Influence of reproductive assets on threat sensitive responses in wild-caught Trinidadian

guppies (*Poecilia reticulata*)

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ABSTRACT

Influence of reproductive assets on threat sensitive response to chemical alarm cues in Trinidadian guppies (*Poecilia reticulata*) from low and high predation risk areas.

Jemma Katwaroo-Andersen

Accrued reproductive assets in Trinidadian guppies may affect both the costs and benefits associated with anti-predator behaviour and may influence their threat sensitive response pattern. While the threat sensitive behaviour in Trinidadian guppies has received considerable attention, the influence of reproductive assets on threat sensitive response patterns has received little attention. This study compares anti-predator behaviour in gravid (high accrued assets) vs. non-gravid (low accrued assets) guppies from both high and low predation risk populations. A significant difference in anti-predator response was found between gravid and non-gravid guppies in the Lower Aripo population (high predation site); gravid guppies exhibited a stronger overall anti-predator response to the same level of ambient predation threat, whilst non-gravid guppies showed weaker responses. However, in the Upper Aripo population (low predation site), a similar response was found in gravid and non-gravid guppies. The greater anti-predator response observed by gravid guppies in the Lower Aripo population supports the hypothesis that accrued reproductive assets influence threat sensitive response in Trinidadian guppies. Furthermore, the difference in response observed between the Lower and Upper Aripo guppy populations suggests

that long-term predation plays an important role in shaping anti-predator responses to predation risk. This experiment suggests that gravid guppies from high predation sites integrate accumulated reproductive assets, immediate predation risk and long-term predation pressure to make decisions regarding their anti-predator responses.

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Influence of reproductive assets on threat sensitive responses in wild-caught

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Introduction

Predation is a major evolutionary force acting on prey individuals, shaping their behaviour, morphology and life history patterns (Lima & Dill 1990; Preisser et al., 2005). As a result, prey are continually faced with the conflicting demands of the detection and avoidance of predation and a suite of other activities such as foraging, mating and/or territorial defense to increase its fitness (Chivers & Smith, 1998; Lima & Bednekoff, 1999). Predator avoidance strategies of prey may thus be best described as a series of trade-offs between anti-predator benefits and the fitness gain associated with other behaviour patterns (Lima & Bednekoff, 1999; Brown 2003).

Ydenberg & Dill (1986) proposed an economic model, which predicts the response of prey to an acute predation risk. The model predicts that prey organisms should constantly choose the behavioural option (fleeing or remaining) that optimizes the cost-benefit ratio. A prey remaining to forage would risk being captured and killed, but gets the benefit of continuing its activity and increasing its potential energy intake. Conversely, a prey fleeing would come at the cost of less foraging and increased expenditure of energy, but benefits by escaping being captured and killed. Thus the decision (fleeing vs. remaining) should be based on a trade-off between the relative costs and benefits associated with either response.

The ability to make such behavioural decisions may be made more difficult under uncertain conditions. Predation risk is spatially and temporally variable due to seasonal

changes in predator and/or prey guild membership, prey and predator movements and/or

transient visits by predators (Sih et al., 2000). Such variability in local predation threats

may interact to shape both the overall intensity and pattern of anti-predator behaviour

(Lima & Bednekoff, 1999). As a result, predation threat can be categorized as being acute

or chronic. Acute predation threat involves immediate or short-term risk, whilst chronic

threat involves a much longer-term risk. Several models have been proposed as extension

of the Ydenberg and Dill (1986) model, predicting the response of prey based on current

state (threat-sensitivity; Helfman 1989) or recent accrued fitness (asset protection; Clark

1994).

Threat-sensitivity and the response to acute predation threats

Since predation risk is variable (Sih et al. 2000), prey individuals should be able to

display behavioural responses that reflect the magnitude of the risk detected, exhibiting

low intensity anti-predator response to low levels of risk and proportionally more intense

responses as perceived risk levels increase (Helfman, 1989). Such a trade-off is referred to

as the threat sensitive predator avoidance hypothesis (Helfman, 1989). Threat sensitive

responses are immediate responses based on perceived predation threat. Recent work

demonstrates that the relative concentration of chemical alarm cue detected is a reliable

indicator of the level of predation threat, since the concentration of alarm cue is directly

related to the proximity of a predation threat (Dupuch et al., 2004; Brown et al., 2006a).

Work done by Brown et al. (2006a) suggests that the threat-sensitive anti-predator trade-

off is dynamic with prey continually adjusting their behavioural response according to

immediate, intermediate or long-term patterns of predation risk. By adjusting the form and

intensity of anti-predator response based on the level of perceived predation threat, prey

individuals can balance predator avoidance with other activities and hence optimize their trade-off (Helfman & Winkleman, 1997). Furthermore, prey displaying threat sensitive responses should be at a selective advantage since they can still obtain fitness benefits while avoiding predation (Helfman, 1989). Threat-sensitive responses have been demonstrated over a wide range of taxa, including terrestrial invertebrates (Persons & Rypstra, 2001), aquatic invertebrates (Rochette et al., 1997), amphibians (Rohr & Madison, 2001), reptiles (Amo et al., 2004), birds (Lima 1992a, 1992b), mammals (Swaisgood et al., 1999; Wirsing et al., 2007) and fishes (Winkelman, 1996; Chivers et al., 2001b; Brown et al., 2006b).

The threat sensitive response pattern may vary from 'graded or pure threat-sensitive' to 'non-graded or hypersensitive' (Helfman & Winkleman, 1997; Brown et al., 2006b). The shift in the threat sensitive response pattern is altered by the relative benefits of predator avoidance versus those associated with other fitness-related activities (Helfman & Winkleman, 1997; Brown et al., 2006b). A graded or pure threat sensitive response is where prey demonstrates anti-predator behaviour that is proportional to the level of perceived predation risk (Ferrari et al., 2005). On the other hand, a non-graded or hypersensitive response is where prey respond at maximum or near maximum when a threat is detected above a certain concentration of chemical alarm cue known as the minimal behavioural response threshold (Marcus & Brown, 2003; Brown et al., 2006b).

Implicit to all models of predator avoidance is the assumption that prey can reliably assess local predation threats (Brown, 2003) which allows them to optimize their trade-offs between predator avoidance and other fitness related benefits (Brown & Chivers, 2005). Within aquatic ecosystems, prey use public information provided by chemical cues which

are an honest and reliable indicator of local predation threat and allow prey to discern information, such as the specific type and size of predators and the types of prey in the predator's diet (Kats & Dill, 1998; Mirza & Chivers, 2003). Chemosensory cues are used by a wide variety of taxonomically diverse freshwater and marine species and have been extensively studied (Brown, 2003; Wisenden & Chivers, 2006; Ferrari et al., 2010). One well studied form of chemosensory cue available to aquatic prey is damage-released chemical alarm cues, which are chemicals produced/and or stored in the epidermis and released into the water column following a predation attempt, in which a prey individual is injured or captured (Chivers & Smith, 1998). Detection of these cues by nearby conspecifics and some heterospecifics has been shown to elicit anti-predator responses such as freezing, dashing, area avoidance, hiding, increased shoaling and reduced foraging and mating (Chivers & Smith, 1998; Brown, 2003).

Asset Protection and the response to longer-term predation threats

Clark (1994) proposed the Asset Protection Model based on the trade-offs that prey must make in order to avoid predation and maintain or increase their reproductive fitness. The asset protection principle states that reproductive asset is valuable and should be protected, such that the larger the current reproductive asset, the more important it is to protect it. Conversely, the smaller the asset, the greater risk that can be taken to increase the asset. This model, unlike the threat sensitivity model takes into consideration past history such as foraging and reproductive status of prey. Thus, the degree of accrued assets should affect the associated costs and benefits and ultimately influence prey's anti-predator response. However, both models predict prey's anti-predator response and the trade-offs

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made between the benefits associated with predator avoidance and other fitness related activities.

According to Clark (1994), the optimal decision of prey individuals should not be solely based on current or immediate response but on both its internal and external states, which in turn may affect the trade-off between the costs and benefits. Several studies have demonstrated that prey with greater accumulated assets respond differently to predators (accumulated assets of an organism include reproductive value, body size, fat reserve accumulated by an organism over a period of time). For example, larger juvenile Coho salmon *(Oncorhynchus kisutch)* accept less predation risk when compared to smaller sized Coho salmons (Reinhardt & Healey, 1999). This acceptance of less predation risk by larger salmon may be due to the protection of their larger reproductive value, body size and fat reserve since the greater the asset, the more important it is to protect it (Clark, 1994). Furthermore, striped parrotfishes (*Scarus iseri*) increase their flight initiation distance (FID), (the distance between the prey and a potential predator at which the prey starts to flee) with body size, which correlates to their reproductive value (Gotanda et al., 2009). This increased FID with increasing body size is attributed to the lower risk-taking associated with the higher reproductive value of larger individuals.

The Trinidadian guppy system

To date, the question of how accrued assets shape the threat-sensitive response patterns of prey remain poorly understood. The goal of this study is to test the potential effects of accrued reproductive assets (Asset Protection) on the threat sensitive behavioural decisions of prey originating from areas of low vs. high predation threats. Many studies

have looked at several factors that shape the threat sensitive response pattern in prey fishes and the trade-off between the benefits associated with foraging and predator avoidance. This study is unique in that by using gravid and non-gravid guppies from low and high predation risk areas, it will allow us to investigate and compare the effects of reproductive assets on threat sensitive response pattern.

The Trinidadian guppy is the ideal species to demonstrate the Asset Protection model and its influence on threat sensitive behaviour due to its abundance in a variety of freshwater habitats in Trinidad's northern mountain range, (Magurran, 2005). Guppies are faced with a variety of predators (Botham et al., 2008) and are sensitive to immediate predation risk; they typically respond to predatory fish by increasing their shoaling, inspecting the predator, reducing their activity and moving to the surface water (Magurran, 2005). The Trinidadian guppy possesses a chemical alarm signal system and uses damage-released chemical alarm cues to assess local predation threats (Brown & Godin, 1999). Furthermore, guppies moderate their anti-predator response and demonstrate threat sensitive behaviour (Brown et al., 2009b). Their behavioural responses to different levels of con-specific alarm cues have been extensively studied (Nordell, 1998; Brown & Godin, 1999; Brown et al., 2009b, 2010).

Guppies are ovoviviparous; the young develop in eggs, nourished by the yolk and are retained by female until hatching time with reproduction continuing throughout the year. Female guppies are more wary of predators; their overall anti-predator responses are on average four times higher than in males (Magurran & Seghers, 1994) and are likely to provide a better estimate of predation risk.

In the Trinidadian guppy, the level of predation risk (acute vs. chronic) plays a major role in shaping its overall anti-predator behaviour. Previous reports (Botham et al., 2008; Brown et al., 2009b) have demonstrated that the levels of predation threat guppies experience shape their threat-sensitive behavioural trade-offs. Guppies exposed to frequent predation (chronic) exhibited a graded threat-sensitive response with anti-predator behaviour proportional to the concentration of alarm cue. Conversely, guppies with infrequent (acute) exposure to predation threat exhibited a non-graded threat-sensitive response pattern. This response pattern is expected because long-term predation pressure shapes threat sensitive response pattern in the Trinidad guppy (Brown et al., 2009b).

Predictions

Many studies have focused on the response of prey to immediate predation threat, but little work has been done on the influence of reproductive asset. To date, it remains unknown if reproductive asset influences the threat sensitive response patterns in Trinidadian guppies. In this study, I exposed gravid vs. non-gravid wild-caught Trinidadian guppies from high vs. low predation sites (ambient risk) to varying concentrations of conspecific alarm cues (acute risk) to assess potential interactions between the predictions of the Asset Protection Model and the Threat-sensitive Predator Avoidance hypothesis.

If the level of accumulated reproductive assets is the only factor governing threat sensitive response behaviour, then I predict that gravid guppies should demonstrate a stronger overall response intensity compared to non-gravid guppies. This prediction is based on the reasoning that according to the asset protection model, the greater the accumulated reproductive asset (gravid), the more important it is to protect it. Gravid guppies should devote more attention to anti-predator response than non-gravid guppies due to higher accumulated assets or 'fitness value' (Clark, 1994).

Conversely, if immediate predation threat is the only factor influencing anti-predator response in the Trinidadian guppies, then I predict that both gravid and non-gravid guppies should demonstrate similar anti-predator response patterns when exposed to the same concentration of alarm cues. This prediction is based on the reasoning that guppies are sensitive to immediate predation risk (Botham, 2008) and threat sensitive behaviour to conspecific chemical alarm cues has already been established in guppies (Brown et al., 2009b).

In addition, I predict that chronic predation risk will influence anti-predator response intensity in both river populations. Lower Aripo guppies would demonstrate a higher response intensity than Upper Aripo guppies because of the increased cost associated with frequent and unpredictable predation threats. This prediction is based on the reasoning that chronic predation risk influences the cost-benefit trade-offs between predator avoidance and fitness related activities and has been established in the Trinidadian guppies (Brown et al., 2009b).

Materials and Method

Test Site

All experimental fish were collected from the Upper and Lower Aripo Rivers, located in the Northern Range Mountains, Trinidad during the period January and May 2012. The rivers differ in the number and variety of fish predators present. The Upper Aripo River is characterized as a low predation site (Magurran, 2005) as it is located above a barrier waterfall, which prevents the upstream migration of predators (Botham et al., 2008). The only known guppy predators in the Upper Aripo River are the Hart's rivulus *(Rivulus hartii)* and a predatory freshwater prawn (*Macrobrachium crenulatum*), both of which prey only on small, juvenile guppies (Brown et al., 2010). The Lower Aripo river is considered a relatively high predation site (Botham et al., 2008; Brown et al., 2010) and contains several predators that prey on guppies including the pike cichlid (*Crenichichla alta*), the blue acara cichlid *(Aequidens pulcher)*, the black acara cichlid (*Cichlasoma bimaculatum*), the brown coscorub (*Cichlasoma taenia*), Hart's rivulus and a predatory characin *(Astyanax bimaculatus)* (Godin, 1995).

Test Fish Population

In total, 160 gravid and 160 non-gravid female guppies (standard length [Ls] ranging from 1.2 cm to 3.0 cm) were collected from each river using a hand seine. Gravid guppies were differentiated from non-gravid guppies by their larger size and location of their gravid spot on the abdomen. The fish were transported in separate containers containing their respective stream water. Upon arrival at the laboratory, all guppies caught were housed in 60-L glass aquaria tanks at approximately 27°C. Gravid and non-gravid guppies from each stream were placed in separate tanks and all fish were fed dry

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commercial flaked food (Tetramin) twice daily. Substrates, consisting of small (1-2 cm)

and medium (2-4 cm) sized rocks were added to each holding tank to imitate natural

stream conditions. The water in the tanks was continuously filtered and aerated with small

corner filters. Each holding tank was secured with a lid made of wire mesh in order to

prevent escape of fish. Aquaria were exposed to a 12 h light (L): 12 h dark (D) cycle for a

minimum of 3 days prior to testing as in Brown & Godin, (1999).

Stimulus preparation

Wild caught non-gravid female guppies from both Upper and Lower Aripo River

were collected and used for the preparation of alarm cue stimulus. In total, 91 Lower Aripo

female (L_s 1.85 cm) and 73 Upper Aripo female (L_s 2.04 cm) were used. Immediately

following their removal from holding tanks, all fish were euthanized humanely with a

single blow to the head (in accordance with Concordia Animal Care Committee Protocol

AC-2011-BROW). The head, tail and all internal visceral tissues were removed and the

remaining tissues (skin and underlying skeletal muscle) were placed in 100 mL of distilled

water and homogenized. Previous studies have shown that skin and skeletal muscles are a

reliable indicator of predation risk and can elicit an anti-predator response, hence function

as an alarm cue (Brown & Godin, 1999; Brown et al., 2009b). The homogenate was

filtered through polyester filter floss and brought to a final concentration of $0.1cm^2$ of skin

per mL. The stock solution was then diluted to three concentrations; 25%, 50% and 100%

with the addition of distilled water. Distilled water was used as a control (0%). The final

concentrations were similar to those used by Brown & Godin (1999) and Brown et al.

(2010). All alarm cue solutions were frozen in 10 mL aliquots at -20°C until needed. For

this study, a total of 210.40 cm^2 of tissue was collected from both the Lower and Upper Aripo donors respectively. A blend of Upper and Lower Aripo guppies were used for alarm cues to control for any population-specific response (Brown et al., 2010).

Experimental Protocol

Laboratory experiments were conducted between Jan 10th-24th and May 3rd - 18th 2012. All fish were allowed to acclimate in holding tanks for 24 hours before being placed in test tanks for observation. Observations were carried out in a series of glass test tanks with the dimensions: 35 cm (l) x 22 cm (w) x 23 cm (h) containing water and a gravel substrate. Each tank was lined with white plastic on three sides for visual isolation between tanks. Each tank contained a single air stone and a 1.5 m length of airline tubing. The airline tubing allowed for injection of the chemical stimuli and distilled water into the experimental tanks, which allowed the rapid diffusion of the stimulus and minimized any mechanical disturbance of the focal fish associated with the stimulus injection. I divided the test tanks into three equal horizontal sections by drawing lines at 5-cm intervals, along the front and back walls, in order to quantify area use. Shoals of three guppies were randomly removed from the holding tank using a hand net and placed into a test tank to acclimate for at least 2 hours prior to testing. Focal study population consisted of shoals of three gravid and three non-gravid guppies from both the Lower and Upper Aripo Rivers separately exposed to different concentration of alarm cue. Shoals of three guppies were used since this falls into its shoal size in nature (Magurran & Seghers, 1990a).

A single observer (Jemma Andersen) stood at an angle in front of the test tanks to avoid creating an overhead shadow and disturbance during trials. Testing consisted of 10-

minute trials divided into a 5-min pre-stimulus (control) and a 5-min post-stimulus (experimental) injection observation period. During the 5-min pre-stimulus period, 60 mL of tank water was withdrawn through the stimulus injection tube and discarded. An additional 60 mL of tank water was removed and retained and anti-predator behaviours observed. Following the pre-stimulus observation, 10 mL of stimulus alarm cue was injected at one of three concentrations (25%, 50% or100%) or 10 mL of distilled water (0%) used as a control. The order of treatments was randomised. The retained 60 mL of tank water was used to slowly flush the stimulus into the test tank. Once the stimulus was injected into the stimulus injection tube, a 5-min post-stimulus injection observation period started.

Behavioural Measures

To assess and compare the intensity of anti-predator response in gravid and non-gravid guppies from the Lower and Upper Aripo river population, three behavioural measures were recorded during each trial. I recorded area use, shoaling index and foraging attempts during both the pre- and post-stimulus observation periods. Area use, shoaling index and foraging attempts were recorded every 15s for 5 minutes. Area use was measured as the position of each guppy within the tank, whose scores range from 1 (bottom third of the tank) to 3 (top third of the tank). Thus, area use scores ranged from 3 (all fish near the substrate) to 9 (all fish near the surface). Shoaling index score ranged from 1to 3 (1= no guppy within one body length of each other, 3 = all guppies within one body length of each other, (Brown & Godin 1999; Brown et al., 2009b). A reduction in area use and an increase in shoaling index are typical anti-predator responses towards

conspecific alarm cues in Trinidadian guppies (Brown & Godin 1999; Brown et al., 2009b). Foraging attempts were measured as a directed lunge towards an object in the water column, at the surface or on the bottom, involving opercula expansion and opening of the mouth. Guppies typically reduce their foraging attempts in the presence of a predator (Fraser et al., 2004). A total of 15-18 replicates were conducted per stimulus concentration for gravid and non-gravid guppies from Lower and Upper Aripo populations. Experimental observations were made blind to the treatment and individual guppies were exposed to only one experimental treatment. At the end of each experimental trial, the subjects were removed from the test aquarium and its standard body length (Ls) and body mass were measured and recorded. The test subjects were removed and transferred to a separate holding tank to avoid being re-tested and subsequently released back to the site of collection.

Statistical Analysis

The change in area use, shoaling index and foraging attempts was calculated between pre- and post-stimulus observation periods (post-stimulus – pre-stimulus) separately and these difference scores used as dependent variables in all subsequent multivariate analyses. All dependent variables met the assumptions for parametric tests. Stream population (Upper vs. Lower Aripo), reproductive status (gravid vs. non-gravid) and stimulus concentration (0% (DW), 25%, 50% or 100%) were used as independent variables. Multivariate GLM were used for all analyses as all three behavioural measures are likely highly correlated. Initially, a MANCOVA (Table 1) was performed to determine the overall main effect and interaction of independent variables (population, reproductive

state and stimulus concentration) on multiple dependent variables with standard length used as covariate.

To further explore population differences, separate two-way MANOVAs (Table 2), for both Upper and Lower Aripo populations were subsequently conducted. Foraging attempts, area use and shoaling index were the dependent variables and concentration of alarm cues and reproductive status were the independent variables. Separate one-way MANOVAs was conducted for gravid and non-gravid guppies, to analyze the effect of stimulus concentration on the anti-predator response. The three behavioural measures were the dependent variables and gravid and non-gravid guppies of the Lower Aripo population were independent variables. All statistical analysis was performed using SPSS 18.0 statistical program with a 0.05 significant level.

Results

Qualitatively, differing effects were found for reproductive state and stimulus concentration on the anti-predator response patterns of female guppies. In particular, the effects of reproductive state and stimulus concentration differed based on ambient predation. Gravid guppies from both river populations demonstrated the strongest response when exposed to the same concentration of alarm cue. Furthermore, both gravid and non-gravid guppies from the Lower Aripo population demonstrated an overall stronger anti-predator response.

The overall Multivariate Analysis of Covariance (MANCOVA) demonstrated a significant effect of stream population, reproductive status and stimulus concentration on behavioural responses in the Trinidadian guppies (MANCOVA; $P < 0.05$; Table 1). A significant interaction of stream population and stimulus concentration was also observed ($P < 0.05$; Table 1). Under low predation risk conditions (Upper Aripo River), guppies exhibited non-graded responses of similar intensity regardless of reproductive state. However, I observed a very different response pattern under high predation risk conditions (Lower Aripo River); gravid females responded with a higher intensity than non-gravid regardless of the level of acute threat detected.

Stream specific comparisons

Lower Aripo

When testing the Lower Aripo guppies alone, I found a significant effect of both stimulus concentration (MANOVA; $F_{(3,119)} = 24.99$; $P < 0.001$) and reproductive status (MANOVA; $F_{(3,117)} = 5.07$; $P = 0.002$; Table 2) on anti-predator response. There was no significant interaction (MANOVA; $F_{(3,117)} = 1.88$; $P = 0.14$).

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Gravid guppies showed greater reduction in area use and number of foraging attempts and increased shoaling index when compared to non-gravid guppies (Figures 1-3). I further performed a one-way MANOVA and results revealed a significant multivariate effect for stimulus concentration on all three behavioral responses in both gravid (MANOVA; $F_{(3,58)} = 16.45$; $P < 0.001$) and non-gravid guppies (MANOVA; $F_{(3,61)} = 10.23$; $P < 0.001$).

Upper Aripo

Overall, results indicated a significant effect of concentration of alarm cue on anti-predator response (MANOVA; $F_{(3,117)} = 15.54$; $P < 0.001$; Table 2). No significant effect of reproductive status ($F_{(3,115)} = 0.68$; $P = 0.57$) nor an interaction ($F_{(3,117)} = 0.31$; $P = 0.82$) were found. The response patterns of gravid and non-gravid guppies under low ambient predation threats were similar.

Table 1. Results of the overall Multivariate Analysis of Covariance (MANCOVA) for the effect of independent variables (stream population, reproductive status and conc. of alarm cues) and their interaction on dependent variables (foraging attempts, area use and shoaling index). N=18.

Factor	F	d.f.	P
Population	3.69	3, 233	0.013
Reproductive status	4.39	3, 233	0.005
Stimulus concentration	34.92	3, 233	< 0.001
Population x RS	1.36	3, 233	0.26
Population x Stimulus	2.85	3, 235	0.038
RS x Stimulus	1.20	3, 235	0.31
Three-way interaction	1.14	3, 235	0.34

Table 2.   Results of MANOVAs for Upper Aripo (low predation) and Lower Aripo (high predation) populations. Significant effects when P< 0.05.

Factor	F	d.f.	P
Lower Aripo River			
Reproductive Status	5.07	3,117	0.002
Stimulus concentration	24.99	3,119	< 0.001
RS x Stimulus	1.88	3,119	0.14
Upper Aripo River			
Reproductive Status	0.68	3,115	0.57
Stimulus concentration	15.54	3,117	< 0.001
RS x Stimulus	**0.31**	**3, 117**	**0.82**

Discussion

Overall my results suggest that reproductive status has a significant effect on anti-predator response in the Lower Aripo population but not in the Upper Aripo population. While both gravid and non-gravid guppies exhibited anti-predator response to conspecific alarm cues, my findings indicate significant differences between gravid and non-gravid guppies in the Lower Aripo (high predation risk) population. Gravid guppies from the Lower Aripo population produced a stronger response in terms of area use, shoaling index and number of foraging attempts than did non-gravid guppies.

In this study accrued reproductive asset (Clark, 1994) may have been operative in affecting prey's anti-predator response in the Lower Aripo population, since gravid guppies displayed greater anti-predator response intensity than non-gravid guppies. The stronger response observed for gravid guppies in the Lower Aripo population may be due to the greater allocation of energy towards protecting and increasing its accumulated reproductive asset. These results are also in accordance to several dynamic models proposed (McNamara & Houston 1986; Brown 1988; Clark, 1994), all of which suggest that larger animals should be less willing to risk predation compared to smaller animals. In fishes, body size influences their vulnerability to predation, larger fish on average showed greater predation risk avoidance after a predation threat than smaller fish (Sogard, 1997). Moreover, extensive research by Reinhardt & Healey (1999) suggests that Coho salmon use their own body size as cues for long-term, state-dependent adjustments of feeding behaviours. The lower risk taking is probably an example of asset protection, whereby larger animals accept less predation risk to protect their greater accumulated fitness value and are similar to the responses found here. In this study, the greater response

demonstrated by gravid guppies may also be attributed to its larger size, which makes it a more profitable item by predators (Pocklington & Dill, 1995).

Furthermore, reproductive status has shown to have a greater effect on the Lower Aripo guppy population than the Upper Aripo population. Results from this study suggest an overall greater response by gravid guppies in the Lower Aripo population when exposed to the same level of predation risk than the Upper Aripo guppies. The greater response by Lower Aripo gravid guppy population can also be attributed to the higher frequency of predation threat faced naturally in the wild. It has been shown that the overall intensity of antipredator response is influenced by current conditions, including variability in predation risk over a period of days to weeks (Brown et al., 2009b). Collectively, these findings support our theory that long-term predation pressure together with accumulation of reproductive asset influences behavioural trade-offs in the Lower Aripo guppy population.

Another intriguing finding from this study is the difference in response intensity for both river guppy populations. Overall, Lower Aripo guppy population showed a greater anti-predator response to the same level of predation threat than the Upper Aripo guppy population. A proposed mechanism commonly citied in current literature to account for these behavioural differences is the role of long-term predation pressure in shaping anti-predator responses to predation risk. Literature suggests that provenance and long-term ambient predation pressure can determine the overall intensity and threat sensitive response pattern in the Trinidadian guppies (Magurran, 2005; Brown et al., 2009b). For example, Brown et al. (2009b) demonstrated that guppies from high predation sites displayed higher response intensity and a graded response pattern, whilst guppies from low predation sites displayed a lower response intensity and a non-graded response pattern to

the same range of alarm cue concentrations. The difference in response intensity and threat-sensitive pattern in Upper and Lower Aripo populations may be due to the cost-benefit trade-offs between successful predator avoidance versus associated fitness value. Any immediate or short-term factor that increases fitness value relative to predator avoidance benefits should favor a graded response pattern, whilst factors that decrease fitness value relative to predator avoidance benefits should favor a hypersensitive response pattern (Brown et al., 2006a).

The moderation of anti-predator response by prey organisms is important since successful predator avoidance is costly in terms of time and energy available for other fitness-related activities (Lima & Dill, 1990). An anti-predator response intensity that is proportional to the level of perceived threat will prevent the expenditure of time and energy on anti-predator behaviour and allow prey to continue to forage. Doing so would presumably allow prey to optimize the benefits associated with predator avoidance and fitness-related activities (Lima & Dill, 1990). Conversely, by exhibiting anti-predator response intensity that is not proportional to the level of perceived threat would result in reduction and loss of fitness activities such as foraging and mating. More importantly, these results suggest that guppies display threat sensitive behaviour, which are consistent with Helfman's (1989) Threat Sensitivity Model and confirm work previously done by (Brown & Godin, 1999).

Literature suggests that the threat sensitive response pattern is altered by the relative benefits of predator avoidance versus those associated with other fitness-related activities (Helfman & Winkleman, 1997; Brown et al., 2006b). According to the threat sensitivity model, under conditions of low perceived risk, a prey is expected to exhibit a

less intense anti-predator response, since the benefits of fitness related activities outweigh those associated with predator avoidance. However, under conditions of higher perceived predation threats, prey should therefore exhibit a greater anti- predator response because of larger predator avoidance benefits. These results confirm that the threat sensitive anti-predator response pattern is determined by the trade-off between the cost and benefits of predator avoidance and other fitness related activities.

Brown et al., (2006b) demonstrated that group size influences the threat sensitive response pattern in convict cichlids by affecting the trade-off between predator avoidance and other fitness related activities. Singletons or those in small shoals trade-off foraging because of the greater benefit of increased predator avoidance and survival and demonstrated a non-graded anti-predator response. However, at the same level of predation threat, cichlids in larger shoals exhibited a strong-graded threat-sensitive response because of the reduced predator avoidance benefits associated with group membership (Hoare et al., 2004). Several other factors have been shown to be associated with a shift in the threat sensitive response pattern and include: hunger level (Brown & Smith, 1996), familiarity among shoal members (Chivers et al., 2007), group size (Brown et al., 2006b), parasite (Seppälä et al., 2008) and social grouping (Brown et al., 2009a).

In the Trinidadian guppies, predation risk has shown to be a strong selective force in the evolution of reproductive adaptations (Maghagen, 1991). Female guppies in high-predation sites mature earlier at a smaller size, have higher fecundity, shorter interbrood intervals and higher reproductive allotment compared to those from low predation sites (Reznick & Endler, 1982). Furthermore, Magurran & Seghers, (1990b) found that under elevated predation risk, males increase coercive mating in order to exploit females that are

preoccupied with observing and evading predators. Predation risk has also been shown to influence the colour in male guppies; males from high predation sites are less brightly colored and perform fewer displays than males from low predation sites (Magurran & Seghers, 1990a). Guppies become brighter, larger, school less and react at a shorter distance to predators with decreasing predation (Endler, 1980). My data add to this, showing that reproduction also shape how prey respond to acute threats, at least under high-risk conditions.

It remains unclear as to what factors determine the intensity of anti-predator response in the Trinidadian guppy. Whether it is genetic or experience or both. Much of the literature suggests that predation experience may mediate anti-predator response in guppies from high-risk areas by creating phenotypic selection in the escape ability of guppies (O'Steen et al., 2002; Kelley & Magurran, 2003). Studies have shown that wild caught guppies from high-risk areas exhibited a higher response than those reared in the laboratory, due to prior experience. However, guppies from low predation sites with no prior experience (except with *Rivulus hartii*) were able to differentiate between predator models and suggest that guppies have an innate ability to respond to particular predator. Hence the mediation of anti-predator response in the Trinidadian guppy may have both an experience and genetic component.

In addition I observed that both gravid and non-gravid guppies were responsive to alarm cue and provide additional evidence that guppies are sensitive to immediate predation threat and are capable of making threat sensitive decisions. Prey capable of making such decision should be at a selective advantage since they can still obtain fitness benefits while reducing their risk of predation (Helfman, 1989). It is also crucial to

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emphasize that accumulated reproductive asset may have an additive effect by influencing the response intensity in the Trinidadian guppies. The observed differences in anti-predator behaviour in both stream populations may suggest that prior experience and long-term predation pressure may play a role in shaping anti-predator behaviour.

This study has demonstrated a difference in the anti-predator response intensity between gravid and non-gravid Lower Aripo Trinidadian guppies, indicating that the level of accumulated reproductive asset may play a significant role in shaping anti-predator response intensity. However, this present study was carried out under laboratory conditions, hence a more in-depth analysis of anti-predator response between gravid and non-gravid guppies over an extended period of time under more natural conditions would be needed to draw any definite conclusions.

Conclusion

Due to accumulated reproductive asset, gravid guppies from the Lower Aripo river population showed heightened anti-predator responses, which suggest protection of current reproductive asset and ultimately their survival. Based on Clark's (1994) reproductive asset protection principle, an organism optimal behaviour is dependent on its reproductive value, which is dependent on age, physiological state, and current environmental conditions. Such factors may likely affect the trade-off between risk and benefit (Clark, 1994). However, in the Upper Aripo population reproductive asset was shown to have no influence in the threat sensitive response intensity.

It is important to note that in this experiment, Lower Aripo gravid guppies integrate accumulated reproductive asset, immediate predation risk and long-term predation pressure to make decisions regarding their anti-predator responses. However, we need a better understanding of how accumulated reproductive asset affect the behaviour. More studies are needed, ideally under natural conditions, directly comparing wild gravid and non-gravid guppies within and between populations to help disentangle the effects of reproductive asset on behaviour.
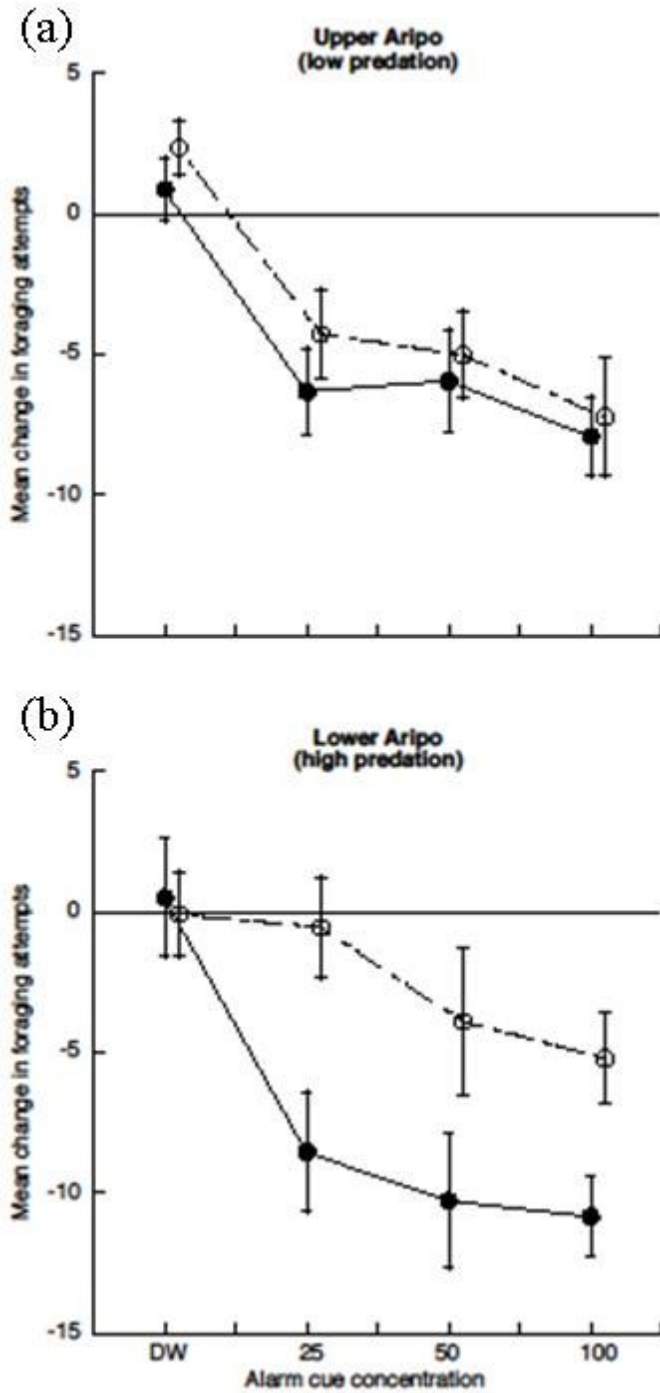
Figure 1.   Mean (±SE) change in foraging attempts for gravid (closed dots) and non-gravid (open dots) female Trinidadian guppies (*Poecilia reticulata*) from the (a) Upper and (b) Lower Aripo River exposed to conspecific chemical alarm cues at 25%, 50% and 100% concentration or a distilled water control.
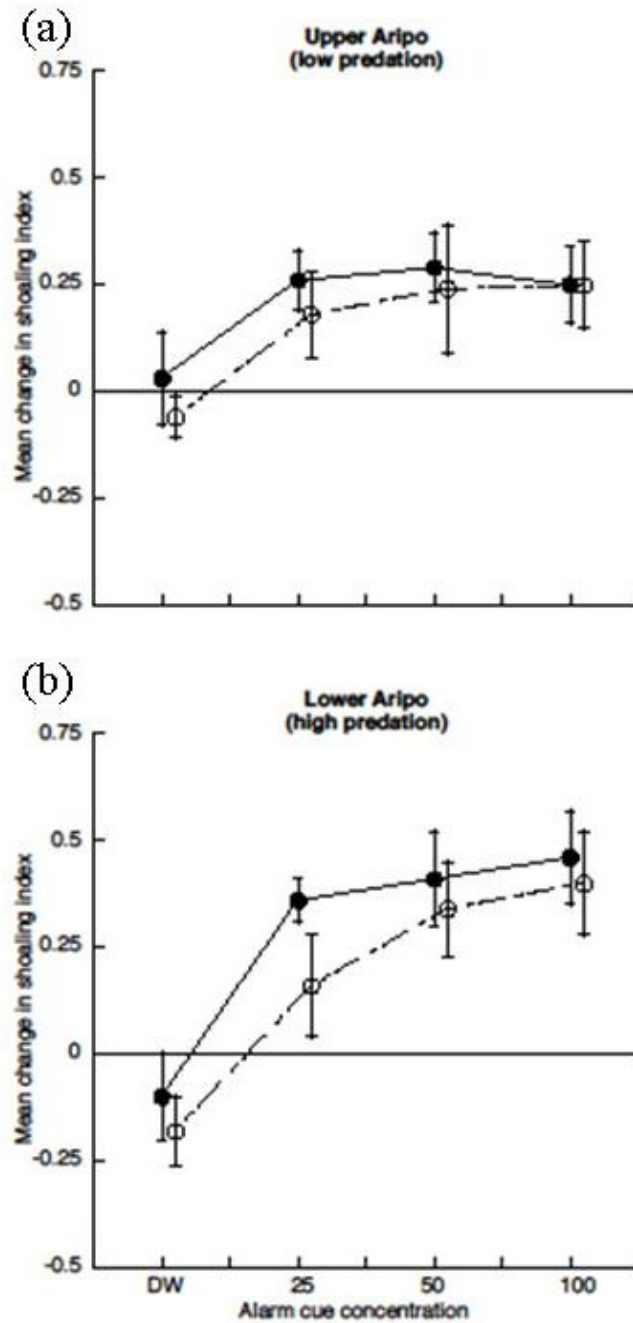
Figure 2.   Mean (±SE) change in shoaling index for gravid (closed dots) and non-gravid (open dots) female Trinidadian guppies (*Poecilia reticulata*) from the (a) Upper and (b) Lower Aripo River exposed to conspecific chemical alarm cues at 25%, 50% and 100% concentration or a distilled water control.
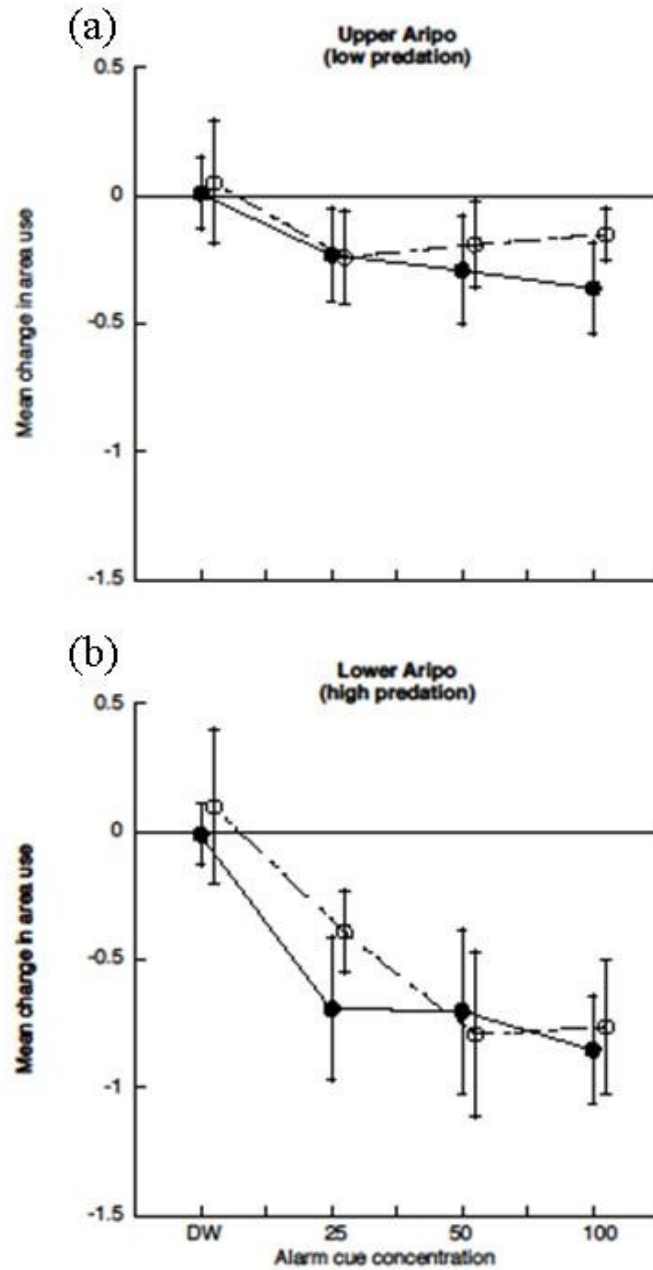
Figure 3.   Mean (±SE) change in area use for gravid (closed dots) and non-gravid (open dots) female Trinidadian guppies (*Poecilia reticulata*) from the (a) Upper and (b) Lower Aripo River exposed to conspecific chemical alarm cues at 25%, 50% and 100% concentration or a distilled water control.

References

Amo, L., Lopez, P., & Martin, J. 2004. Wall lizards combine chemical and visual cues of ambush snake predators to avoid overestimating risk inside refuges. Animal Behaviour **67**: 647-653.

Botham, M.S., Hayward, R.K., Morrell, L.J., Croft, D.P., Ward, J.R., Ramnarine, I., & Krause, J. 2008. Risk-sensitive anti-predator behaviour in the Trinidadian guppy, *Poecilia reticulata*. Ecology **89**: 3174-3185.

Brown, G.E., Elvidge, C.K., Macnaughton, C.J., Ramnarine, I., & Godin, J.G.J. 2010. Cross-population responses to conspecific chemical alarm cues in wild Trinidadian guppies, *Poecilia reticulata*: evidence for local conservation of cue production. Canadian Journal of Zoology **88**: 138-146.

Brown, G.E., Harvey, M.C., Leduc A.O.H.C., Ferrari M.C.O., & Chivers D.P. 2009a. Social context, competitive interactions and the dynamic nature of anti-predator responses of juvenile rainbow trout *Oncorhynchus mykiss*. Journal of Fish Biology **75**: 552-562.

Brown, G.E., Macnaughton, C.J., Elvidge, C.K., Ramnarine, I., & Godin, J.G.J. 2009b. Provenance and threat-sensitive predator avoidance patterns in wild-caught Trinidadian guppies. Behavioral Ecology and Sociobiology **63**: 699-706.

Brown, G.E., Rive, A.C., Ferrari, M.C.O., & Chivers, D.P. 2006a. The dynamic nature of anti-predator behaviour: prey fish integrate threat-sensitive anti-predator responses within background levels of predation risk. Behavioral Ecology and Sociobiology **61**: 9-16.

Brown, G.E., Bongiorno, T., DiCapua, D.M., Ivan, L.I., & Roh, E. 2006b. Effects of group size on the threat-sensitive response to varying concentrations of chemical alarm cues by juvenile convict cichlids. Canadian Journal of Zoology **84**: 1-8.

Brown, G.E., & Chivers, D.P. 2005. Learning as an adaptive response to predation. *In*: Ecology of Predator–Prey Interactions. *Edited by* P. Barbosa & I. Castellanos. Oxford University Press, Oxford, pp. 34-54.

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., & Godin, J.G.J. 1999. Chemical alarm signals in Trinidadian guppies: laboratory and field evidence. Canadian Journal of Zoology **77**: 562-570.

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

Brown, J.S. 1988. Patch use as an indicator of habitat preference, predation risk, and competition. Behavioral Ecology and Sociobiology **22**: 37-47.

Chivers, D.P., Zhao, X., & Ferrari, M.C.O. 2007. Linking morphological and behavioural defenses: prey fish detect the morphology of conspecifics in the odour signature of their predators. Ethology **113:** 733-739.

Chivers, D.P., Mirza, R.S., Bryer, P.J., & Kiesecker, J.M. 2001b. 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 signalling in aquatic predator-prey systems: a review and prospectus. Ecoscience **5**: 338-352.

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

Dupuch, A., Magnan, P., & Dill, L.M. 2004. Sensitivity of northern red belly dace, *Phoxinus eos*, to chemical alarm cues. Canadian Journal of Zoology **82**: 407-415.

Endler, J.A. 1980. Natural selection on color patterns in *Poecilia reticulata*. Evolution **34**: 76-91.

Ferrari, M.C.O., Elvidge, C.K., Jackson, C.D., Chivers, D.P., & Brown, G.E. 2010. The responses of prey fish to temporal variation is risk: sensory habituation or risk assessment? Behavioral Ecology **21**: 532-536.

Ferrari, M.C.O., Trowell J.J., & Brown G.E. 2005. The role of learning in the development of threat-sensitive predator avoidance by fathead minnows. Animal Behaviour **70**: 777-784.

Fraser, D.F., Gilliam, J.F., Akkara, J.T., Albanese, B.W., & Snider, S.B. 2004. Night feeding by guppies under predator release: effects on growth and daytime courtship. Ecology **85**: 312-319.

Godin, J.G.J. 1995. Predation risk and alternative mating tactics in male Trinidadian guppies *(Poecilia reticulata)*. Oecologia **103**: 224-229.

Gotanda, K., Turgeon, K., & Kramer, D. 2009. Body size and reserve protection affect flight initiation distance in parrotfishes. Behavioural Ecology and Sociobiology **63**: 1563-1572.

Helfman, G.S., & Winkelman, D.L. 1997. Threat sensitivity in bicolor damselfish: effects of sociality and body size. Ethology **103**: 369-383.

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

Hoare, D.J., Couzin, I.D., Godin, J.G.J., & Krause, J. 2004. Context-dependent group size choice in fish. Animal Behaviour **67**: 155-164.

Kats, L.B., & Dill, L.M. 1998. The scent of death: Chemosensory assessment of predation risk by prey animals. Ecoscience **5**: 361-394.

Kelley, J.L., & Magurran, A.E. 2003. Learned predator recognition and anti-predator responses in fishes. Fish and Fisheries **4**: 216-226.

Lima, S.L., & Bednekoff, P.A. 1999. Temporal variation in danger drives anti-predator behaviour: the predator risk allocation hypothesis. American Naturalist **153**: 649-659.

Lima, S.L. 1992a. Strong preferences for apparently dangerous habitats: a consequence of differential escape from predators. Oikos **64**: 597-600.

Lima, S.L. 1992b. Vigilance and foraging substrate: anti-predator considerations in a nonstandard environment. Behavioral Ecology and Sociobiology **30**: 283-289.

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

Magnhagen, C. 1991. Predation as a cost of reproduction. Trends in Ecology & Evolution **6**: 183-186.

Magurran, A.E. 2005. Evolutionary Ecology: The Trinidadian Guppy. Oxford: Oxford University Press, pp. 95-99.

Magurran, A.E., & Seghers, B.H. 1994. Sexual conflict as a consequence of ecology: evidence from guppy, *Poecilia reticulata* populations in Trinidad. Proceedings of the Royal Society of London B **255**: 31-36.

Magurran, A.E., & Seghers B.H. 1990a. Population differences in the schooling behaviour of newborn guppies, *Poecilia reticulata*. Ethology **84**: 334-342.

Magurran, A.E., & Seghers, B.H. 1990b. Risk sensitive courtship in the guppy (*Poecilia reticulata*). Behavior **112**: 194-201.

Marcus, J., & Brown, G.E. 2003. Response of pumpkinseed sunfish to conspecific chemical alarm cues: an interaction between ontogeny and stimulus concentration. Canadian Journal of Zoology **81**: 1671-1677.

McNamara, J. M., & Houston, A.I. 1986. The common currency for behavioural decisions. American Naturalist **127**: 358-378.

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

Nordell, S.E. 1998. The response of female guppies, *Poecilia reticulata*, to chemical stimuli from injured conspecifics. Environmental Biology of Fishes **51**: 331-338.

O'Steen, S., Cullum, A.J., & Bennett, A.F. 2002. Rapid evolution of escape ability in Trinidadian guppies (*Poecilia reticulata*). Evolution **56**: 776-784.

Persons, M.H., & Rypstra, A.L. 2001. Wolf spiders show graded anti-predator behaviour in the presence of chemical cues from different sized predators. Journal of Chemical Ecology **27**: 2493-2504.

Pocklington, R., & Dill, L.M. 1995. Predation on females or males: who pays for bright male traits? Animal Behaviour **49**: 1122-1124.

Preisser, E.L., Bolnick, D.I., & Bernard, M.F. 2005. Scared to death? The effects of intimidation and consumption in predator-prey interactions. Ecology **86**: 501-509.

Reinhardt, U.G., & Healey, M.C. 1999. Size-dependent foraging behaviour and use of cover in juvenile Coho salmon under predation risk. Canadian Journal of Zoology **75**: 1642-1651.

Reznick, D., & J.A. Endler. 1982. The impact of predation on life history evolution in Trinidadian guppies (*Poecilia reticulata*). Evolution **36**: 160-177.

Rochette, R., Dill, L.M., & Himmelman, J.H. 1997. A field test of threat sensitivity in a marine gastropod. Animal Behaviour **54**: 1053-1062.

Rohr, J.R., & Madison, D.M. 2001. A chemically mediated tradeoff between predation risk and mate search in newts. Animal Behaviour **62**: 863-869.

Seppälä O., Valtonen E.T., & Benesh D.P. 2008. Host manipulation by parasites in the world of dead-end predators: adaptation to enhance transmission? Proceedings of the Royal Society B **275**: 1611-1615.

Sih, A., Ziemba R., & Harding, K. C. 2000. New insights on how temporal variation in predation risk shapes prey behaviour. Trends in Ecology and Evolution **15**: 3-4.

Sogard, S.M. 1997. Size-selective mortality in the juvenile stage of teleost fishes: a review. Bulletin of Marine Sciences **60**: 1129-1157.

Swaisgood, R.R., Owings, D.H., & Rowe, M.P. 1999. Conflict and assessment in a predator–prey system: Ground squirrels versus rattlesnakes. Animal Behaviour **57**: 1033-1044.

Winkelman, D.L. 1996. Reproduction under predatory threat: Trade-offs between nest guarding and predator avoidance in male dollar sunfish (*Lepomis marginatus*). Copeia **4**: 845-851.

Wirsing, A.J., Heithaus, M.R., & Dill, L.M. 2007. Living on the edge: dugongs prefer to forage in microhabitats that allow escape from rather than avoidance of predators. Animal Behaviour **74**: 93-101.

Wisenden, B.D., & Chivers, D.P. 2006. Fish Chemoreception. *In* The role of public chemical information in anti-predator behaviour. *Edited by* F. Ladich, S.P. Collins, P. Moller, & B.G. Kapoor. Science Publisher: Enfield, pp. 259-278.

Ydenberg, R. C., & Dill, L. M. 1986. The economics of fleeing from predators. Advances in the Study of Behaviour **16**: 229-249.