Effects of Social Status and Food Availability on Predator Avoidance Behaviour in
Young-of-the-Year Rainbow Trout, *Oncorhynchus mykiss*.

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A Thesis

in

The Department

of

Biology

Presented in Partial Fulfillment of the Requirements
for the Degree of Master of Science at
Concordia University
Montreal, Quebec, Canada

May 2005

Canada

Abstract

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Prey animals are often faced with a Hobson's choice: risk starvation or risk predation. Individuals capable of optimizing this trade-off through the use of context specific predator avoidance behaviour should be at a selective advantage. Food availability and social status have been shown to exert a strong influence on this trade-off, however, it remains unknown if these factors interact (i.e., do dominants and subordinates vary their behavioural decisions based on food availability). The purpose of this study was to examine the effects of food availability and social status, as well as their interaction, on the individual behaviour by young-of-the-year rainbow trout (*Oncorhynchus mykiss*) in response to perceived predation threats. Pairs of dominants and subordinates were assigned to either low, intermediate or high food availability treatments and exposed to either conspecific alarm cue (predation threat) or distilled water (no threat). The results suggest that an individual's response to a perceived predation threat is indeed dependent upon both social status and food availability. When tested together or alone, the main factor influencing predator avoidance behaviour differed between social classes. Predator avoidance by dominants was determined by food availability, whereas avoidance behaviour by subordinates was determined by aggression. Thus these results support the hypothesis of context dependant predator avoidance strategies.

Acknowledgments

First, I would like to thank those trout who so honourably dedicated their lives to the search for greater knowledge. None of this would have been possible were it not for your selfless sacrifice. I would also like to thank my supervisor Dr. Grant Brown for his generosity, support, and horribly bad sense of humour, danke sehr herr doktor. I also wish to show my gratitude to my committee members, Dr. James Grant and Dr. Emma Despland for their suggestions and constructive criticisms.

To Antoine, Ellie, Justin, Pat, Nathalie, Jae-Woo, Ivan, and Reehan I wish to give my heartfelt thanks for sharing in my pain and suffering over the last two years. I would also like to express gratitude to my dear friends Arianne, Kevin, and Holly for your moral support and for believing in me. To my parents, grandparents, Andrew and Jerm I wish to humbly say that I am forever in you debt, the love, patience, and guidance you have shown me will never be forgotten. Finally, to anyone and everyone I have ever had the pleasure to explore the beautiful outdoors with, thank you, such adventures have provided the motivation to keep pushing forward these past two years. And remember "Great things are done when men and mountains meet, this is not done by jostling in the street".

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Introduction

Trade-offs

There are two unifying characteristics of prey animals, the need to forage and the need to evade predation. As both somatic and gonadal growth are directly linked to an individual's energy intake, natural selection would be predicted to favor animals that maximize foraging benefits. For many temperate fish species increased foraging efficiency, rate and/or intake may be especially important since survival is highly size dependent (Oliver et al., 1979; Olson, 1996; Hyvärinen & Vehanen, 2004). However, behaviour patterns associated with improved foraging, such as increased searching, reduced refuge use and vigilance, also expose an individual to a greater risk of predation (Horat & Semlitsch, 1994). Since failure to detect and respond to a predation threat can lead to a devastating reduction in future fitness prey animals should also be selected to maximize predator avoidance (Lima & Dill, 1990). Given that predation is a ubiquitous selection pressure, prey animals are often faced with a Hobson's choice: risk starvation or risk predation (Clark, 1994). Foraging decisions made by prey under threat of predation are therefore based on a series of threat-sensitive trade-offs between the need to forage and the need to evade predation (Brown, 2003). Selection should favor those individuals capable of optimizing these trade-offs through the execution of effective context specific predator avoidance behaviour (Helfman, 1989; Lima & Bednekoff, 1999; Chivers et al., 2001).

Differential Predator Avoidance

Despite the ubiquitous nature of predation, individual predator avoidance behaviour is heterogeneous within a given population. This variation may be attributed to differences in prey susceptibility, energy stores, absolute energy requirements, and parasite loads (Godin & Sproul, 1988; Reinhardt, 1999; Bernot, 2003), and in males, the presence of a female or female estrous odours (Godin & Dugatkin, 1996; Kavaliers et al., 2001). For juvenile salmonids the former three factors likely represent the most important sources of variation in observed predator avoidance behaviour patterns. Within an age class larger individuals are likely to be at a greater risk of predation than their smaller counterparts, as they are both more conspicuous and profitable prey (Martel, 1996; Quinn et al., 2001). Larger animals also tend to have greater stored energy reserves and are therefore protecting greater potential fitness (see below), so that the benefits of antipredator behaviour should be high (Clark, 1994; Reinhardt & Healey, 1999; Reinhardt, 2002). Conversely, larger individuals generally have higher energy requirements, which should also make them more risk prone. As predation is both spatially and temporally variable (Lima & Bednekoff, 1999; Novotny, 1999) and the willingness to take risks is influenced by multiple factors, the costs and benefits of a given trade-off will vary. Thus there is no single optimal behavioural decision covering all situations. Predicting individual behaviour in response to a predation threat can, therefore, be quite difficult.

Asset-Protection Principle

Recently, Clark (1994) proposed that the optimal trade-off between the benefits of predator avoidance and the benefits of foraging may be influenced by accumulated fitness assets. Fitness assets are defined as a combination of both current nutritional status and stored energy reserves. According to Clark's (1994) asset-protection principle, as fitness assets increase, individuals benefit less from each unit gained relative to the assets that would be lost if captured by a predator. Furthermore, the benefits of foraging increase over time as the prospect for making up lost foraging opportunities decreases, thus foraging benefits are more immediate (Reinhardt & Healy, 1999). The asset-protection principle therefore makes two predictions: 1) individuals with greater assets should be less willing to risk predation, and 2) if the time available to accumulate assets is finite then individuals should increase risk-taking with time (Reinhardt & Healy, 1999; Reinhardt, 2002).

Work by Grant & Noakes (1987), Reinhardt & Healey (1997, 1999), and Dowling & Godin (2002) provide empirical evidence supporting the asset-protection principle in brook charr (*Salvelinus fontinalis*), coho salmon (*Oncorhynchus kisutch*), and banded killifish (*Fundulus diaphanous*), respectively. In young-of-the-year (YOY) brook charr willingness to risk predation decreased with body size; larger individuals exhibited greater reactive distances, latencies to first foraging attempt, and refuge use (Grant & Noakes, 1987). Similarly, following exposure to a model predator, smaller coho salmon resumed feeding significantly sooner than larger individuals (Reinhardt & Healy, 1999). Furthermore, smaller, more risk-prone, individuals were able to maintain their growth rates under predation risk, whereas

larger, more risk-averse, salmon experienced reduced growth rates (Reinhardt &
Healy, 1997). In addition, for small salmon, individuals that had been well fed prior
to exposure to a predator resumed feeding significantly later than those that were not
well fed. Likewise, smaller banded killifish emerged from refuge significantly sooner
than larger individuals, however, current nutritional state had no influence on latency
to emergence (Dowling & Godin, 2002). This discrepancy between Reinhardt &
Healy (1999) and Dowling & Godin (2002) may be explained by the fact that in the
latter study the test fish were food deprived for only 24 hours prior to testing,
Reinhardt & Healy (1999), however, had maintained the experimental diet for 14
days. A 24-hour starvation period may have been insufficient to induce a
compensatory growth response, as many teleost fishes can survive extended periods
of starvation (Pettersson & Brönmark, 1993; Dowling & Godin, 2002). In all cases
the individuals with the greatest accumulated assets were most averse to the threat of
predation.

While direct testing of the asset-protection principle has been limited to fish
there is indirect evidence that it is also applicable to a wide array of animal groups
including arachnids (Walker & Rypstra, 2003), amphibians (Whitham & Mathis,
2000) and birds (Moore, 1994). Under the risk of predation hungry wolf spiders
(*Pardosa milvina*) foraged at levels similar to spiders not under threat. Moreover,
satiated spiders significantly reduced feeding under the risk of predation (Walker &
Rypstra, 2003). Hungry graybelly salamanders (*Eurycea multiplicata griseogaster*)
returned to foraging significantly sooner than salamanders that had recently eaten,
when presented with the chemical cues of a predatory sculpin (*Cottus carolinae*)

(Whitham & Mathis, 2000). Similarly, yellow-rumped warblers (*Dendroica coronata*) in migratory disposition resumed foraging sooner and consumed more prey under the threat of predation than warblers not in migratory disposition (Moore, 1994).

However, work on Atlantic salmon (*Salmo salar*) and rainbow trout (*Oncorhynchus mykiss*) appears to contradict the asset-protection principle (Gotceitas & Godin, 1991; Johnsson, 1993). When socially dominant and subordinate juvenile salmon were fed similar rations, dominant individuals appeared to accept greater risk and resumed foraging sooner (Gotceitas & Godin, 1991). Larger rainbow trout were also more willing to risk predation to gain access to food than were smaller trout (Johnsson, 1993). These apparent discrepancies with the predictions of the asset-protection principle may lie in the fact that life-history strategies in salmonids are dependent on growth rates. The larger individuals may be choosing to either: 1) become sexually mature as parr or 2) smolt the following spring. In either case the decision is based on growth rates or fat deposition rates (Reinhardt, 1999; Maclean & Metcalfe, 2001). Both of these strategies place time restrictions on the accumulation of assets and would necessitate that the larger fish maintain high growth rates and hence risk predation. Therefore, it may be argued that these two studies provide support for the second prediction of asset-protection.

Social Hierarchies

Size-determined social hierarchies provide an intriguing opportunity for studying the asset-protection principle. Social hierarchies, formed in a wide range of

taxonomic groups (birds (Oden et al., 2004), arthropods (Monnin et al., 2003), reptiles (Alberts et al., 2002), primates (Wittig & Boesch, 2003), fish (Huntingford et al., 1990)), facilitate a reduction in the costs associated with aggressive interactions, time and energy expenditure and increased risk of injury and predation (see Grant, 1997 for a review of the costs of aggression). Dominant individuals, typically of larger body-size, gain preferential access to resources often precluding subordinates (Grant, 1990; Huntingford et al., 1990). Thus dominance is usually associated with higher growth rates and higher future reproductive fitness (Gotceitas & Godin, 1991; Pusey et al. 1997; Barroso et al., 2000; but see Martin-Smith & Armstrong, 2002; Hardwood et al., 2003). As dominant individuals generally have greater stored fat reserves and higher nutritional statuses they should be more risk-averse. Since aggressive interactions increase visibility, and hence vulnerability to predation, dominants should reduce territory defense in response to an increased perceived risk of predation (Martel & Dill, 1995; Martel, 1996). Subordinates, however, should be relatively more risk-prone owing to their lower accumulated assets. Predation, therefore, has the potential to alter dominance hierarchies, allowing subordinates to forage on more profitable patches and experience higher growth rates. Reinhardt (1999) directly tested this prediction in coho salmon by presenting size-determined dominance hierarchies with an electrified model predator. Exposure to the predator resulted in a significant reduction in aggressive interactions, allowing smaller individuals to gain access to more profitable foraging positions. Smaller coho also experienced higher growth rates under 'risky' conditions (Reinhardt, 1999).

Chemical Alarm Cues

The asset-protection principle as well as most other dynamic optimization models of foraging necessitate that prey individuals be capable of accurately assessing the local threat of predation. Prey individuals may obtain information about the level of risk in their environment using several different sensory modalities (Smith, 1999); in aquatic systems vision and chemoreception likely represent the most important modalities involved in risk assessment. While visual cues are both spatially and temporally reliable, they could potentially be manipulated by predators and are considered risky, as both predator and prey must be in close proximity (Brown, 2003). Chemical cues, while being less reliable in time and space, are considered to be honest signals and are associated with lower risk, as predator and prey need not be in visual range (Smith, 1999; Brown, 2003). Furthermore, the availability of visual cues is often limited by a variety of factors including time of day, vegetative cover, and water turbidity (Gelowitz et al., 1993; Mirza & Chivers, 2001; Golub et al., 2005). Chemical cues therefore represent an important, and occasionally the only, source of information regarding the threat of predation available to prey individuals.

Damage-released alarm cues have been demonstrated in a vast number of aquatic vertebrate and invertebrate taxa (Chivers & Smith, 1998; Bryer et al., 2001; Brown, 2003). These chemical cues require mechanical damage to the skin and serve to "alert" nearby conspecifics and sympatric heterospecifics to a potential predation threat (Brown, 2003; Mirza & Chivers, 2003). Exposure to chemical alarm cues can trigger a series of species-typical predator avoidance behaviour patterns, including

reductions in movement, foraging and aggression, as well as increases in refuge use and shoaling (Mirza & Chivers, 2003; Brown & Smith, 1997; Leduc et al., 2004b). By providing a reliable indication of local predation risk, alarm cues allow individuals to increase survival under the threat of predation and maximize foraging-predator avoidance trade-offs (Mirza & Chivers, 2003). Therefore chemical alarm cues offer researchers a dependable means of presenting a predation threat.

Purpose and Predictions

Several studies suggest that both current nutritional state (Reinhardt & Healy, 1997; Whitham & Mathis, 2000; Walker & Rypstra, 2003) and body size/dominance/stored energy reserves (Grant & Noakes, 1987; Reinhardt & Healy, 1999; Dowling & Godin, 2002) play an important role in influencing individual predator avoidance behaviour. Likewise there is an abundance of studies demonstrating the use of chemical alarm cues in mediating predation threats (Brown & Smith, 1997; Brown et al., 2003; Chivers et al., 1995; Mirza & Chivers, 2002). However, to date no study has examined the effects of accumulated assets and social status on the response of individuals to a chemically induced predation threat.

It is the intent of this thesis to examine how current nutritional state influences the behavioural decisions made by dominant and subordinate individuals under a perceived risk of predation and whether the observed behavioural patterns are in accordance with the asset-protection principle. As maintained by the asset-protection principle current nutritional state and size should alter the costs and benefits of predator avoidance behaviour. Individuals with a high current nutritional state and

large size should be the most risk-averse, conversely individuals with a low current nutritional status and small size should be the most risk-taking.

It is predicted that if individual antipredator behaviour is solely determined by asset protection, then dominant and subordinate individuals should show similar behavioural response patterns (Table 1). Individuals with low nutritional status should be more willing to risk predation and thus show reduced predator avoidance in response to alarm cues. When food is scarce individuals are unable to meet their minimum energetic requirements and their nutritional state will be low. The benefits associated with gaining foraging opportunities (e.g. reducing risk of starvation) should outweigh the benefits of predator avoidance. When nutritional status is intermediate or high it is predicted that willingness to risk predation will be reduced and thus animals should show increased predator avoidance to alarm cues.

If individual antipredator behaviour is solely determined by social status, then dominants and subordinates should show opposite response patterns (Table 1). When nutritional status is low, dominant individuals should experience increased foraging benefits relative to the benefits of predator avoidance. Therefore, aggression should remain high, as dominants are not expected to respond to a predation threat. As a result the benefits of foraging for subordinates are likely to be outweighed by the combined costs of predation and aggression. For dominant individuals, with intermediate or high nutritional statuses, the benefits of predator avoidance should greatly outweigh the benefits associated with foraging. Dominants are thus expected to increase predator avoidance, and as a result aggression levels should drop. Consequently, subordinates gain access to previously unavailable food resources,

thereby increasing the benefits of foraging under threat. Subordinates should therefore, continue to forage following exposure to a predation risk.

Given the large volume of research demonstrating that both social status (Grant & Noakes, 1987; Reinhardt & Healy, 1999) and food availability (Reinhardt & Healy, 1997; Whitham & Mathis, 2000; Walker & Rypstra, 2003) influence avoidance behaviour it is unlikely that individual behavioural patterns can be explained by either asset protection or social status alone. Hence, it is predicted that an individual's response to the threat of predation will be based on an interaction of food availability (a proxy for current nutritional status) and social status.

Experiment 1

Materials and Methods

Test Fish

Young-of-the-year rainbow trout were obtained from Arpents Verts hatcheries, Ste-Edwidge-de-Clifton, Quebec, Canada. The trout were held in 110 L glass aquaria, filled with continuously filtered dechlorinated tap water on a 12 hr light: 12 hr dark cycle. Water temperature (~17°C) and pH (~ 7.0) were both well within the preferred ranges for rainbow trout (Kerr & Lasenby, 2000). The fish were fed ad libitum with ground up trout chow (Vigor #4, Corey Feed mills).

Stimulus Preparation

Trout skin extract was prepared from 13 donors ($\bar{X} \pm SD$ = 42.85 ± 3.37 mm fork length (FL)). Donor fish were humanely killed by cervical dislocation (in

accordance with the Concordia University Animal Care Committee Protocol #AC-2002-BROW). Skin fillets were removed from both sides of the donor and immediately placed into 100 mL of chilled glass-distilled water. A total of 40.23 cm^2 of skin was collected, which was then homogenized and filtered through polyester filter floss. The final volume was adjusted to 457 mL with glass-distilled water producing a concentration known to elicit predator avoidance behaviour (Leduc et al., 2004 a, b). The resulting skin extract was then frozen at -20°C, in 12 mL aliquots until needed. The distilled water control was similarly stored in 35 mL aliquots. The fact that the trout may simply be showing a generalized response to the odor of an injured fish was not controlled for as it has previously demonstrated that rainbow trout do not respond to either the skin extract of green swordtails (*Xiphophorus helleri*) or hypoxanthine-3-*N*-oxide, the reputed cyprinid alarm cue (Brown & Smith, 1997; Mirza & Chivers, 2001; Brown et al., 2003). The food stimulus used to promote activity was prepared 1 hour before each set of trials by adding 0.5 g of ground up trout chow to 100 mL of distilled water, the mixture was then stirred for at least 10 min to produce a homogenous solution. The solution was then filtered through polyester filter floss to remove remaining food particles.

Test Tanks

The trials were conducted in a series of 37 L glass aquaria equipped with a gravel substrate and a single air-stone attached to an end wall. Two supplementary lengths of tubing (acrylic airline) were attached to the air-stone permitting the introduction of the chemical and food stimuli without disturbing the test fish. Three

sides of the test tanks were wrapped in black plastic to prevent the visual transmission of an antipredator response from one test tank to another (Magurran & Higham, 1988; Suboski et al., 1990; Ryer & Olla, 1991). The temperature and light regimes for the test tanks were identical to those of the holding tanks.

Experimental Protocol

Two trout differing slightly in size ($\bar{X}$ difference ± SD: FL = 5.3 ± 2.2 mm, body weight (BW) = 0.31 ± 0.13 g; $\bar{X}$ % difference ± SD: FL = 12.2 ± 4.8%, BW = 34.1 ± 10.7 %) were placed into each test tank. Fish of unequal size were chosen to quickly induce a social hierarchy, as relative size is often a strong indicator of dominance. A weight difference of as low as 5% is sufficient to confer dominance status to the heavier fish in steelhead trout (Abbott et al., 1985). However, during the first few months following emergence size does not appear to be a good predictor of social status in Atlantic salmon (Huntingford et al., 1990; Metcalfe et al., 1995). Social status may also be determined through differences in colouration. In Atlantic salmon submission is usually associated with darkening of the sclera of the eye and body colour (O'Connor et al., 1999). However, because colouration is also not an infallible indicator of social status, dominance was determined through a combination of size, colouration and aggressiveness. Fork length and weight were measured before and after running the trials. FL and weight at time of testing were 43.2 ± 3.2 mm ($\bar{X}$ ± SD) and 0.91 ± 0.22 g, respectively, for dominants and 38.0 ± 3.4 mm and 0.60 ± 0.18 g for subordinates. A total of 15 pairs of control and experimental trials were conducted for each treatment combination (N = 90).

Pairs of trout were randomly assigned to one of three feeding regimes (1, 5, or 10% of the combined bodyweight of the dominant and subordinate) and one of two stimuli (alarm cue (AC) or distilled water (DW)). The rations were selected on the basis of previous work with rainbow trout demonstrating that 1% and 5% rations represent maintenance and growth rations (Alsop & Wood, 1997; Gregory & Wood, 1999; Kamunde & Wood, 2003). The 10% ration was selected as it likely approached a satiation ration. Assignment of pairs was such that an equal number of all treatments were run in each test period to minimize day affects upon any one treatment. The test fish were allowed 48 hrs to acclimate and establish dominance prior to testing. Feeding took place once a day at ~ 11:00 for a total of three feedings, with food being introduced at the water surface. The food was completely consumed before the start of trials. All trials consisted of live observations and were run between 12:00 and 4:30 pm.

The trials were divided into 5 min pre- and 5 min post-stimulus injection observation periods separated by a 1 min stimulus injection period. Preceding the pre-stimulus observation period, 60 ml of tank water was withdrawn and discarded from both the stimulus and food odour injection tubes to remove any stagnant water. Then an additional 60 ml was withdrawn from the stimulus tube and 120 ml from the food odour tube and retained. Five ml of food odour was administered at the start of both observation periods and 5 ml of either trout skin extract or distilled water at the end of the pre-stimulus period. The stimuli and food odour were slowly flushed it into the tanks using the retained water. Both observation periods started upon the complete introduction of 5 ml of food stimulus.

During the observation periods, time spent moving, the number of feeding attempts, latency to first feeding attempt, and the number of aggressive interactions, were recorded (see Table 2 for operational definitions). A decrease in time spent moving, number of feeding attempts, and the number of aggressive interactions as well as an increase in latency to first feeding attempt are all indicative of an antipredator response (Brown & Smith, 1997; Leduc et al., 2004a, b; Vehanen, 2003; Martel & Dill, 1993).

Statistical Analysis

The data for time spent moving, number of feeding attempts, and latency to first feeding attempt were first analyzed using a repeated-measures ANOVA to account for the fact that data were collected for each test fish over two observation periods. The independent variables examined were observation period (the repeated measure), social status, ration, and stimulus. Since the repeated-measures ANOVA revealed a significant effect of observation period and as no difference in behaviour between alarm cue and distilled water treatments is expected for the pre-stimulus observation period all further statistical tests analyzed only data from the post-stimulus observation period. Next, a paired samples t-test was performed to evaluate the effect of social status on time spent moving, number of feeding attempts and latency to first feeding attempt. Since the paired-samples t-test revealed a significant effect of social status, all further statistical analysis treated data for dominants and subordinates separately. Finally, two-way ANOVAs were used to test for differences in each behavioural measure in response to the different stimuli under the different

rations. The independent variables examined were ration and stimulus. Data for

aggression were analyzed using a repeated-measures ANOVA, with observation

period (the repeated-measure), ration, and stimulus being the independent variables.

Social status was not included since aggression was confined almost solely to

dominant individuals. A two-way ANOVA was then used to test for differences in

dominant aggression in response to the different stimuli under the different rations.

Subsequent post-hoc comparisons were performed for number of aggressive

interactions using the Fisher's Protected Least Square Differences test. Finally, to

determine the magnitude of the effect of the experimental stimulus, the difference

between pre- and post-observation periods was explored for all behavioural measures

using independent sample t-tests to compare the response to alarm cue with the

response to distilled water. All statistics were calculated using SPSS 10.1.

Results

The results suggest that an individual's response to a chemically mediated

predation-threat is dependent upon the individual's social status and food availability.

In general, dominant individuals were more active than subordinates. Dominants

displayed a strong antipredator response to alarm cues under the 5% and 10% ration

treatments but only a weak response under the 1% treatment (Fig. 1-3). Subordinates,

however, responded to alarm cues under the 1% and 5% ration treatments, but not the

10% treatment (Fig. 1-3). Furthermore, aggression levels decreased with increasing

food availability (Fig. 4), suggesting aggression levels as a mechanism involved in

determining the response of subordinate individuals.

Repeated-measures ANOVA

The repeated–measures ANOVA revealed a significant effect of observation period for time spent moving, number of feeding attempts and latency to first feeding attempt, but not aggression (Table 4). Individuals were more active in the pre- than post-stimulus injection observation period, spending more time moving as well as foraging more often and sooner. Similarly a significant interaction of observation period and cue was found for all behavioural measures except aggression (Table 4). Individuals increased predator avoidance behaviour in the post-observation period upon exposure to alarm cue but not distilled water. There was also a significant interaction between observation period and social status for time spent moving, with subordinates spending significantly less time moving during the post-observation period (Table 4). Finally, a marginally significant interaction between observation period and ration was found for latency to first feeding attempt, with individuals foraging later in the post-observation period under the 5% ration (Table 4).

Paired-samples t-test

The paired-samples t-test revealed a significant effect of social status on time spent moving and latency to first feeding attempt ($t_{(89)} = 11.767$, $p < 0.001$; $t_{(89)} = -2.600$, $p = 0.011$). Dominant individuals spent more time moving and foraged sooner than subordinate individuals. However, there was no effect of social status on number of feeding attempts ($t_{(89)} = 1.194$, $p = 0.236$).

2-way ANOVA

The 2-way ANOVAs revealed a significant effect of cue on time spent moving, number of feeding attempts and latency to first feeding attempt for dominant individuals, but not subordinates (Table 5). Dominant individuals spent more time moving, and foraged more often and sooner in the distilled water treatments. A further effect of ration was found for number of aggressive interactions by dominants (Table 5). Dominant individuals were significantly less aggressive under the 10% ration (1% to 10% FPLSD, p = 0.001; 5% to 10% FPLSD, p = 0.005), with no difference in aggression between 1% and 5% rations (FPLSD, p = 0.654). For subordinate individuals there was a significant effect of cue on time spent moving, with subordinates spending more time moving in the distilled water treatments (Table 5). There was also a significant interaction of ration and cue for latency to first feeding attempt (Table 5).

Independent-samples t-test

In the 1% ration treatment, the experimental stimulus (alarm cue) elicited strong predator avoidance behaviour in subordinate individuals when compared to the control stimulus (distilled water). Subordinates significantly decreased time spent moving and number of foraging attempts, as well as increased latency to first foraging attempt (Table 3; Fig. 1-3). Dominants decreased time spent moving in response to alarm cue, but showed no change in number of feeding attempts or latency to first feeding attempt (Table 3; Fig. 1-3). Neither dominants nor subordinates decreased aggression levels in the presence of the alarm cue (Table 3; Fig. 4).

In the 5% ration treatment, dominant individuals showed an increase in predator avoidance behaviour in response to alarm cue when compared to distilled water. Dominants significantly decreased time spent moving and number of feeding attempts, as well as increased latency to first feeding attempt (Table 3; Fig. 1-3). Subordinates significantly increased latency to first feeding attempt and marginally decreased time spent moving in response to alarm cue, but showed no change in number of feeding attempts (Table 3; Fig. 1-3). There were no significant changes in aggression levels on presentation of alarm cue by either dominants or subordinates (Table 3; Fig. 4).

In the 10% ration treatment, dominants, but not subordinates, significantly reduced time spent moving and increased latency to first foraging attempt in presence of alarm cue (Table 3; Fig. 1 & 3). There were no changes in aggression levels or foraging attempts by either dominants or subordinates in response to alarm cue (Table 3; Fig. 2 & 4).

Experiment 2

The findings of the first experiment suggest that the levels of aggression determined the behavioural response patterns displayed by subordinate individuals. In order to verify this hypothesis the second experiment tested dominant and subordinate individuals separately. If, as expected, aggression was responsible for determining subordinate behaviour, then when tested separately subordinate individuals should respond in accordance to the asset-protection principle.

Materials and Methods

Test Fish

The rainbow trout used originated from the same population as those in experiment 1, however, fish were not retested. The trout were held under the same conditions as in experiment 1; see experiment 1 for a detailed description.

Stimulus Preparation

Trout skin extract was prepared from 3 donors ($\bar{X} \pm SD = 67.0 \pm 5.8$ mm FL); a total of 23.8 cm^2 of skin was collected and processed as described in experiment 1. The final volume was adjusted to 270 mL with glass-distilled water. The relative concentration (cm^2 per ml water) of the skin extract was the same as in the first experiment. New skin extract was merited as the trout had grown considerably ($\sim$ 19% for dominants and 17% for subordinates) since the completion of the first experiment. Mirza and Chivers (2002) have previously demonstrated that brook charr are able to distinguish between the alarm cues of different size classes of conspecifics. This could have potentially led to a reduction in the intensity of the response to the experimental stimulus.

Test Tanks

The test tanks used were identical to those used in the first experiment. See experiment 1 for detailed description of the experimental set up.

Experimental protocol

Two trout differing slightly in size ($\bar{X}$ difference ± SD: FL = 6.2 ± 4.2 mm,

BW = 1.1 ± 0.80 g) were placed into a secondary holding tank. FL and BW at time of

testing were 72.1 ± 10.4 mm ($\bar{X}$ ± SD) and 4.7 ± 2.1 g, respectively, for dominants

and 66.0 ± 8.9 mm and 3.6 ± 1.5 g for subordinates. A total of 10 pairs of control and

experimental trials were conducted for each treatment combination (N = 80).

Pairs of trout were randomly assigned to one of two feeding regimes (1 or

10% of the combined bodyweight) and one of two stimuli (AC or DW). The test fish

were allowed 45 hrs to establish dominance. Three hrs prior to testing the dominant

and subordinate were separated and randomly placed individually into test tanks.

Feeding took place once a day at ~ 11:00 for the two acclimation days and at ~ 8:30

on test day. Differences in feeding times were necessary to allow the dominant and

subordinate pairs to be fed together on test day and still run trials at the same time of

day as experiment 1. The remainder of the experimental protocol was identical to that

used in experiment 1; see experiment 1 for a detailed description.

Statistical analysis

The data for dominants and subordinates were analyzed separately as they

were tested individually. For both dominants and subordinates the dependent

variables, time spent moving, number of feeding attempts, and latency to first feeding

attempt, were analyzed using a repeated-measures ANOVA to account for the fact

that data were collected for each test fish over two observation periods. Independent

variables examined were observation period (the repeated measure), ration, and

stimulus. Since the repeated-measures ANOVA revealed a significant effect of observation period and as no difference in behaviour between alarm cue and distilled water treatments is expected for the pre-stimulus observation period all further statistical tests analyzed only data from the post-stimulus observation period. Subsequent two-way ANOVAs were used to test for differences in each behavioural measure in response to the different stimuli under the different rations. The independent variables used were stimulus and ration. Finally, to determine the magnitude of the effect of the experimental stimulus, the difference between pre- and post-observation periods was explored using independent sample t-tests to compare the response to alarm cue with the response to distilled water.

Results

Contrary to the first experiment, both dominants and subordinates showed strong predator avoidance behaviour in response to a chemically mediated predation threat under the 1% ration treatment (Fig. 6-8). However, dominant individuals displayed strong predator avoidance under the 10% ration, while subordinates displayed only weak avoidance behaviour (Fig. 6-8). Furthermore, activity levels appeared to be similar for both social groups. These results suggest that in the first experiment the behaviour of subordinates, both prior to and after exposure to a predation-threat, may have indeed been the result of aggressive interactions by dominant individuals. The results also suggest that the stress experienced by subordinates in the presence of dominants may persist, continuing to determine their behavioural response following separation.

Repeated-measures ANOVA

The repeated–measures ANOVA revealed a significant effect of observation period for time spent moving, number of feeding attempts and latency to first feeding attempt for both dominant and subordinate individuals (Table 7). Individuals were more active in the pre- than post-stimulus injection observation period, spending more time moving as well as foraging more often and sooner. Similarly a significant interaction of observation period and cue was found for all behavioural measures for both dominants and subordinates (Table 7). Individuals increased predator avoidance behaviour in the post-observation period upon exposure to alarm cue but not distilled water.

2-way ANOVA

The 2-way ANOVAs revealed a significant effect of ration on time spent moving, number of feeding attempts and latency to first feeding attempt for both dominants and subordinates (Table 8). Individuals spent a greater amount of time moving as well as foraged more often and earlier under the 1% ration treatment. A further effect of cue was found for time spent moving, number of feeding attempts and latency to first feeding attempt for dominant individuals, with dominants moving more, and foraging more often and sooner in distilled water treatments (Table 8). Additionally, for dominant individuals the interaction between ration and cue was found to be significant for latency to first feeding attempt (Table 8). Dominants foraged sooner under the 1% ration in the distilled water treatment.

Independent-samples t-test

A significant reduction in time spent moving was observed for both dominants and subordinates in response to the introduction of alarm cue under both feeding regimes, when compared to distilled water (Table 6; Fig. 6). Dominants and subordinates also both showed a significant decrease in number of foraging attempts and an increase in latency to first feeding attempt in response to alarm cues under the 1% ration (Table 6; Fig. 7 & 8). Dominants, but not subordinates, also increased latency to first feeding attempt in response to alarm cues under the 10% ration (Table 6; Fig. 8).

Discussion

As anticipated, the predator avoidance behaviour demonstrated by YOY rainbow trout in response to a chemically-mediated predation threat was governed by an interaction between social status and food availability. Previous studies in brook charr (Grant & Noakes, 1987) and coho salmon (Reinhardt & Healy, 1999) have shown that dominant individuals are more risk-averse, showing stronger antipredator behaviour than subordinates. Although Grant & Noakes (1987) did not directly assess social status, brook charr form size determined social hierarchies (Newman, 1956); therefore it is not unreasonable to assume that larger charr were dominant. Likewise, several studies have shown that hungry individuals are more risk-prone, reducing or showing no antipredator behaviour in response to a threat (Moore, 1994; Whitham & Mathis, 2000; Walker & Rypstra, 2003). While it is apparent that both

social status and food availability are important in determining individual behavioural responses to threats, this is the first study to examine the interaction between these factors.

The findings of the first experiment suggest that the main factor influencing predator avoidance behaviour differed between dominants and subordinates. Food availability, as a proxy for current nutritional status, appears to determine avoidance behaviour in dominant individuals. In contrast, the behaviour of subordinate individuals appears to be determined by aggression levels.

By continuing to forage dominant individuals exhibited only a weak response to alarm cues under low food availability. However, under intermediate to high food availabilities exposure to these same cues elicited significant increases in antipredator behaviour, causing a decrease in movement and foraging activity. When food is scarce, meeting daily energetic requirements becomes difficult, thus leading to a reduction in current nutritional status. As individuals with a low nutritional status experience reduced growth rates and are at greater risk of starvation, the benefits of gaining extra foraging opportunities are likely to be high and outweigh the benefits of predator avoidance. Yet, when food is abundant, current nutritional status is expected to be high, thus the benefits associated with foraging under risk are likely to be low and outweighed by the benefits of predator avoidance. These results are consistent with the predictions of the asset-protection principle (Clark, 1994), suggesting that, for dominants, the decision of whether to respond to a threat is based upon current fitness assets.

Subordinate individuals responded to the threat of predation with an increase in antipredator behaviour under low and intermediate, but not high food treatments. This response pattern is likely due to the aggression levels exhibited by the dominants. When food availability was low to intermediate dominant individuals were highly aggressive. Furthermore, aggression was not reduced following the presentation of a predation threat. While subordinates are expected to experience considerable benefits from gaining extra foraging opportunities, the combined costs of predation and aggression are likely to be greater, thus individuals should increase antipredator behaviour. However, when food availability was high dominants significantly decreased aggression; once again there was no observed reduction of aggression in response to alarm cues. When aggression levels are low, the benefits associated with foraging are expected to outweigh the costs; as a result selection should favor subordinates who continue to gain foraging opportunities under threat. These results are consistent with the prediction that social status determines subordinate behavioural response patterns.

A lack of difference in aggression levels in response to alarm cues was unexpected. As movement increases visibility, aggressive individuals are likely to be more vulnerable to predation; therefore aggression and territory defense should be reduced in response to a perceived risk (Martel & Dill 1993; Martel & Dill, 1995). However, dominant individuals showed no significant change in aggression under threat. Similarly, on days of simulated predation threat Reinhardt (1999) observed an increase in the relative frequency of chases to aggressive displays. The apparent lack of response may have been the consequence of a need by dominant individuals to

quickly re-establish their position following exposure to a threat (Reinhardt, 1999). This may also have been the case in the present study with dominant individuals becoming more aggressive as time since exposure passed, however, interval aggression rates, which would have allowed for verification of this hypothesis, were not measured.

The findings of the second experiment are in agreement with those of the first experiment, suggesting that predator avoidance behaviour of: 1) dominant individuals is determined by current nutritional status and 2) subordinate individuals is determined by previous social status. Contrary to the first experiment, the activity levels of dominants and subordinates were similar, suggesting that aggression and its associated stress are a likely mechanism by which subordinate response patterns are determined. The weak response exhibited by subordinates under the 10% ration treatment (in contrast to no response in experiment 1), may be due to a reduction in the stress experienced during recent interactions with a dominant. If this is the case then it is possible that as stress is further reduced, subordinates would exhibit stronger avoidance behaviour and, like dominants, their response patterns would become determined by current nutritional status.

Given the results of the first experiment it is clear that food availability under the 1% ration treatment was below the maintenance ration for both dominants and subordinates (Fig. 5). It would therefore be expected that if current nutritional status was responsible in determining prey behaviour under threat, that dominants and subordinates should have responded less or not at all to alarm cues in the second experiment under the 1% ration. This discrepancy between the predicted and

observed responses may be explained by one of three mechanisms: 1) a shift in trade-offs, 2) a change in relative energetic requirements, or 3) a change in perceived predation threat. First, the trout used in experiment 2, although of the same age-class as those used in experiment 1, were considerably larger in both body weight and length. Therefore, according to the asset-protection principle the test fish in the second experiment would likely gain less relative fitness benefits per unit food than the smaller fish of the first experiment (Clark, 1994). This reduction in benefits associated with foraging should lead to a shift in the trade-offs towards greater risk-aversion. Second, as animals grow their relative energetic requirements decrease according to the relationship $B = cM^{0.75}$, where B is basal metabolic rate and M is mass (Burger & Johnson, 1991; Dodds et al., 2001). Therefore, a 1% ration may have been sufficient to meet the energetic requirements of the fish in experiment 2, consequently the higher nutritional status would have made the larger individuals more risk averse. Third, the single fish may have perceived a greater threat of predation. Gregarious behaviour is often associated with a reduction in individual risk by decreasing the probability that a given individual will be attacked, increasing the difficulty of a predator following a single prey or increasing likelihood of predator detection through the summed vigilance of group members (Boland, 2003, Fernández et al., 2003). An individual is therefore likely to perceive a greater level of threat when alone than when paired or in a group, leading to an increase in predator avoidance. All three of these mechanisms are quite plausible and indeed, may be working synergistically, thus further work is necessary to elucidate the cause of this discrepancy in results.

The present study does not provide any evidence that chemically mediated predation threats are capable of temporarily disrupting social hierarchies. These findings are somewhat surprising given that Reinhardt (1999) has previously demonstrated that visual cues of a predator can cause an alteration in the dynamics of social hierarchies. There are two possible explanations for this disagreement in results: 1) chemical cues may simply not be capable of altering hierarchies or 2) the social hierarchies tested in the present study were too weak. First, given the vast body of literature that underlines the importance of chemical alarm cues as a source of information concerning predation threats (Smith, 1992), it seems unlikely that alarm cues are incapable of altering hierarchies. Furthermore, there is a plethora of studies demonstrating that chemical alarm cues elicit a strong reduction in aggression levels (Vehanen, 2003; Martel & Dill, 1993; Martel & Dill, 1995). It would therefore appear that chemical alarm cues should be capable of causing the dissolution of social hierarchies. Second, Reinhardt (1999) tested hierarchies of twenty fish whereas the current study only tested two. It is possible that a dominant individual may be able to maintain its status throughout a predation threat without greatly increasing the costs of aggression when only one other individual is present. However, as the number of subordinates increases the costs of aggression under threat are likely to rise drastically. Therefore, predation threat may only be capable of altering hierarchies once a critical threshold in the size of hierarchies has been reached.

While social hierarchies appear to be stable under a chemically-mediated threat of predation, the results of this study reaffirm that food availability is capable of causing the dissolution of social hierarchies. As food availability was increased

from intermediate to high levels, aggression significantly decreased. As the abundance of food increases, the defendability of this resource decreases (Grant, 1997). Therefore, when food abundance is high aggression levels are reduced (Slaney & Northcote, 1974; Grant et al., 2002) and the dynamics of social hierarchies are altered allowing subordinates to gain access to food. Under the high food treatment subordinates were able to gain access to previously unavailable food resources and continued to forage under the risk of predation, while dominants increased predator avoidance behaviour.

When taken as a whole this study suggests that trade-offs are likely extremely important in determining the future fitness of YOY rainbow trout, this is especially true of the trade-off between predator avoidance and foraging. In fact, balancing the need to forage against the need to avoid predation may be one of the single most important trade-offs faced by YOY, as growth rates are integral in determining life-history strategies as well as overwinter survival (Maclean & Metcalfe, 2001; Reinhardt, 1999; Oliver et al., 1979; Olson, 1996; Hyvärinen & Vehanen, 2004). The results support the prediction that YOY rainbow trout are indeed capable of balancing the conflicting demands of foraging and predator avoidance. Furthermore, this trade-off is influenced by multiple factors, including social status and food availability, and that the costs and benefits of said trade-off are heterogeneous within a population of prey individuals. Therefore, predicting individual predator avoidance behaviour can be quite difficult and it is important to consider all possible variables, as there is no optimal trade-off for all situations.

Future research should replicate the first experiment testing larger social hierarchies to determine if chemical alarm cues are capable of altering hierarchies, as it is likely that the lack of response in current study was an artifact of the experimental design. If, as is expected, chemical cues can alter hierarchies then periodic exposure to predation threat may allow subordinates to gain extra foraging opportunities and hence reduce size heterogeneity within a population. The use of periodic exposures to chemical alarm cues could potentially benefit fisheries by increasing size homogeneity, and if the cues are combined with predator odours, increased post-release survival through the learning of potential predators (Suboski & Templeton, 1989).

Figure 1. Mean (± SE) time spent moving for experiment 1 under 1% (A), 5% (B), & 10% (C) body weight feeding regimes for dominant and subordinate individuals exposed to alarm cue or distilled water stimuli. Solid bars = pre-stimulus injection observation period, open bars = post-stimulus injection observation period.

1% Body Weight

5% Body Weight

10% Body Weight

Mean time spent moving (s)

PreStimulus
PostStimulus

Alarm cue   Distilled water   Alarm cue   Distilled water
Dominant   Subordinate

Figure 2. Mean (± SE) number of feeding attempts for experiment 1 under 1% (A),

5% (B), & 10% (C) body weight feeding regimes for dominant and subordinate

individuals exposed to alarm cue or distilled water stimuli. Solid bars = pre-stimulus

injection observation period, open bars = post-stimulus injection observation period.

PreStimulus
PostStimulus

1% Body Weight

5% Body Weight

Mean # feeding attempts

10% Body Weight

Alarm cue   Distilled water   Alarm cue   Distilled water
Dominant   Subordinate

Figure 3. Mean (± SE) latency to first feeding attempt for experiment 1 under 1%

(A), 5% (B), & 10% (C) body weight feeding regimes for dominant and subordinate

individuals exposed to alarm cue or distilled water stimuli. Solid bars = pre-stimulus

injection observation period, open bars = post-stimulus injection observation period.

Mean latency to first feeding attempt (s)

Legend: PreStimulus / PostStimulus

1% Body Weight

5% Body Weight

10% Body Weight

Alarm cue | Distilled water | Alarm cue | Distilled water
Dominant | Subordinate
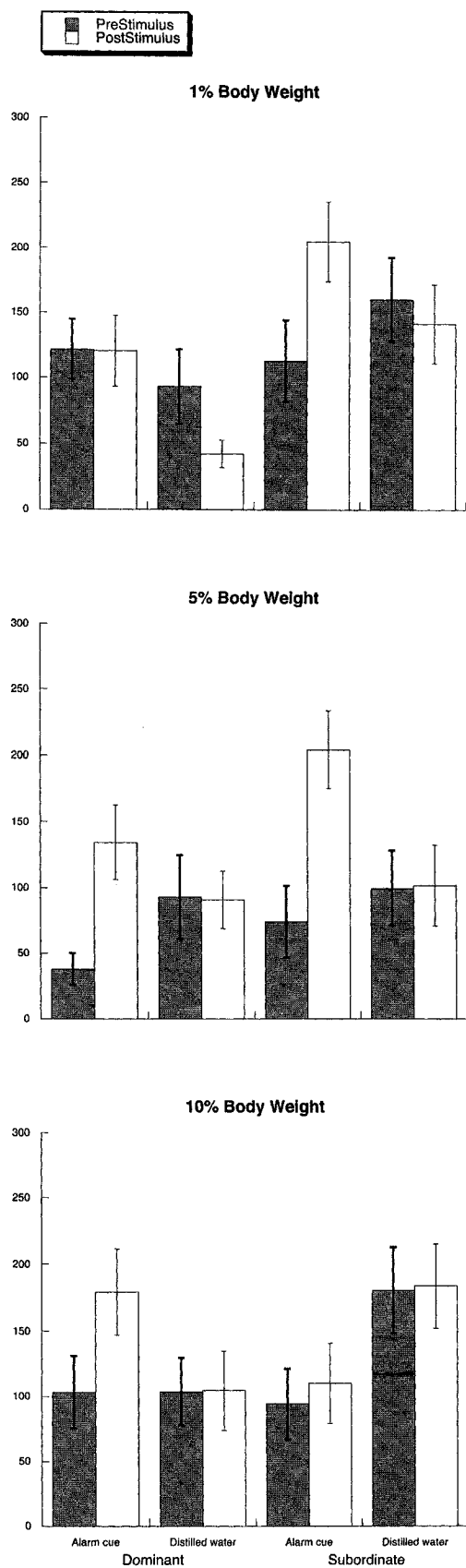
Figure 4. Mean (± SE) number of aggressive interactions for experiment 1 under

1% (A), 5% (B), & 10% (C) body weight feeding regimes for dominant and

subordinate individuals exposed to alarm cue or distilled water stimuli. Solid bars =

pre-stimulus injection observation period, open bars = post-stimulus injection

observation period.

Mean # aggressive interactions
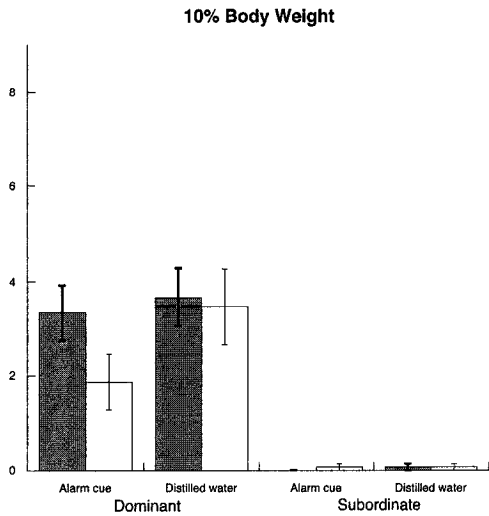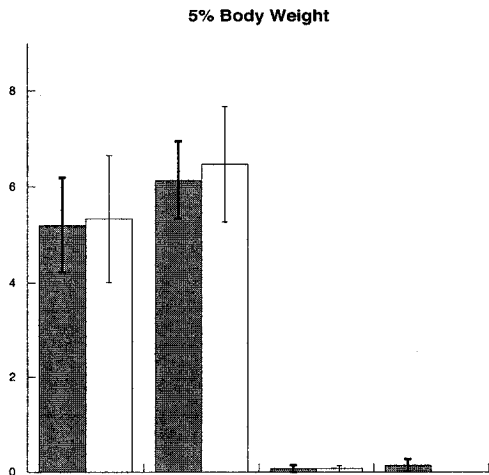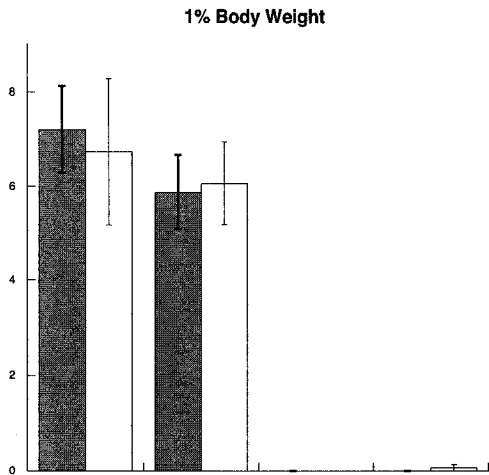
Figure 5. Mean (± SE) change in body weight over the duration of experiment 1

under 1%, 5%, & 10% body weight feeding regimes for dominant and subordinate

individuals. Solid bars = alarm cue stimulus, open bars = distilled water stimulus.

Mean Change in Body Weight
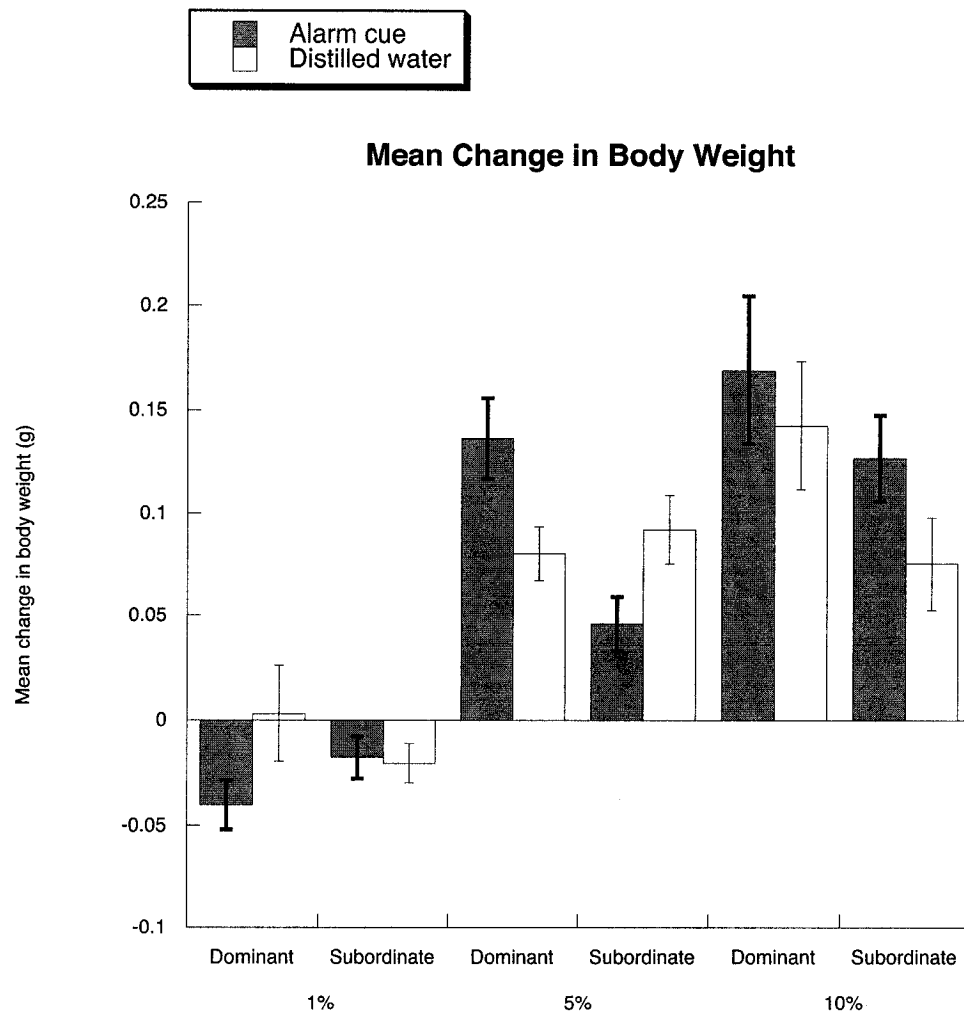
Figure 6. Mean (± SE) time spent moving for experiment 2 under 1% (A) & 10%

(B) body weight feeding regimes for dominant and subordinate individuals exposed

to alarm cue or distilled water stimuli. Solid bars = pre-stimulus injection

observation period, open bars = post-stimulus injection observation period.

1% Body Weight

10% Body Weight

Mean time spent moving (s)

PreStimulus
PostStimulus

Alarm cue   Distilled water   Alarm cue   Distilled water
Dominant   Subordinate
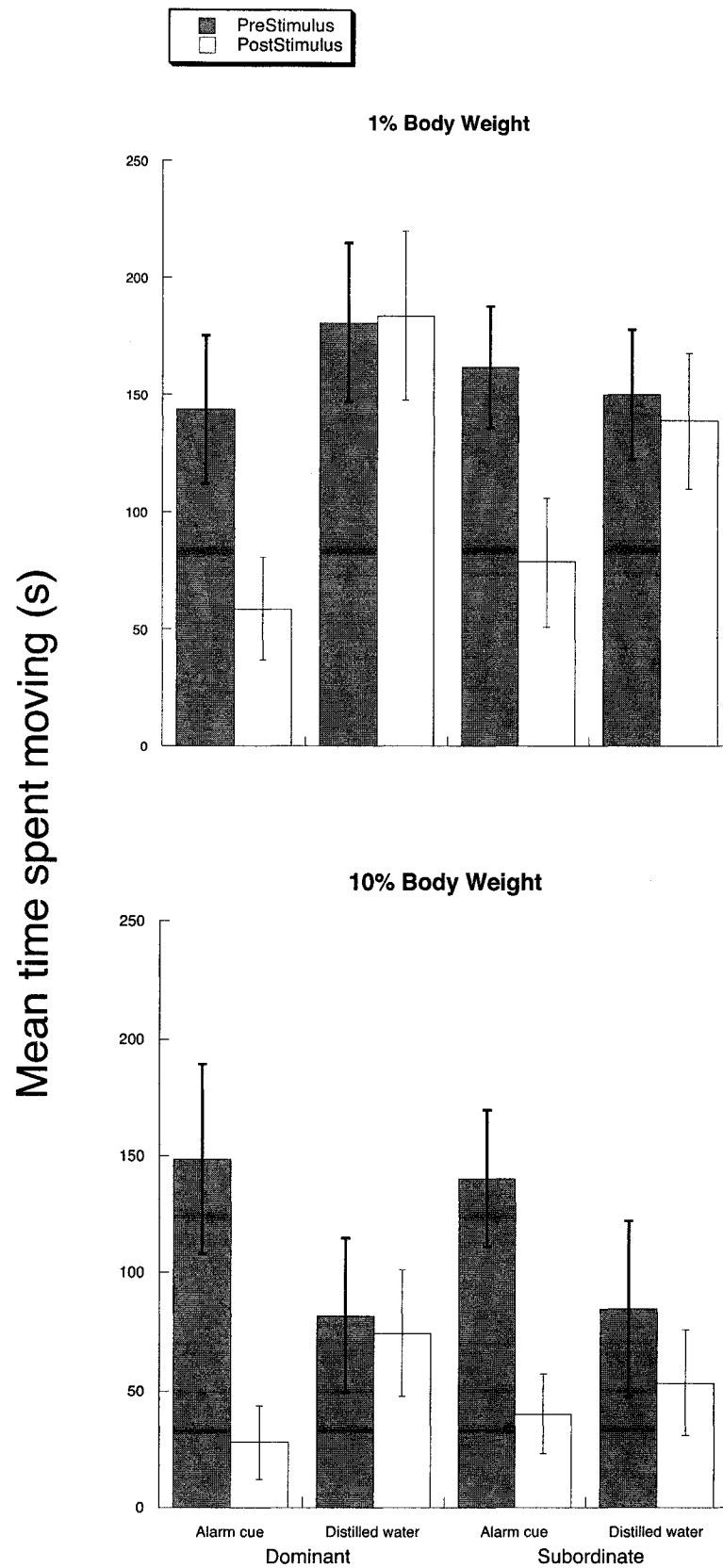
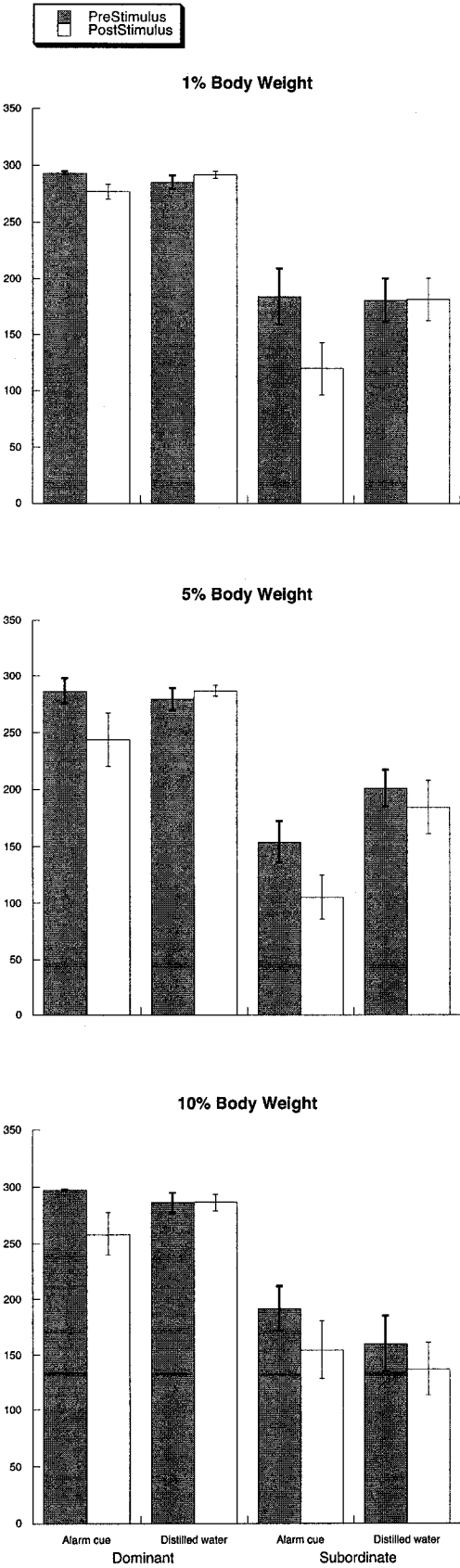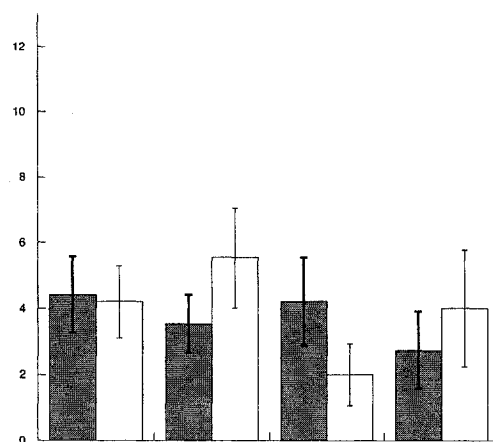**Figure 7.** Mean (± SE) number of feeding attempts for experiment 2 under 1% (A)

& 10% (B) body weight feeding regimes for dominant and subordinate individuals

exposed to alarm cue or distilled water stimuli. Solid bars = pre-stimulus injection

observation period, open bars = post-stimulus injection observation period.

1% Body Weight

10% Body Weight

Mean # feeding attempts

Alarm cue   Distilled water   Alarm cue   Distilled water

Dominant   Subordinate
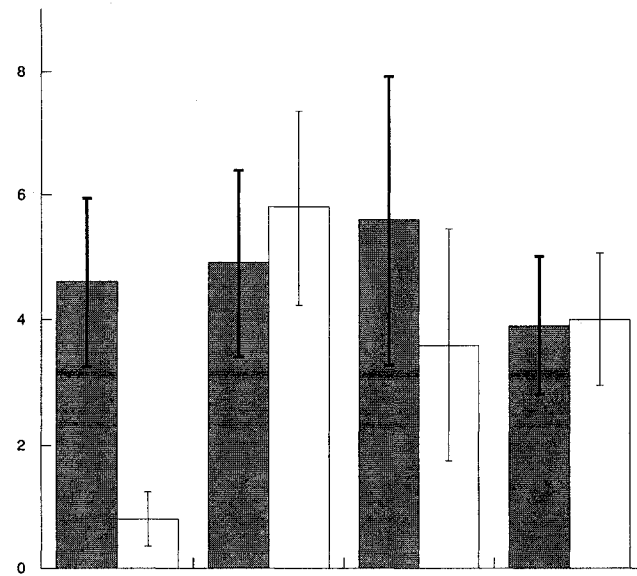
PreStimulus
PostStimulus
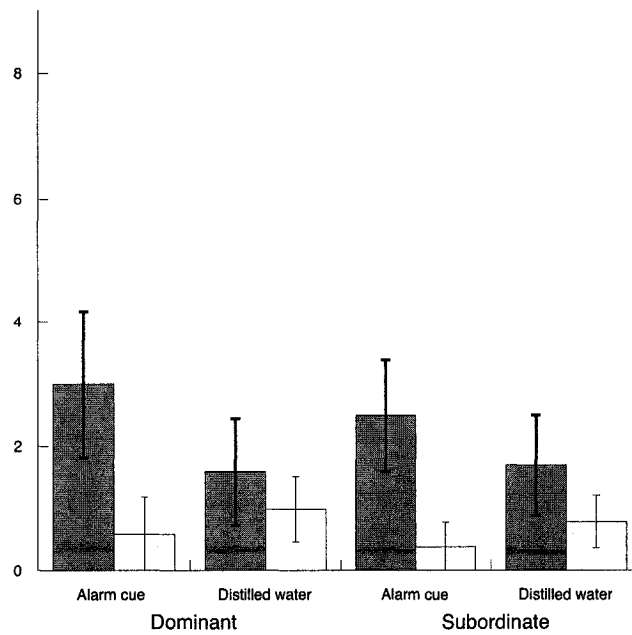
Figure 8. Mean (± SE) latency to first feeding attempt for experiment 2 under 1%

(A), 10% (B) body weight feeding regimes for dominant and subordinate individuals

exposed to alarm cue or distilled water stimuli. Solid bars = pre-stimulus injection

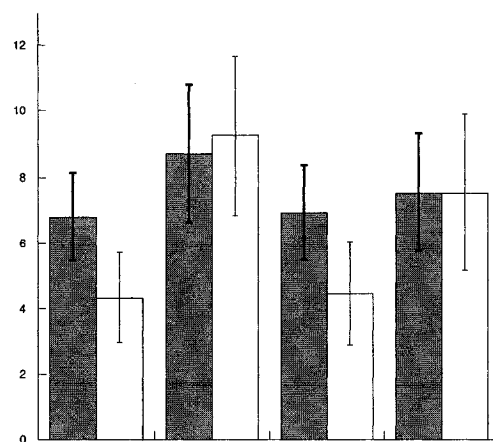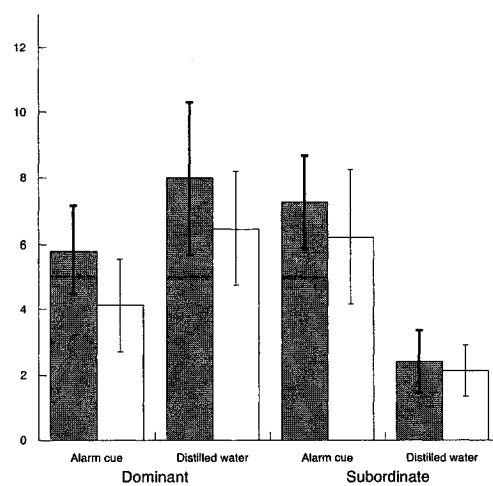observation period, open bars = post-stimulus injection observation period.

Mean Latency to first feeding attempt (s)

Legend: Prestimulus / Poststimulus

1% Body Weight

10% Body Weight

Alarm cue | Distilled water | Alarm cue | Distilled water
Dominant | Subordinate
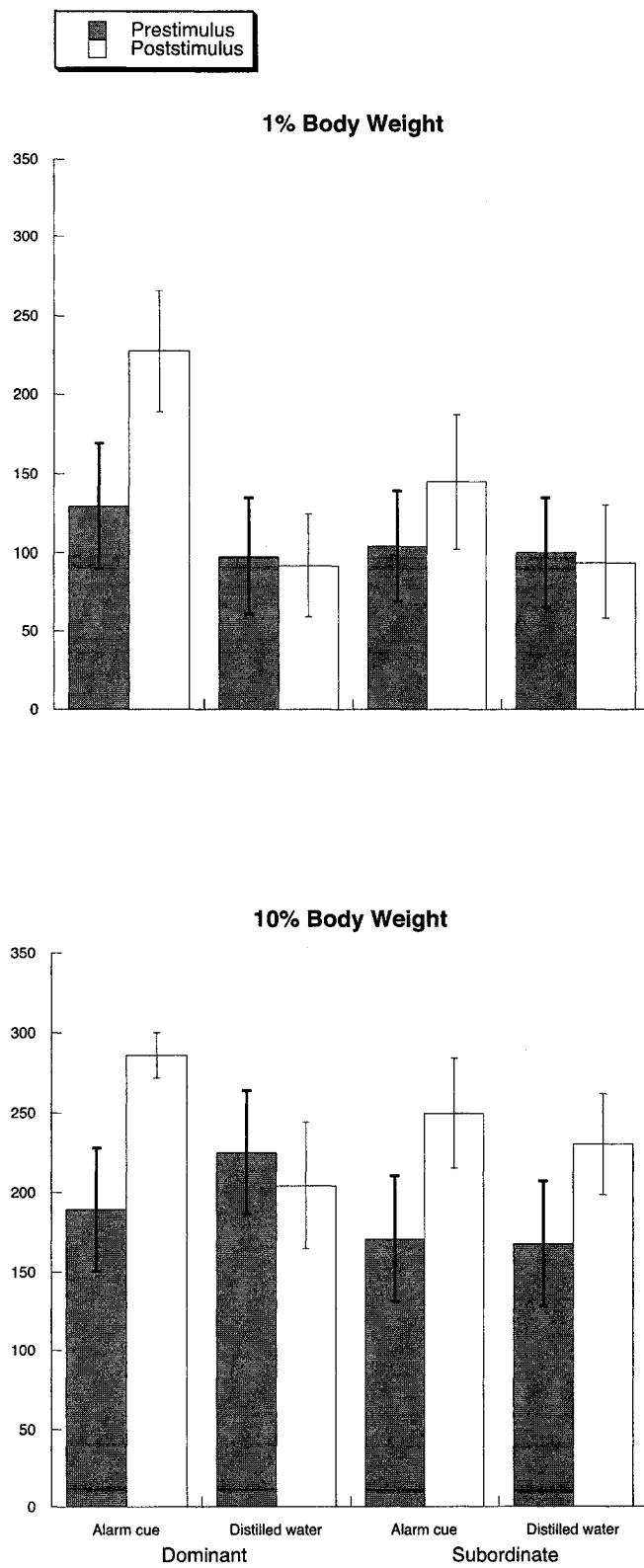
Table 1. Predicted behavioural patterns of dominant (D) and subordinate (S) individuals in response to a predation threat according to the asset-protection and social status hypotheses under 1%, 5%, and 10% ration feeding regimes. ↓ = reduction in behavioural measure, ↑ = increase in behavioural measure, - = no change in behavioural measure.

<u>Experiment 1</u>

	Asset Protection			Social Status		
	1%	5%	10%	1%	5%	10%
Behavioural measure	D/S	D/S	D/S	D/S	D/S	D/S
Time spent moving	-/-	↓/↓	↓/↓	-/↓	↓/-	↓/-
Number of feeding attempts	-/-	↓/↓	↓/↓	-/↓	↓/-	↓/-
Latency to 1st feeding attempt	-/-	↑/↑	↑/↑	-/↑	↑/-	↑/-
Number of aggressive interactions	-/-	↓/↓	↓/↓	-/↓	↓/-	↓/-

<u>Experiment 2</u>

	Asset Protection			Social Status		
	1%	5%	10%	1%	5%	10%
Behavioural measure	D/S	D/S	D/S	D/S	D/S	D/S
Time spent moving	-/-	↓/↓	↓/↓	-/↓	↓/-	↓/-
Number of feeding attempts	-/-	↓/↓	↓/↓	-/↓	↓/-	↓/-
Latency to 1st feeding attempt	-/-	↑/↑	↑/↑	-/↑	↑/-	↑/-

Table 2. Operational definitions of the three foraging and three aggressive Modal Action Patterns assessed in this study.

Foraging MAPs	Definition
Time spent moving	Combined duration of any displacements of greater than half a body length.
Feeding attempts	Displacement of greater than half a body length toward an object in the water column or on the substrate involving an obvious strike at the object.
Latency to first feeding attempt	Time lapse between the beginning of an observation period and an individual's first feeding attempt.
Aggressive MAPs	
Chases	Pursuit of an individual over a distance of greater than two body lengths.
Approaches	Advancement of greater than two body lengths towards a stationary individual.
Biting	A strike directed towards an individual.

Table 3. Results of independent samples t-test for experiment 1 comparing differences in time spent moving, number of feeding attempts, latency to first feeding attempt, and number of aggressive interactions between alarm cue and distilled water treatments under 1%, 5%, and 10% ration feeding regimes. Significant differences are denoted in **bold** face, marginal differences are denoted in *italic* face.

Variables	t	df	p
1% Ration			
Moving			
Dominant	3.040	28	**0.003**
Subordinate	2.850	28	**0.004**
Feeding			
Dominant	1.452	28	0.079
Subordinate	2.875	28	**0.004**
Latency			
Dominant	-1.191	28	0.122
Subordinate	-2.169	28	**0.020**
Aggression			
Dominant	-0.437	28	0.333
Subordinate	-1.000	28	0.326
5% Ration			
Moving			
Dominant	2.345	28	**0.013**
Subordinate	1.647	28	*0.056*
Feeding			
Dominant	2.775	28	**0.005**
Subordinate	1.103	28	0.140
Latency			
Dominant	-2.890	28	**0.004**
Subordinate	-3.987	28	**<0.001**
Aggression			
Dominant	0.114	28	0.455
Subordinate	0.807	28	0.426
10% Ration			
Moving			
Dominant	1.935	28	**0.032**
Subordinate	0.593	28	0.279
Feeding			
Dominant	0.088	28	0.465
Subordinate	0.486	28	0.316

Table 3.   Continued…

Latency

Dominant	-2.121	28	**0.022**
Subordinate	-0.274	28	0.393

Aggression

Dominant	-1.232	28	0.114
Subordinate	0.564	28	0.577

Table 4. Results of repeated-measures ANOVA for experiment 1 assessing the effects of observation period, ration, and cue, and their interactions on time spent moving, number feeding attempts, and latency to first feeding attempt. Observation period was the repeated-measures factor for individual fish. Significant differences denoted in **bold** face, marginal differences denoted in *italic* face.

Effect	F	df	p
Moving			
Period	30.013	1, 168	**<0.001**
Period*Social	4.982	1, 168	**0.027**
Period*Ration	0.364	2, 168	0.695
Period*Cue	20.379	1, 168	**<0.001**
Period*Social*Ration	0.447	2, 168	0.640
Period*Social*Cue	0.002	1, 168	0.966
Period*Ration*Cue	0.382	2, 168	0.683
Period*Social*Ration*Cue	1.911	2, 168	0.151
Feeding			
Period	4.360	1, 168	**0.038**
Period*Social	0.131	1, 168	0.718
Period*Ration	1.907	2, 168	0.152
Period*Cue	9.757	1, 168	**0.002**
Period*Social*Ration	1.064	2, 168	0.347
Period*Social*Cue	0.131	1, 168	0.718
Period*Ration*Cue	1.441	2, 168	0.240
Period*Social*Ration*Cue	0.169	2, 168	0.845
Latency			
Period	12.895	1, 168	**<0.001**
Period*Social	0.878	1, 168	0.350
Period*Ration	2.974	2, 168	*0.054*
Period*Cue	24.107	1, 168	**<0.001**
Period*Social*Ration	2.233	2, 168	0.110
Period*Social*Cue	0.026	1, 168	0.873
Period*Ration*Cue	1.497	2, 168	0.227
Period*Social*Ration*Cue	1.093	2, 168	0.338
Aggression			
Period	0.334	1, 84	0.565
Period*Ration	0.547	2, 84	0.581
Period*Cue	0.706	1, 84	0.403
Period*Ration*Cue	0.133	2, 84	0.876

Table 5. Results of 2-Way ANOVAs for experiment 1 assessing the effects of ration and cue, and their interactions on time spent moving, number of feeding attempts, latency to first feeding attempt, and number of aggressive interactions during the post-stimulus observation period. Significant differences denoted in **bold** face.

Effect	F	df	p
Dominant			
Moving			
Ration	1.309	2, 84	0.276
Cue	6.541	1, 84	**0.012**
Ration*Cue	0.728	2, 84	0.486
Feeding			
Ration	0.764	2, 84	0.469
Cue	4.574	1, 84	**0.035**
Ration*Cue	0.641	2, 84	0.529
Latency			
Ration	2.347	2, 84	0.102
Cue	10.222	1, 84	**0.002**
Ration*Cue	0.356	2, 84	0.702
Aggression			
Ration	6.658	2, 84	**0.002**
Cue	0.577	1, 84	0.450
Ration*Cue	0.581	2, 84	0.562
Subordinate			
Moving			
Ration	0.040	2, 84	0.961
Cue	4.938	1, 84	**0.029**
Ration*Cue	2.545	2, 84	0.085
Feeding			
Ration	1.632	2, 84	0.202
Cue	0.059	1, 84	0.808
Ration*Cue	2.641	2, 84	0.077
Latency			
Ration	0.398	2, 84	0.673
Cue	1.515	1, 84	0.222
Ration*Cue	4.588	2, 84	**0.013**

Table 6. Results of independent samples t-test for experiment 2 comparing differences in time spent moving, number of feeding attempts, and latency to first feeding attempt between alarm cue and distilled water treatments under 1% and 10% ration feeding regimes. Significant differences are denoted in **bold** face.

Variables	t	df	p
1% Ration			
Moving			
Dominant	-2.604	18	**0.009**
Subordinate	-2.175	18	**0.024**
Feeding			
Dominant	-3.044	18	**0.004**
Subordinate	-2.641	18	**0.009**
Latency			
Dominant	1.923	18	**0.035**
Subordinate	-1.803	18	**0.044**
10% Ration			
Moving			
Dominant	-3.120	18	**0.003**
Subordinate	-2.152	18	**0.023**
Feeding			
Dominant	1.516	18	0.074
Subordinate	-1.425	18	0.086
Latency			
Dominant	2.561	18	**0.010**
Subordinate	0.958	18	0.176

Table 7. Results of repeated-measures ANOVA for experiment 2 assessing the effects of observation period, ration, and cue, and their interactions on time spent moving, number feeding attempts, and latency to first feeding attempt. Observation period was the repeated-measures factor for individual fish. Significant differences denoted in **bold** face.

Effect	F	df	p
Dominant			
Moving			
Period	17.937	1, 36	**<0.001**
Period*Ration	0.852	1, 36	0.362
Period*Cue	16.466	1, 36	**<0.001**
Period*Ration*Cue	0.254	1, 36	0.618
Feeding			
Period	9.179	1, 36	**0.005**
Period*Ration	0.003	1, 36	0.959
Period*Cue	11.141	1, 36	**0.002**
Period*Ration*Cue	2.218	1, 36	0.145
Latency			
Period	5.620	1, 36	**0.023**
Period*Ration	0.055	1, 36	0.815
Period*Cue	9.758	1, 36	**0.004**
Period*Ration*Cue	0.036	1, 36	0.850
Subordinate			
Moving			
Period	24.026	1, 36	**<0.001**
Period*Ration	0.619	1, 36	0.437
Period*Cue	9.359	1, 36	**0.004**
Period*Ration*Cue	0.005	1, 36	0.942
Feeding			
Period	17.903	1, 36	**<0.001**
Period*Ration	0.902	1, 36	0.349
Period*Cue	8.120	1, 36	**0.007**
Period*Ration*Cue	0.604	1, 36	0.442
Latency			
Period	9.796	1, 36	**0.003**
Period*Ration	3.548	1, 36	0.068
Period*Cue	4.792	1, 36	**0.035**
Period*Ration*Cue	0.289	1, 36	0.594

Table 8. Results of 2-Way ANOVAs for experiment 2 assessing the effects of ration and cue, and their interactions on time spent moving, number of feeding attempts, and latency to first feeding attempt during post-stimulus observation period. Significant differences denoted in **bold** face, marginal differences denoted in *italic* face.

Effect	F	df	p
Dominant			
Moving			
Ration	7.108	1, 36	**0.011**
Cue	10.735	1, 36	**0.002**
Ration*Cue	2.270	1, 36	0.141
Feeding			
Ration	6.719	1, 36	**0.014**
Cue	10.910	1, 36	**0.002**
Ration*Cue	0.678	1, 36	0.416
Latency			
Ration	7.612	1, 36	**0.009**
Cue	8.878	1, 36	**0.005**
Ration*Cue	6.442	1, 36	**0.016**
Subordinate			
Moving			
Ration	6.341	1, 36	**0.016**
Cue	2.241	1, 36	0.143
Ration*Cue	0.930	1, 36	0.341
Feeding			
Ration	10.934	1, 36	**0.002**
Cue	0.932	1, 36	0.341
Ration*Cue	0.182	1, 36	0.672
Latency			
Ration	8.409	1, 36	**0.006**
Cue	0.131	1, 36	0.719
Ration*Cue	0.000	1, 36	1.000

References

Abbott, J.C., Dunbrack, R.L. & Orr, C.D. 1985. The interaction between size and experience in dominance relationships of juvenile steelhead trout (*Salmo gairdneri*). Behaviour, 92: 241-253.

Alberts, A.C., Lemm, J.M., Perry, A.M., Morici, L.A., & Phillips, J.A. 2002. Temporary alteration of local social structure in a threatened population of Cuban iguanas (*Cyclura nubila*). Behavioral Ecology and Sociobiology, 51: 324–335.

Alsop, D.H. & Wood, C.M. 1997. The interactive effects of feeding and exercise on oxygen consumption, swimming performance and protein usage in juvenile rainbow trout (*Oncorhynchus mykiss*). The Journal of Experimental Biology, 200: 2337–2346.

Barroso, F.G., Alados, C.L., & Boza, J. 2000. Social hierarchy in the domestic goat: effect on food habits and production. Applied Animal Behaviour Science, 69: 35-53.

Bernot, R.J. 2003. Trematode infection alters the antipredator behavior of a pulmonate snail. Journal of the North American Benthological Society, 22: 241-248.

Boland, C.R.J. 2003. An experimental test of predator detection rates using groups of free-living emus. Ethology, 109: 209-222.

Brown, G.E. 2003. Learning about danger: chemical alarm cues and local risk assessment in prey fishes. Fish and Fisheries, 4: 227-234.

Brown, G.E. & Smith, R.J.F. 1997. Conspecific skin extracts elicit antipredator responses in juvenile rainbow trout (*Oncorhynchus mykiss*). Canadian Journal of Zoology, 75: 1916-1922.

Brown, G.E., Adrian, J.C., Jr., Naderi, N.T., Harvey, M.C., & Kelly, J.M. 2003. Nitrogen oxides elicit antipredator responses in juvenile channel catfish, but not in convict cichlids or rainbow trout: conservation of the Ostariophysan alarm cue. Journal of Chemical Ecology, 29: 1781-1796.

Bryer, P.J., Mirza, R.S., & Chivers, D.P. 2001. Chemosensory assessment of predation risk by slimy sculpins (*Cottus cognatus*): responses to alarm, disturbance, and predator cues. Journal of Chemical Ecology, 27: 533-546.

Burger, I.H. & Johnson, J.V. 1991. Dogs large and small – the allometry of energy-requirements within a single species. Journal of Nutrition, 121: 18-21.

Chivers, D.P., Brown, G.E. & Smith, R.J.F. 1995. Acquired recognition of chemical stimuli from pike, *Esox lucius*, by brook sticklebacks, *Culea inconstans* (Osteichthyes, Gasterosteidae). Ethology, 99: 234-242.

Chivers, D.P., Mirza, R.S., Bryer, P.J. & Kiesecker, J.M. 2001. Threat-sensitive predator avoidance by slimy sculpins: understanding the importance of visual versus chemical information. Canadian Journal of Zoology, 79: 867-873.

Chivers, D.P. & Smith, R.J.F. 1998. Chemical alarm signaling in aquatic predator-prey systems: a review and prospectus. Écoscience, 5: 315-321.

Clark, C.W. 1994. Antipredator behaviour and the asset-protection principle. Behavioral Ecology, 5: 159-170.

Dodds, P. S., Rothman, D. H., & Weitz, J. S. 2001. Re-examination of the "3/4-law" of Metabolism. Journal of Theoretical Biology, 209: 9-27.

Dowling, L.M., & Godin, J-G. J. 2002. Refuge use in a killifish: influence of body size and nutritional state. Canadian Journal of Zoology, 80: 782-788.

Fernández, G.J., Capurro, A.F., & Reboreda, J.C. 2003. Effect of group size on individual and collective vigilance in Greater Rheas. Ethology, 109: 413-425.

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

Godin, J.-G., & Dugatkin, L. A. 1996. Female mating preferences for bold males in the guppy, *Poecilia reticulata*. Proceedings of the National Academy of Science USA, 93: 10262–10267.

Godin, J.-G.J. & Sproul, C.D. 1988. Risk taking in parasitized sticklebacks under threat of predation: effects of energetic need and food availability. Canadian Journal of Zoology, 66: 2360-2367.

Golub, J.L., Vermette, V., & Brown, G.E. 2005. Response to conspecific and heterospecific alarm cues by pumpkinseeds (*Lepomis gibbosus*): field verification of the ontogenetic shift. Journal of Fish Biology. 66: 1073-1081.

Gotceitas, V. & Godin, J.-G.J. 1991. Foraging under the risk of predation in juvenile Atlantic salmon (*Salmo salar* L.): effects of social status and hunger. Behavioral Ecology and Sociobiology, 29: 255-261.

Grant, J.W.A. 1990. Aggressiveness and the foraging behaviour of Young-of-the-Year brook charr (*Salvelinus fontinalis*). Canadian Journal of Fisheries and Aquatic Sciences, 47: 915-920.

Grant, J.W.A. 1997. Territoriality. In *Behavioural Ecology of Teleost Fishes* (ed. J.-G.J. Godin), pp. 81-103. Oxford University Press, Oxford.

Grant, J.W.A. & Noakes, D.L.G. 1987. Escape behaviour and use of cover by Young-of-the-Year brook trout, *Salvelinus fontinalis*. Canadian Journal of Fisheries and Aquatic Sciences, 44: 1390-1396.

Grant, J.W.A., Girard, I.L., Breau, C., & Weir, L.K. 2002. Influence of food abundance on aggression in juvenile convict cichlids. Animal Behaviour, 63:323-330.

Gregory, T.R. & Wood, C.M. 1999. Interactions between individual feeding behaviour, growth, and swimming performance in juvenile rainbow trout (*Oncorhynchus mykiss*) fed different rations. Canadian Journal of Fisheries and Aquatic Sciences, 56: 479-486.

Harwood, A.J., Armstrong, J.D., Metcalfe, N.B., & Griffiths, S.W. 2003. Does dominance status correlate with growth in wild stream-dwelling Atlantic salmon (*Salmo salar*)? Behavioral Ecology, 14: 902-908.

Helfman, G.S. 1989. Threat-sensitive predator avoidance in damselfish-trumpetfish interactions. Behavioral Ecology and Sociobiology, 24: 47-58.

Horat, P. & Semlitsch, R.D. 1994. Effects of predation risk and hunger on the behaviour of two species of tadpoles. Behavioral Ecology and Sociobiology, 34: 393-401.

Huntingford, F.A., Metcalfe, N.B., Thorpe, J.E., Graham, W.D., & Adams, C.E. 1990. Social dominance and body size in Atlantic salmon parr, *Salmo salar* L. Journal of Fish Biology, 36: 877-881.

Hyvärinen, P. & Vehanen, T. 2004. Effect of trout body size on post-stocking survival and pike predation. Ecology of Freshwater Fish, 13: 77-84.

Johnsson, J.J. 1993. Big and brave: Size selection affects foraging under risk of predation in juvenile rainbow trout, *Oncorhynchus mykiss*. Animal Behaviour, 45: 1219-1225.

Kamunde, C. & Wood, C.M. 2003. The influence of ration size on copper homeostasis during sublethal dietary copper exposure in juvenile rainbow trout, *Oncorhynchus mykiss*. Aquatic Toxicology, 62: 235-254.

Kavaliers, M., Choleris, E., & Colwell, D.D. 2001. Brief exposure to female odors "emboldens" male mice by reducing predator-induced behavioral and hormonal responses. Hormones and Behavior, 40: 497-509.

Kerr, S. J. & Lasenby, T. A. 2000. Rainbow trout stocking in inland lakes and streams: an annotated bibliography and literature review. Fish and Wildlife Branch, Ontario Ministry of Natural Resources, Peterborough, Ontario. 220 p. + appendices.

Leduc, A.O.H.C., Ferrari, M.C.O., Kelly, J.M., & Brown, G.E. 2004a. Learning to recognize novel predators under weakly acidic the effects of reduced pH on acquired predator recognition by juvenile rainbow trout. Chemoecology, 14: 107 - 112.

Leduc, A.O.H.C., Kelly, J.M., & Brown, G.E. 2004b. Detection of conspecific alarm cues by juvenile salmonids under neutral and weakly acidic conditions: laboratory and field tests. Oecologia, 139: 318 - 324.

Lima, S.L. & Bednekoff, P.A. 1999. Temporal variation in danger drives antipredator behavior: the predation risk allocation hypothesis. American Naturalist, 153: 649-659.

Lima, S.L. & Dill, L.M. 1990. Behavioral descisions made under the risk of predation: a review and prospectus. Canadian Journal of Zoology, 68: 619-640.

Maclean, A. & Metcalfe, N.B. 2001. Social status, access to food, and compensatory growth in juvenile Atlantic salmon. Journal of Fish Biology, 58: 1331-1346.

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

Martel, G. 1996. Growth rate and influence of predation risk on territoriality in juvenile coho salmon (Oncorhynchus kisutch). Canadian Journal of Fisheries and Aquatic Sciences, 53: 660-669.

Martel, G. & Dill, L.M. 1993. Feeding and aggressive behaviours in juvenile coho salmon (Oncorhynchus kisutch) under chemically-mediated risk of predation. Behavioral Ecology and Sociobiology, 32: 365-370.

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

Martin-Smith, K.M. & Armstrong, J.D. 2002. Growth rates of wild stream-dwelling Atlantic salmon correlate with activity and sex but not dominance. Journal of Animal Ecology, 3: 413-423.

Metcalfe, N.B., Taylor, A.C., & Thorpe, J.E. 1995. Metabolic rate, social status and life-history strategies in Atlantic salmon. Animal Behavior, 49: 431–436.

Mirza, R.S. & Chivers, D.P. 2001. Are alarm cues conserved within salmonid fishes? Journal of Chemical Ecology, 27: 1641-1655.

Mirza, R.S. & Chivers, D.P. 2002. Brook char (*Salvelinus fontinalis*) can differentiate chemical alarm cues produced by different age/size classes of conspecifics. Journal of Chemical Ecology, 28: 555-564.

Mirza, R.S. & Chivers, D.P. 2003. Response of juvenile rainbow trout to varying concentrations of chemical alarm cue: response thresholds and survival during encounters with predators. Canadian Journal of Zoology, 81: 88-95.

Monnin, T., Ratnieks, F.L.W., & Brandao, C.R.F. 2003. Reproductive conflict in animal societies: hierarchy length increases with colony size in queenless ponerine ants. Behavioral Ecology and Sociobiology, 54: 71-79.

Moore, F.R. 1994. Resumption of feeding under the risk of predation: effect of migratory condition. Animal Behaviour, 48: 975-977.

Newman, M.A. 1956. Social behavior and interspecific competition in two trout species. Physiological Zoology, 29: 64-81.

Novotny, V., Basset, Y., Auga, J., Boen, W., Dal, C., Drozd, P., Kasbal, M., Isua, B., Kutil, R., Manumbor, M., & Molem, K. 1999. Predation risk for herbivorous insects on tropical vegetation: A search for enemy-free space and time. Australian Journal of Ecology, 24: 477-483.

O'Connor, K.I., Metcalfe, N.B. & Taylor, A.C. 1999. Does darkening signal submission in territorial contests between juvenile Atlantic salmon, *Salmo salar*? Animal Behaviour, 58: 1269–1276.

Oden, K., Berg, C., Gunnarsson, S., Algers, B. 2004. Male rank order, space use and female attachment in large flocks of laying hens. Applied Animal Behaviour Science, 87: 83-94.

Oliver, T.D., Holeton, G.F., & Chua, K.E. 1979. Overwinter mortality of fingerling smallmouth bass in relation to size, relative energy stores, and environmental temperatures. Transactions of the American Fisheries Society, 108: 130-136.

Olson, M.H. 1996. Ontogenetic niche shifts in largemouth bass: variability and consequences for first-year growth. Ecology, 77: 179-190.

Pettersson, L.B. & Brönmark, C. 1993. Trading off safety against food: state dependent habitat choice and foraging in crucian carp. Oecologia, 95: 353-357.

Pusey, A., Williams, J., & Goodall, J. 1997. The influence of dominance rank on the reproductive success of female chimpanzees. Science, 277: 828–831.

Quinn, T.P., Wetzel, L., Bishop, S., Overberg, K., & Rogers, D.E. 2001. Influence of breeding habitat on bear predation and age at maturity and sexual dimorphism of sockeye salmon populations. Canadian Journal of Zoology, 79: 1782-1793.

Reinhardt, U.G. 1999. Predation risk breaks size-dependent dominance in juvenile coho salmon (*Oncorhynchus kisutch*) and provides growth opportunities for risk-prone individuals. Canadian Journal of Fisheries and Aquatic Sciences, 56: 1206-1212.

Reinhardt, U.G. 2002. Asset protection in juvenile salmon: how adding biological realism changes a dynamic foraging model. Behavioral Ecology, 13: 94-100.

Reinhardt, U.G. & Healey, M.C. 1997. Size-dependent foraging behaviour and use of cover under predation risk. Canadian Journal of Zoology, 75: 1642-1651.

Reinhardt, U.G. & Healey, M.C. 1999. Season- and size-dependent risk taking in juvenile coho salmon: experimental evaluation of asset protection. Animal Behaviour, 57: 923-933.

Ryer, C.H. & Olla, B.L. 1991. Information transfer and the facilitation and inhibition of feeding in a schooling fish. Environmental Biology of Fishes, 30: 317-323.

Slaney, P.A. & Northcote, T.G. 1974. Effects of prey abundance on density and territorial behaviour of young rainbow trout (*Salmo gairdneri*) in laboratory stream channels. Journal of the Fisheries Research Board of Canada, 31: 1201-1209.

Smith, R.J.F. 1992. Alarm signals in fishes. Reviews in Fish Biology and Fisheries, 2: 33-63.

Smith, R.J.F. 1999. What good is smelly stuff in the skin? Cross function and cross taxa effects in fish "alarm substances". In *Advances in Chemical Signals in Vertebrates* (eds. Johnston, R.E., Müller-Schwarze, D. & Sorensen, P.W.), pp. 475-487. Kluwer Academic, New York.

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

Suboski, M.D. & Templeton, J.J. 1989. Life skills training for hatchery fish: social learning and survival. Fisheries Research, 7: 343-352.

Vehanen, T. 2003. Adaptive flexibility in the behaviour of juvenile Atlantic salmon:

short-term responses to food availability and threat from predation. Journal of Fish Biology, 63: 1034-1045.

Walker, S.E. & Rypstra, A.L. 2003. Hungry spiders aren't afraid of the big bad wolf spider. The Journal of Arachnology, 31: 425–427.

Whitham, J. & Mathis, A. 2000. Effects of hunger and predation risk on foraging behavior of graybelly salamanders, *Eurycea multiplicata*. Journal of Chemical Ecology, 26: 1659-1665.

Wittig, R.M. & Boesch, C. 2003. Food competition and linear dominance hierarchy among female chimpanzees of the Taï National Park. International Journal of Primatology, 24: 847-867.