and density on schooling behaviour was assessed by examining the aggregation of the prey and their orientation in the presence of either or both types of predators (Table 3.1). Aggregation was assessed using the mean inter-prey distance (IPD), and orientation by the mean inter-prey angle difference (IAD) as described above. The assumptions of the GLM were not met for these variables as the residuals were non-normally distributed. Randomizations were undertaken (1000 iterations) and these p-values are reported. No interaction was found between the presence of cod and sculpin as predators on the IPD ($F_{1,28} = 2.19$, $p = 0.14$ by randomization) and there were no significant main effects ($F_{1,28} = 1.69$, $p = 0.199$ for sculpin by randomization and $F_{1,28} = 0.38$, $p = 0.532$ for cod by randomization, see Figure 3.1). Overall, a 7-13 cm difference in the IPD is observed, although it is not significant (85.5±4.0 cm SEM for one sculpin, 79.9±2.9 cm SEM for one cod, 92.1±3.1 cm SEM for cod and sculpin). In terms of prey orientation, no significant interaction was found between the presence of sculpin and cod ($F_{1,28} = 0.25$, $p = 0.642$ by randomization). A main effect of the cod predator was initially observed for the IAD, but statistical significance was lost upon randomization ($F_{1,28} = 4.42$, $p = 0.053$, by randomization).

However, prey were less commonly oriented in the presence of cod than either sculpin alone or both sculpin and cod (Figure 3.2). In concrete terms, the effect of cod presence on the IAD represents a 1.4 degree difference in orientation (72.7±2.7 deg SEM for one cod versus 71.3±3.2 deg SEM for cod and sculpin).

To control for possible density effects, prey were tested with two of the same type of predator (Figure 3.1) so that it would be possible to differentiate between the effects due to two predators or two different types of predators. The IPD values for prey exposed to two sculpin or two cod were similar (88.8±3.4 cm SEM and 87.7±2.7 cm SEM, respectively), and were lower than the mixed predator trial (92.1±3.1 cm SEM). This indicates that observed differences are not simply due to an increase in the number of predators present, but the type of predators present. For the IAD the two-predator conditions were all very similar (two sculpin 74.5±2.8 cm SEM, two cod 71.3±3.1 cm SEM, mixed 71.3±3.2 cm SEM).

3.3.2 Distance to predator

Distance to predator was used as a measure of prey response to predatory presence or threat. In the case of two predators being present, the distance to each predator was measured. Instances where the location of a predator could not be properly identified were excluded from the analysis. This occurred when the predator hid in the area of the tank in shadow, and resulted in some trials being excluded entirely. The normality assumption was violated, so p-values were obtained by randomization (1000 iterations). No interactive effect ($F_{2,42} = 0.129$, $p = 0.122$, by randomization) between predator type and number of predators was found. An almost significant main effect of predator type is observed ($F_{2,42} = 2.92$, $p = 0.058$, by randomization), but the number of predators present is insignificant ($F_{1,42} = 0.39$, $p = 0.537$, by randomization). It is possible that my statistical design is not sensitive to biological differences. I suggest this because when the number of predators of only one type increased, I observed a reduction in the distance to the predator; however, in the mixed trials, with both cod and sculpin together as predators, I found an increase in the distance from the predator (Figure 3.3). This increase in the mean is largely attributable to the presence of the cod in the mixed trials (117.29 cm±4.24 SEM and 102.96 cm±3.45 SEM, for cod and sculpin predators, respectively). This is in contrast to the mostly similar means of sculpin and cod in the unmixed two-predator trials of 97.30 cm±3.56 SEM for sculpin and 92.20 cm±2.88 SEM for cod. In a tank less than 2 metres wide, 15 centimeters would likely be of biological significance.

3.3.3 Cod predation trials

For the mortality data, no statistical tests were conducted because I felt the number of mortalities was a more tangibly relevant biological number to discuss and also because the appropriate test required a larger sample size. This problem is typical of mortality studies, since mortality (due to any number of factors) is a "rare and random" event.

Predation was observed in 50% of trials where a predator was present. No predation was observed in the cod-predator trials; all predation was by sculpin, even in the mixed trials (Figure 3.4). Expected values for the two-predator treatments were obtained by summing the number of predation events in the appropriate single-predator treatments (e.g. the number of mortalities in the two sculpin treatment was compared to the value of the one sculpin treatment times two). The validity of using this method is rationalized in the discussion.

3.4 Discussion

Determining whether or not emergent multiple predator effects (MPEs) exist involves comparing the observed value for a prey variable against a theoretical value, or in this case, finding a significant interaction term that indicates prey are responding differently to both predators than either predator alone. This theoretical, or expected, value is estimated based on knowledge of the system. The typical theoretical model is the additive model, where the observed value is compared to the sum of variable values in the presence of each predator alone. Most MPE studies measure only mortality where the additive model can be problematic (Sih *et al.*, 1998). Unrealistic comparisons arise when predation is measured in proportions and predators consume a high number of prey resulting in prey depletion (Soluk and Collins, 1988). Relyea (2003) lists this as a major shortcoming of MPE studies and advocates preventing prey depletion, and Soluk and Collins (1988) suggest using a multiplicative model instead of an additive model. Preventing prey depletion by exposing prey to predators in a non-contact situation to prevent prey depletion would have undoubtedly affected prey behaviour as prey are able to discern predation risk (Lima and Dill, 1990). To avoid compromising the behavioural portion of my study, predators were free-swimming and some prey depletion did occur. However, as the depletion was minimal (average rate of depletion was 10%) and the total number of mortalities was used instead of a proportion, an additive model was used for the theoretical value of mortality in the present system.

Differences in schooling behaviour, as measured by the IPD and IAD, were not entirely clear. No differences were observed in aggregation (IPD), although comparisons of the means of the mixed predator treatment with the two-cod and two-sculpin treatments show that existing decreases in aggregation are due to the combination of predator type, not simply the increase in predator density. No interactive effect was found for orientation either, but the IAD was almost significantly higher when the cod predator was present, regardless of sculpin presence. This suggests that prey were less commonly orienting and aggregation (schooling) when an adult cod was present. Conclusions regarding schooling behaviour as a result of these differences indicate that a decrease in schooling is possibly occurring when an adult cod is present.

The schooling results contrast the results for the distance to the predator, no significant interaction was found, but the mean differences (almost 15cm) are biologically relevant and it is possible that we are committing an error in accepting there is no significance. The increase in distance to the predator in the mixed condition was mostly attributable to an increase in the distance kept from the adult cod in the mixed trials. The almost significant effect of prey type on the distance to the predator reflects this. Thus prey were less commonly oriented in the presence of an adult cod, but kept a greater distance from them, which is seemingly contradictory. The lack of a significant interaction for the IPD, IAD and distance to the predator precludes the conclusion that emergent effects of multiple predators exist in this system when tested in a laboratory situation, but the inconsistent results indicate that the question merits further investigation.

Emergent MPEs do exist in terms of predation risk, as measured by total mortality, for prey in this study. The total number of mortalities in the mixed predator condition is less than would have been predicted by the number of mortalities in the lone-predator conditions. This is also the case for predator density in the case of sculpin, as the addition of second sculpin predator did not result in a concurrent increase in mortality. However, no mortality was ever attributed to the cod predators, so this trend was not observed with the adult cod. It is possible that naïve cod have a search image; that is, a familiarity, for only bigger cod from their experience in the hatchery and that this skewed the trend of increased predation by one type of predator would not be observed with wild prey as they have presumably encountered both predator species in the wild.

A concern recently raised regarding multiple predators studies involves the pairing of a predator that poses a high risk of predation with one that poses a low risk (Relyea, 2003). This pairing can result in a situation where the combined trial mainly reflects the influence of the stronger predator, but results in an interaction, over-inflating the occurrence of non-additive effects. The term "hierarchy control" (McIntosh and Peckarsky, 1999) describes a prey response when one of two predators presents a higher risk than the other. In this situation, greater predator avoidance should be observed. While sculpin were responsible for all of the predation in this study, the mixed trial shows a decrease in total mortalities, reflecting a real change in the interaction between predators and prey, not merely the same level of predation when sculpins were the sole predator. Additionally, adult cod still had an effect on prey behaviour as reflected in the increased distance kept from the predator in the mixed trial that is mostly due to the presence of adult cod. It may also be that a "hierarchy of response" exists whereby anti-predator behaviours are ranked by their effectiveness. Krause *et al.* (1998) suggested that shoaling is an ineffective strategy for averting surprise (ambush) attacks. This difference in attack strategy may explain the susceptibility of the prey to predation by sculpins, but not to the adult cod that use a pursuit strategy. In Chapter 2, naïve prey were found to use schooling less than their wild counterparts. For the reason that naïve prey were used for this study, distance to the predator may be the best measure of anti-predator behaviour, followed by schooling. Of the schooling components, inter-prey distance is likely the best measure of schooling behaviour, since the differences in angle of orientation were more of degree and not kind. However, I conclude that both predator threat and prey response interact to produce these conflicting results, but in general the predators chosen for this study were equally threatening overall.

Few multiple predator studies have involved fish, and most of those that have did not involve marine organisms (Rahel and Stein, 1988; Martin *et al.* 1989; Soluk, 1993; Crowder *et al.*, 1997; Ekloev and VanKooten, 2001). Ekloev and VanKooten (2001) found higher mortality for mixed predator treatments, reflecting facilitation between predators, while Crowder *et al.* (1997) found reduced mortality for the two-predator condition. However, Crowder *et al.* suggest it is not predator interference, but changes in prey behaviour that mitigate the potential increased risk of two predators. Martin *et al.* (1989) found non-additive effects on the behaviour of snails when exposed to both a crab and a fish at the same time. These results demonstrate that both predator interactions and prey interactions have an effect on the outcome of multiple predator environments. This is important, given that prey behavioural responses to predators are often ignored, even though they have an impact on predator-prey interactions (Sih, 1979). Evidence for non-emergent multiple effects on behaviour was found in this study.

Prey defenses against predators can strongly impact community structure (Matsuda *et al.*, 1994). For instance, predator presence and motivation influences habitat use, diel foraging and activity levels of prey (Gotceitas *et al.*, 1995; Grant and Brown, 1998). The plasticity of anti-predator behaviours is reflected in these defenses, which are becoming better understood through multiple predator designs (Sih, 1987). My study was the first to investigate multiple predator effects in Atlantic cod, and one of the first marine studies in the MPE literature. Non-additive effects on the relative mortality of juvenile Atlantic cod under multiple predator threat were found in this system, while the effects on schooling behaviour were less clear. These emergent effects mean that to determine the effect of multiple predators on prey behaviours and on prey mortality, an additive model that simply sums the results of single-predator studies on mortality is insufficient and that behaviourally it is important to consider the contribution of each predator to the prey response. This study contributes to a growing body of work that suggests both prey behaviours and predator interactions co-vary both linearly and nonlinearly to produce results that cannot be predicted by merely observing a one-predator threat.

Table 3.1 Results of GLM for the mean inter-prey distance, mean inter-angle difference and distance to predator for naïve prey under different predatory threats. * Number in parentheses is the p-value obtained by randomization (1000 iterations).

Response variable	Source	df	MS (adj)	F-value	p-value*
Mean inter-prey distance	Sculpin presence	1	214.9	1.69	0.204 (0.199)
	Cod presence	1	48.1	0.38	0.544 (0.532)
	Sculpin presence*Cod presence	1	278.8	2.19	0.150 (0.140)
	Error	28	127.4		
Mean inter-angle difference	Sculpin presence	1	74.8	0.66	0.423 (0.403)
	Cod presence	1	500.0	4.42	0.045 (0.053)
	Sculpin presence*Cod presence	1	28.2	0.25	0.621 (0.642)
	Error	28	113.2		
Distance to predator	Predator type	2	472.8	2.92	0.065 (0.058)
	# of predators	1	63.8	0.39	0.533 (0.537)
	Predator type*# of predators	2	348.5	2.15	0.129 (0.122)
	Error	42	161.7		

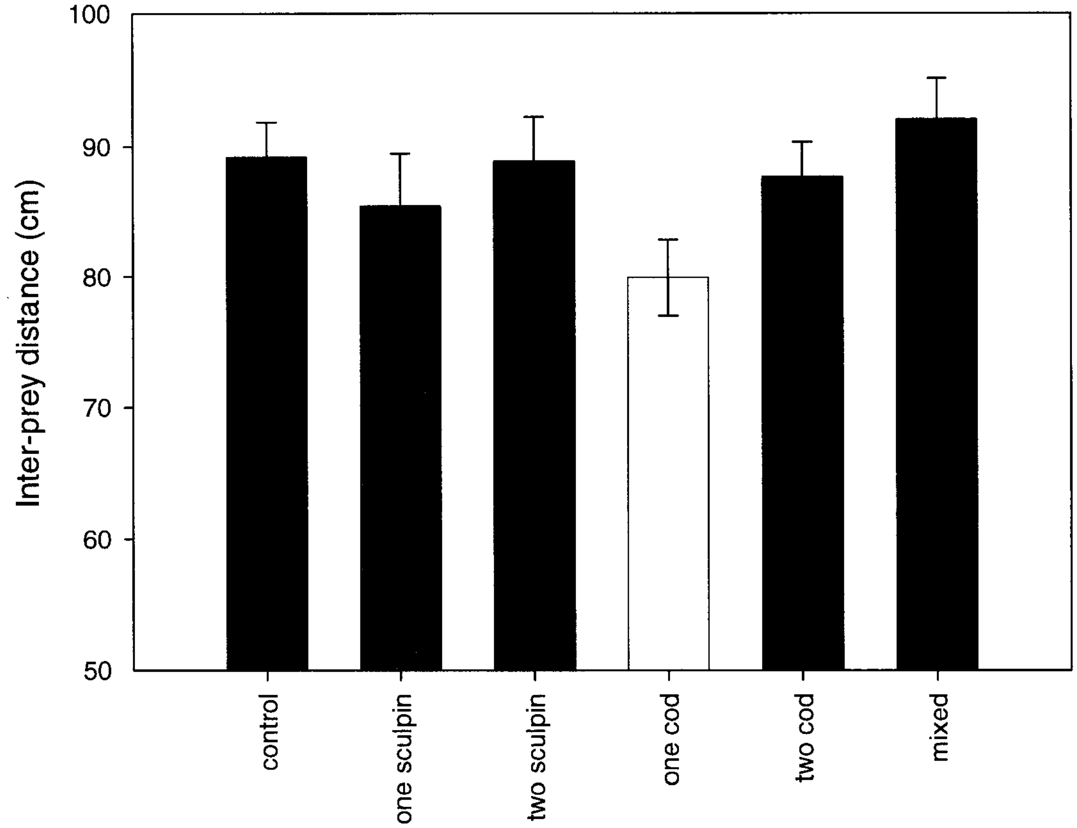

Figure 3.1 Differences in the mean inter-prey distance (cm) of naïve cod in the presence of different types and densities of predators. Columns represent group means ± SEM. See Table 1 for details. Note: y-axis scale does not begin at zero.

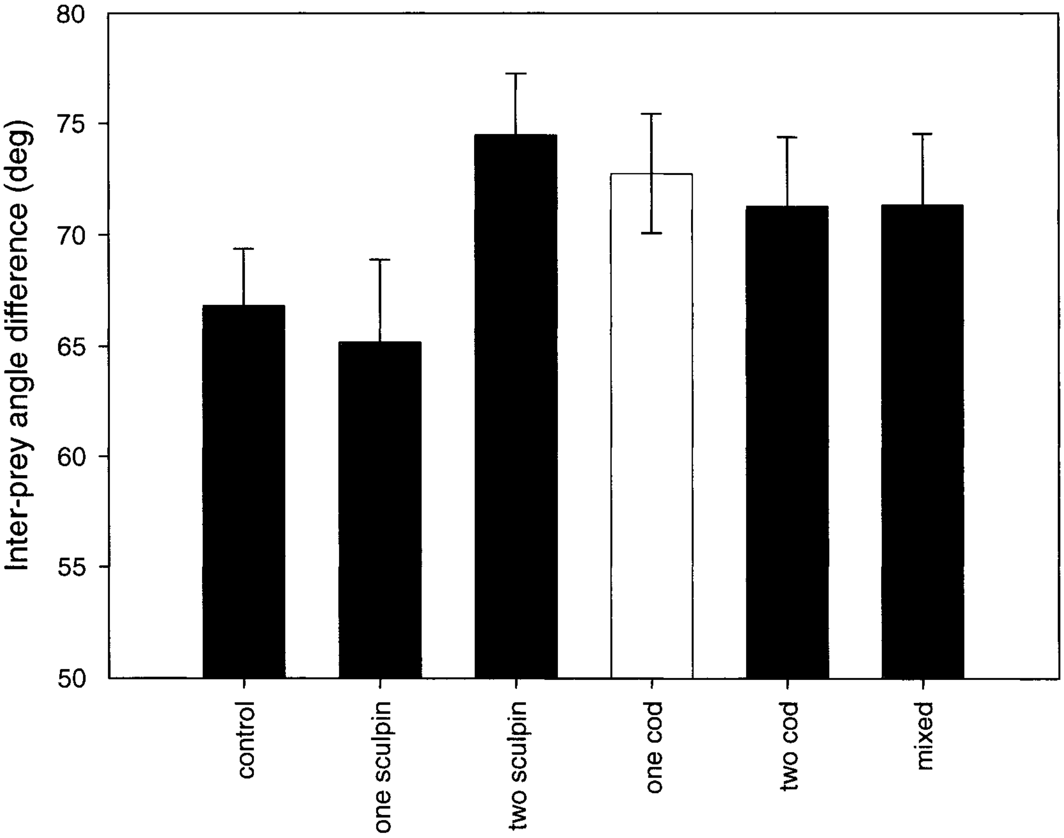

Figure 3.2 Differences in orientation of naïve cod under in the presence of different types and densities of predators as measured by the mean inter-prey angle difference. Columns represent group means ± SEM. See Table 1 for details. Note: y-axis scale does not begin at zero.

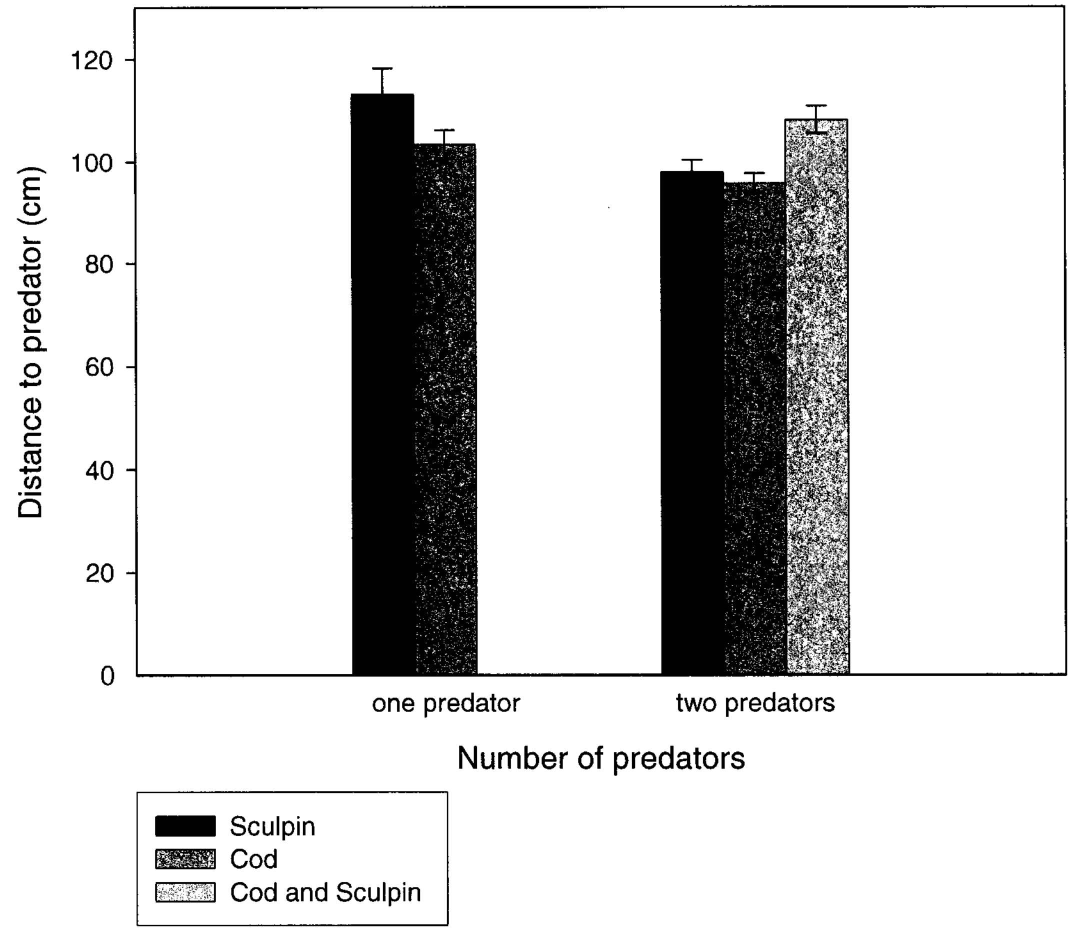

Figure 3.3 Differences in distance to predator (cm) in the presence of different types and densities of predators. Columns represent group means ± SEM, and for the two-predator conditions, the mean distance to each predator. See Table 1 for details.

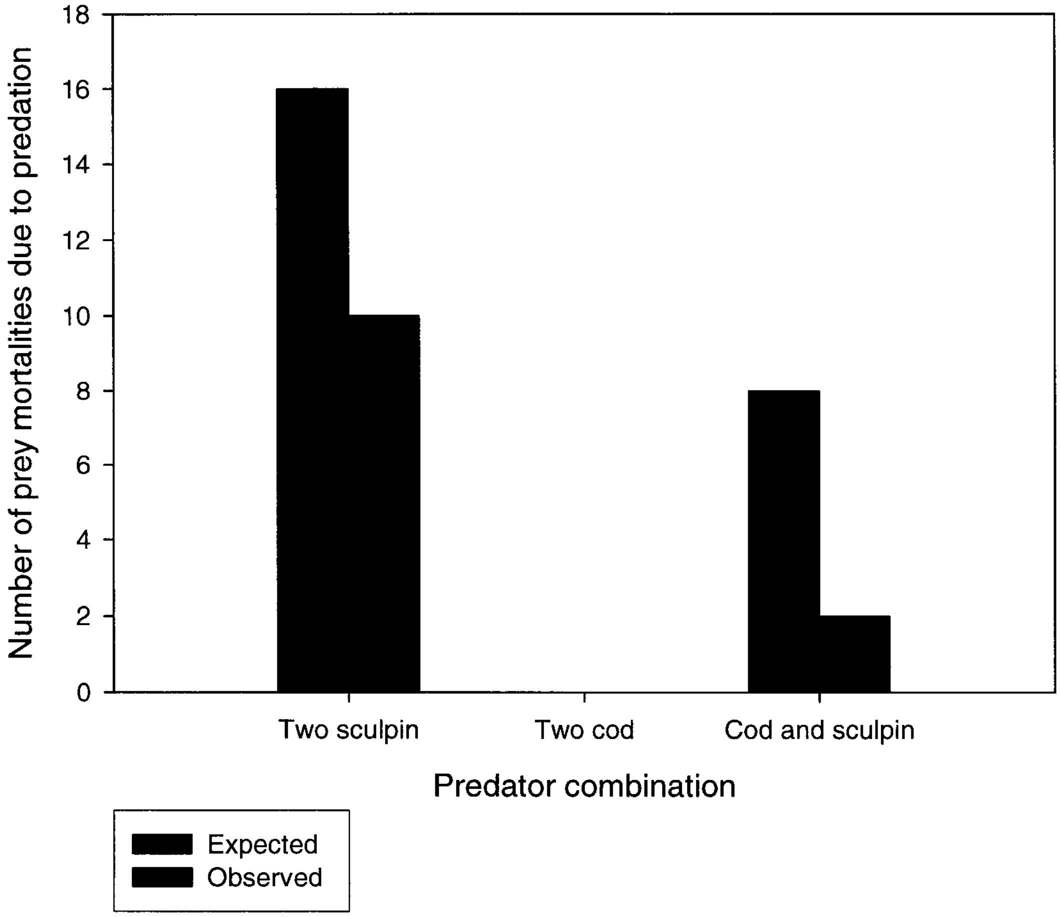

Figure 3.4 Expected and observed values for mortality due to predation. Columns represent the sum total of mortalities. Expected values were calculated by summing the number of mortalities in the individual predator conditions.

CHAPTER 4

Summary

The influence of predator-prey interactions on prey behaviours is shown in both the variety of prey responses, and the flexibility of those responses. In this thesis I examined how previous prey experience with predators affected anti-predator behaviours and survival, and how multiple predators influenced prey behaviour and predation risk.

In Chapter 2, I found that experience did improve anti-predator behaviours. Naïve prey initially schooled less than either wild species, although this behaviour improved with experience. Reduced schooling behaviour may have been the cause of observing predation in 50% of naïve prey trials. I concluded that it may not necessarily be the so-called "sensory-deprived" hatchery environment that results in these behavioural deficits, but social deprivation. Naïve prey may need the appropriate anti-predator behaviours and recognition of actively foraging predators to be modeled by other fish. The results of the experience trials support this suggestion, where even a small increase in schooling resulted in a 100% decrease in predation. It is possible that naïve prey that have witnessed predation events "copied" the appropriate behaviours from other prey. These results indicate that in the future both predator training and wild conspecific exposure may increase the potential for survival of naïve fish in rearing programs intended for release, although an analysis must include a review of the potential costs to be appropriate for an aquaculture program.

The fitness and behavioural implications of living in a multiple predator environment were investigated in Chapter 3. This study was the first to investigate multiple predator effects in Atlantic cod, and one of the first marine studies in the MPE literature. Non-additive effects on the relative mortality of juvenile Atlantic cod under multiple predator threat were found in this system, while the effects on schooling behaviour were less clear. These results have important implications for how we evaluate anti-predator behaviours in the presence of single and multiple predators. In the case of cod, and other schooling species, the effectiveness of schooling as an anti-predator behaviour may depend on what predators are present, as schooling did not appear to mitigate predation by sculpin, an ambush predator, in this study.

Future research may investigate the effect of different predator species, or different levels of predator motivation on anti-predator behaviours in cod. It is possible to improve anti-predator behavioural skills by training, but different types of training and the long-term implications have not been investigated. In terms of the MPE model, future work should include comparing the results of emergent effects in a naïve prey system to a wild prey system, where more natural results may be observed.

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