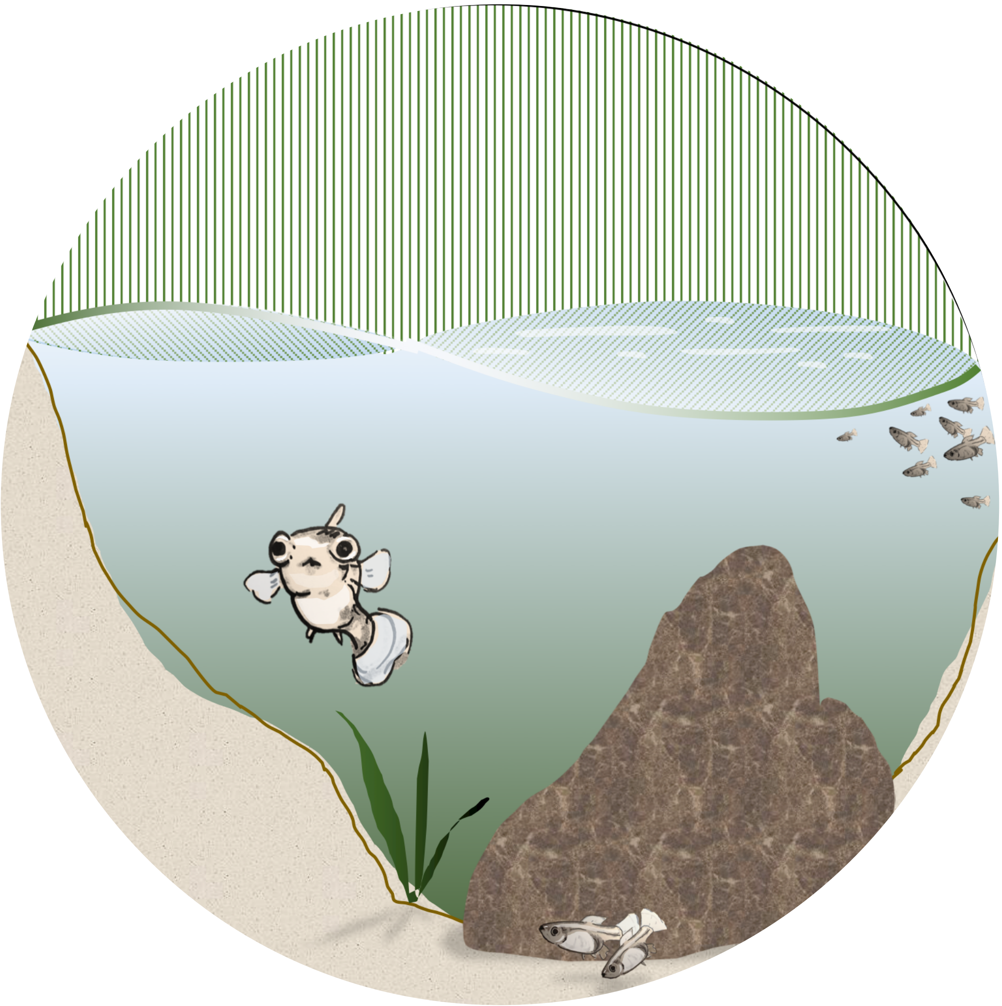
# Uncertainty of risk and information limitations shape neophobic antipredator responses in Trinidadian guppies

*Laurence Eliane An Feyten*



*A Thesis In the Department of Biology*
*Presented in Partial Fulfillment of the Requirement For the Degree of*
*Doctor of Philosophy (Biology)*

*Concordia University*
*Montreal, Quebec, Canada*
*March 2023*

## CONCORDIA UNIVERSITY
## SCHOOL OF GRADUATE STUDIES

This is to certify that the thesis prepared

By: Laurence Eliane An Feyten

Entitled: Uncertainty of risk and information limitations shape neophobic antipredator responses in Trinidadian guppies

And submitted in partial fulfillment of the requirements for the degree of

Doctor of Philosophy (Biology)

complies with the regulations of the University and meets the accepted standards with respect to originality and quality.

Signed by the final examining committee:

____________________ Chair
Dr.

____________________ External Examiner
Dr. Kiyoko Gotanda

____________________ External to Program
Dr. Carly Ziter

____________________ Examiner
Dr. Emma Despland

____________________ Examiner
Dr. James W. A. Grant

____________________ Thesis Supervisor
Dr. Grant E. Brown

Approved by

____________________
Dr. Grant E. Brown, Graduate Program Director

____________________
Dr. Pascale Sicotte, Dean of Faculty

# ABSTRACT

## Uncertainty of risk and information limitations shape neophobic antipredator responses in Trinidadian guppies

**Laurence Eliane An Feyten, Ph.D.**

**Concordia University, 2023**

The combined effects of changing climates, anthropogenic disturbances, and invasive species lead to short and long-term ecosystem changes. As a result, prey populations may be faced with increased uncertainty of risk (i.e., inability to predict predation events due to limitations on the quantity or quality of information). This uncertainty impacts decision-making and risk-assessment abilities among prey. Therefore, it is critical to understand the ecological factors driving uncertainty, and how prey deal with information limitations. Recently, phenotypically plastic neophobic predator avoidance (NPA, increased vigilance towards novel stimuli) has been suggested as a response of prey to uncertainty, without the costs associated with learning specific predator cues. Trinidadian guppies (*Poecilia reticulata*) from high-predation populations exhibit NPA, and NPA can be induced in predator-naïve populations after increasing mean predation risk. However, it remains unclear what specific factors drive uncertainty and the resulting NPA. Using Trinidadian guppies as a model system, I conduct a combination of laboratory and field experiments to identify potential drivers of uncertainty of risk within prey populations. I use an information ecology framework to argue that uncertainty of risk may arise from prey experience during risk information detection, from the risk information source (i.e., conspecifics and/or predators), and from the environment through which risk information must travel. I demonstrate that NPA increases when prey experience multiple novel cues, simultaneously or over time. After repeated encounters with a single novel cue, NPA is lost unless the cue is spatially unpredictable. Furthermore, NPA to a novel cue is mediated when paired with a social safety cue. I also assess predator guild composition and demonstrate that increased predator density (i.e., mean risk), and to a weaker extent predator diversity, increases NPA. Lastly, I show that NPA is greater in microhabitats with greater water velocity and habitat complexity, smaller dimensions, and decreased substrate heterogeneity and substrate diversity. Uncertainty of risk may have deleterious impacts on the distribution and abundance of predator and prey species. My thesis identifies factors contributing to uncertainty, enables prediction of prey responses to such conditions, and can contribute to conservation and management efforts of socio-economically important, endangered, or invasive species.

## *Acknowledgements*

This work was financially supported by Concordia University (Faculty of Arts and Science Graduate Fellowship, Stanley G. French Award, International Tuition Award of Excellence, Garnet Strong Scholarship, Graduate Student Conference & Exposition Award, Graduate Student Mobility Award, and teaching assistantship contracts), Fonds de Recherche du Québec- Nature et Technologies (Doctoral Scholarship), AOII Foundation (Laura Gilliam McDowell Scholarship), and the Natural Sciences and Engineering Research Council of Canada (Discovery Grant to G.E. Brown). All work reported herein was conducted in accordance with Concordia University Animal Research Ethics Protocol AREC-30000255.

I would like to thank my supervisor Dr. Grant E. Brown for his continual support and mentorship, under whose guidance I've greatly improved as a critical thinker, writer, and biologist. I'd also like to thank the members of my committee over the years, Dr. James W. A. Grant, Dr. Emma Despland, Dr. Dylan J. Fraser, Dr. Robert Weladji, and Dr. Pedro Peres-Neto for their advice and suggestions regarding my experimental methods and analyses. In this thesis, I worked with wild populations of Trinidadian guppies in both laboratory and field settings. This was made possible with the valuable assistance of Dr. Indar W. Ramnarine and Kharran Deonarinesingh from the University of West Indies, St. Augustine, Trinidad and Tobago. I also thank the Director of Fisheries in the Trinidadian Ministry of Agriculture, Land and Marine Resources for permission to study and collect individuals from rivers in the Northern Range mountains. For further support in the lab and field, and I would like to thank Alix Brusseau, Dr. Jean-Guy Godin, Dr. Heather Auld, Dr. Sophie Potter, and Isabelle Désormeaux. I would like to thank Dr. Adam L. Crane for his contribution to the experimental design, data collection, and editing for Chapter 3. I thank Dr. Eric Pederson and Gabriel Khattar for their suggestions regarding data analysis of Chapter 5. I'd also like to thank Ebony Demers for her generous contribution to my thesis data collection, for encouraging me to pursue a PhD, and for emotional support throughout the transition into my PhD. I also can't express enough thanks to Gabrielle Généreux, Katherine Hébert, Pedro Henrique Pereira Braga, and Dr. Heather Stewart for countless hours of emotional support, constructive feedback on my statistics and thesis, and for keeping me sane throughout my graduate school experience. Moreover, I'd like to acknowledge my colleagues and lab mates at Concordia throughout the years for fostering an environment that helped me grow as a person and scientist, and for offering a home away from home. Finally, I would also like to thank Céline, Mama, Papa, Kris, and Portia for supporting me unconditionally, always believing in me, and lending your perspectives. So long, and thanks for all the fish.

## *Contribution of Authors*

I contributed to the experimental design, data collection, data analysis, data interpretation, and writing of all chapters of the thesis. Dr. Grant Brown contributed to the experimental design, data collection, data analysis interpretation, and editing of all chapters in the thesis. Dr. Indar W. Ramnarine contributed to editing Chapters 1 to 5. Dr. Maude C.O. Ferrari and Dr. Douglas P. Chivers assisted in experimental conceptualization and editing Chapter 2. Dr. Adam L. Crane contributed to experimental design, data collection, data analysis interpretation, and editing of Chapter 3. Ebony Demers assisted with data collection of Chapters 1, 2, 4, and 5 and editing of Chapters 1, 2, and 4. Chapters 1 to 4 have been published in peer-reviewed journals (specified below), and Chapter 5 is under review for publication in a peer-reviewed ecology journal.

A shortened version of Chapter 1 was published as a letter to the editor:
Feyten, LEA, Demers, EEM, Ramnarine, IW, and Brown, GE (2018) Predation risk assessment based on uncertain information: interacting effects of known and unknown cues. Current Zoology 65(1): 75-76. DOI: 10.1093/cz/zoy083.

Chapter 2 has been published as:
Feyten, LEA, Demers, EEM, Ramnarine, IW, Chivers, DP, Ferrari, MCO, and Brown, GE (2019) Who's where? Ecological uncertainty shapes neophobic predator avoidance in Trinidadian guppies. Behavioral Ecology and Sociobiology 73 (5): 1-10. DOI: 10.1007/s00265-019-2687-7.

Chapter 3 has been published as:
Feyten, LEA, Crane, AL, Ramnarine, IW, and Brown, GE (2021) Predation risk shapes the use of conflicting personal risk and social safety information in guppies. Behavioral Ecology 32 (6): 1296-1305. DOI: 10.1093/beheco/arab096.

Chapter 4 has been published as:
Feyten, LEA, Demers, EEM, Ramnarine, IW, and Brown, GE (2022) Assessing effects of predator density and diversity on neophobia in Trinidadian guppies. Behavioural Processes 201: 104717. DOI: 10.1016/j.beproc.2022.104717.

Chapter 5 is in review as:
Feyten, LEA, Ramnarine, IW, and Brown, GE. Microhabitat conditions drive uncertainty of risk and shape neophobic responses in Trinidadian guppies, *Poecilia reticulata*.

***Table of Contents***

*List of Figures*

**Figure 2.3** Change in shoaling index (A) and change in area use (B) for shoals of guppies conditioned to lemon odour in a spatially predictable or unpredictable fashion (vs. an unexposed control) and tested for the response to lemon odour (shaded boxes) or a distilled water control (open boxes). $n = 10$ per treatment combination. Significant *post hoc* comparison-based independent sample $t$ tests are denoted as NS (non-significant; $p > 0.05$) or an asterisk ($p < 0.05$). An increase in shoaling index and a decrease in area use are indicative of a predator avoidance response. Box plots show median (line in box), 10th and 90th percentile (whiskers), and outliers (open circles).

**Figure 3.1** Experimental design (Experiment 1) for guppies in their natural populations with either high or low ambient predation risk. Trials consisted of the presence or absence of a shoal of 3 conspecifics combined with personal exposure to a chemical cue (alarm cue, novel cue, or a stream water control).

**Figure 3.2** Testing arena used for *in situ* observations of guppies in natural stream populations.

**Figure 3.3** Mean ± SE latencies to enter the foraging arena (a and b) and forage (c and d) for guppies in low-predation or high-predation populations, after the introduction of a personal cue (alarm cue, novel cue, or stream water), with the presence or absence of a social safety cue. Significant Tukey *post hoc* comparison effects denoted by horizontal bars (* = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$).

**Figure 3.4** Mean ± SE number of lines crossed (a) and proportion of line crosses that were calm (b) for experimental shoals from high-predation or low-predation populations. Significant effects of population are denoted by horizontal bars (** = $p < 0.01$, N.S. = $p > 0.05$).

**Figure 4.1** 3D printed predator models (14cm in length) used in this experiment, all of which serve as novel visual predator cues to low-predation guppies.

**Figure 4.2** Experimental flow chart demonstrating a round of conditioning and testing treatments. Conditioning treatments consisted of either high (HD) or low (LD) diversity predator model presentation, combined with either high (HR), intermediate (IR), or low (LR) mean risk treatments. Guppies from conditioned tanks were then split into shoals of 3 and tested for neophobia with either a novel odour or a water control. Each round resulted in a minimum of $n = 6$ for each conditioning and experimental treatment combination. This set up was repeated 3 times, using a total of 720 guppies, to get a minimum final $n = 18$.

**Figure 4.3** Mean ± SE of proportional change in area use across high (a) and low (b) predator diversity treatments, for each risk treatment (high, intermediate, or low), in response to each testing stimulus (water control indicated by blue triangles, novel cue indicated by yellow points). Values above the dashed line indicate an increase in area use after the stimulus introduction, below the dashed line indicate a decrease in area use, and values around the dashed line indicate no proportional change. The more negative the area use value, the stronger the antipredator response.

**Figure 4.4** Mean ± SE of transformed proportional change in shoaling index across high (a) and low (b) predator diversity treatments, for each risk treatment (high, intermediate, or low), in response to each testing stimulus (water control indicated by blue triangles, novel cue indicated by yellow points). Values above the dashed line indicate an increase in shoaling index after the stimulus introduction, below the dashed line indicate a decrease in shoaling index, and values around the dashed line indicate no proportional change. The greater the shoaling index value, the stronger the antipredator response.

**Figure 4.5** Stacked bar plot showing the proportion of increase, no change, or decrease in freezing (post – pre stimulus observation periods), across high (a) and low predator diversity (b) treatments, for each risk treatment (high, intermediate, or low), to each stimulus (water control and novel cue).

**Figure 5.1** Variables within each microhabitat which may affect the availability of information to prey and predictability of risk, including a) water velocity, b) habitat complexity (i.e., rugosity), c) substrate diversity and heterogeneity, and d) pool dimensions.

**Figure 5.2** Map showing the location of our study site on the Acono (triangle) and Lopinot (dot) rivers in the Northern Range, Republic of Trinidad and Tobago.

**Figure 5.3** Schematic diagram of the 3D printed novel heterospecific predator model, 14 cm in length, attached to a dowel and placed gently along the banks of each testing pool.

**Figure 5.4** Schematic of novel foraging arena used for measuring latencies to enter a novel foraging arena, showing the delineation determined as an entry into the arena (dashed line) and the novel food (fish flakes) inside.

**Figure 5.5** Relationship (± SE) between the BoxCox-transformed latency to inspect a novel predator model (s) and the Velocity-Complexity Component. The shape and colour of the data points indicate whether the observation came from the Lopinot (grey dot) or the Acono (black triangle) population.

**Figure 5.6** Relationship (± SE) between the latency to enter, when guppies entered, a novel foraging arena (s) and the Substrate-Diversity Component. The shape and colour of the data points indicate whether the observation came from the Lopinot (grey dot) or the Acono (black triangle) population.

**Figure S2.1** Experimental flow chart for Experiments 1A, 1B, and 2 demonstrating the total number of guppies collected and used, and sample sizes for each conditioning treatment × test stimulus combination.

**Figure S5.1** Schematic of the grid frame (20 cm × 20 cm) used for testing, along with the substrate classifications used.

**Figure S5.2** Schematic of rugosity measure, where a chain of original length (L) of 1 m was placed in a pool, and the linear length of the chain was measured (D). Rugosity was calculated as 1 - D/L. In this example, Rugosity would equal 0.3.

**Figure S5.3** Biplot in Scaling Type 2 of the "average" environmental variables in PC1-PC2.Variables include the transformed average pool width (width.tr), transformed average pool depth (depth.tr), transformed pool length (length.tr), average substrate complexity (rugosity), transformed average surface water velocity (surf.vel.tr), and transformed average mid-depth water velocity (mid.vel.tr).

**Figure S5.4** Biplot in Scaling Type 2 of the "variance" environmental variables in PC1-PC2.Variables include transformed pool width variance (width.var.tr), transformed pool depth variance (depth.var.tr), substrate complexity variance (rugosity.var), surface water velocity variance (surf.vel.var), and mid-depth water velocity variance (mid.vel.var), transformed substrate heterogeneity (hpie.tr), and substrate alpha diversity (alpha).

## *List of Tables*

**Table 4.2** *Post hoc* pairwise comparisons on proportional change in area use, for differences in response to a novel cue (NC) and water control (W), for Risk × Diversity treatments including: High Risk × High Diversity, High Risk × Low Diversity, Low Risk × High Diversity, and Low Risk × Low Diversity. Degrees of freedom and *p* values estimated using Satterthwaite's approximations, with Holm corrections. Significant terms are in bold type.

**Table 4.3** Linear mixed-effect model output for the transformed proportional change in shoaling index, including Satterthwaite *F* tests on fixed effects (a), statistics for random effects (b), and Tukey *post hoc* pairwise comparisons of risk treatments in response to a water control (c), and novel cue (d). Risk treatment included high-risk (HR), intermediate-risk (IR), or low-risk (LR), diversity treatment was either high- or low-diversity, and stimulus was either a novel cue or water control. Significant terms are in bold type, marginally non-significant terms are denoted with " · ". Degrees of freedom and *p* values estimated using Satterthwaite's approximations.

**Table 4.4** Cumulative mixed-effect model output for change in freezing, including Likelihood Ratio $\chi^2$ tests on fixed effects (a), statistics for random effects (b), and thresholds for decreasing to no change in freezing, and no change to increasing freezing. Estimates for thresholds correspond to a latent variable scale produced by the model. Significant terms are in bold type. Degrees of freedom and *p* values estimated using Laplace approximations.

**Table 4.5** Holm-corrected *post hoc* pairwise comparisons of change in freezing responses to a novel cue (NC) and water control (W) for each risk × diversity treatment. Estimates correspond to a latent variable scale produced by the model. Significant terms are in bold type.

**Table 5.1** Eigenvalues of the first 3 Principal Components given by PCAs for the a) "average" and b) "variance" environmental variable category.

**Table 5.2** Summary of the loadings, square loadings (quality representation of variable within PC), and contribution of each response variable in the first 2 PCs for a) "average" and b) "variance" environmental variable categories. In bold are the variable loadings of interest (where the square loadings $\geq 0.24$) for the components retained for subsequent models.

**Table 5.3** Linear mixed-effect model output for the latency to inspect a novel predator model (BoxCox-transformed), including Satterthwaite *t* tests on (a) fixed effects, and (b) statistics for random effects. Velocity-Complexity and Pool Dimension correspond to the first and second components of the "average" environmental variable category, respectively. Substrate Diversity and Dimension Variance correspond to the first and second components of the "variance" environmental variable category, respectively. Significant terms are in bold type. Degrees of freedom and *p* values estimated using Satterthwaite's approximations.

**Table 5.4** Generalized linear mixed-effect model (Binomial distribution) output for whether guppies entered a novel foraging arena, fit by maximum likelihood (Laplace approximation) on (a) fixed effects, and (b) statistics for random effects. Velocity-Complexity and Pool Dimension correspond to the first and second components of the "average" environmental variable category, respectively. Substrate Diversity and Dimension Variance correspond to the first and second components of the "variance" environmental variable category, respectively. Significant terms are in bold type.

***Glossary***

| Term | Definition | Source |
|---|---|---|
| *Abundant/complex information:* | When large amounts of information are difficult to perceive and process. This should include diverse information; Shannon information theory posits greater diversity of information results in lower predictability of what will be encountered next. | Zimmerman 2000;<br>Shannon 1948;<br>Crane et al. *in review* |
| *Conflicting information* | When multiple sources of information are detected, conveying differential or opposing information. | Zimmerman 2000;<br>Crane et al. *in review* |
| *Incomplete Information* | When information obtained/detected represents only a part of the key information (i.e., to identify the source and severity of risk). | Crane et al. *in review* |
| *Known Information* | Information that is reliable, where individuals have an innate or learned response to this cue. | Feyten & Brown 2018;<br>Crane et al. *in review* |
| *Noise* | Background patterns or stimuli which interfere with the detection or response to cues of interest, and which can occur in several modalities. These unwanted or unintended additions to information, including distortions or transmission errors, generate greater uncertainty. | Koops 1998;<br>McNicol 2004;<br>Brumm 2013;<br>Shannon 1948 |
| *Non-social Information* | Variably termed private or personal information in the literature.<br>Information that is acquired by direct interaction with the environment; Information not extracted from interactions with, or observations of, other animals. | Dall et al. 2005;<br>Wagner & Danchin 2010 |
| *NPA* | Neophobic predator avoidance, the active avoidance of novel stimuli that convey potential predation risk. It is a phenotypically plastic behaviour. | Brown et al. 2013;<br>Elvidge et al. 2016;<br>Crane and Ferrari 2017 |
| *Personal Knowledge* | The sum of information possessed by an individual. | Wagner & Danchin 2010 |

| | | |
|---|---|---|
| *Public Information* | Any cue from the abiotic or biotic environment that is potentially available to any organism. | Wagner & Danchin 2010;<br>Schmidt et al. 2010 |
| *Reliability (of information)* | How consistently correlated a cue is with an event (i.e., immediate predation risk). | Searcy & Nowicki 2005 |
| *Sensory Complementarity* | Integrating multiple sources of information within or across modalities. | Dall et al. 2005;<br>Lima & Steury 2005;<br>Muñoz & Blumstein 2012 |
| *Social Information* | Information gathered by interacting with, or observing the behaviour of, other organisms (e.g., conspecifics, or heterospecifics with similar ecological requirements). | Wagner & Danchin 2010;<br>Schmidt et al. 2010 |
| *Unavailable information* | When information is lacking, or undetectable by an individual. | Zimmerman 2000;<br>Crane et al. *in review* |
| *Uncertainty (of predation risk)* | The inability to predict the probability of the outcome of an event (e.g., predation), due to limitations in risk information. | Dall & Johnstone 2002;<br>Wagner & Danchin 2010;<br>Johnson et al. 2013 |
| *Unknown Information* | Information that is novel and unreliable, where prey have no innate or learned responses to this cue. | Feyten & Brown 2018;<br>Crane, Brown, et al. 2020;<br>Crane et al. *in review* |

# I. General Introduction

## *i. Behavioural trade-offs & risk assessment*

As a result of the non-consumptive effects of predation (Preisser et al. 2005; Johnson et al. 2013), prey are forced to balance predator avoidance with a suite of other activities such as foraging, territory defense, and/or courtship (Lima and Dill 1990; Sih 1992). Therefore, prey must make behavioural decisions (trade-offs) regarding these often-conflicting demands on time and energy. Prey can benefit from increased detection and avoidance of predators (i.e., survivorship), at the expense of those associated with engaging in other activities (Lima and Bednekoff 1999). However, the costs associated with these trade-offs are asymmetrical. If prey fail to respond with predator avoidance to a threat (i.e., false negative; Johnson et al. 2013), their risk of mortality increases (Lima and Dill 1990). Conversely, if prey respond when there is no relevant threat (i.e., false positive; Johnson et al. 2013), they may miss opportunities for other activities (Lima and Dill 1990). Predation context may dictate whether false positive errors outweigh false negative errors (Bouskila and Blumstein 1992; Abrams 1994; Rhoades and Blumstein 2007). In low-predation habitats, the costs of false positives (missed opportunities) may surpass the costs of false negatives (failing to respond), leading to less risk averse behaviour compared to individuals in high-predation habitats. In high-predation habitats (i.e., elevated mean risk), the costs of false negatives would surpass the costs of false positives, resulting in greater risk averse behaviour. For example, reef fishes decrease their foraging behaviour (feeding rate and amount of food consumed) with increased proximity to a potential predator (Catano et al. 2016). The ability to reliably recognize and assess local risks is critical to making behavioural decisions appropriate for the predation context (Brown, Ferrari, and Chivers 2011).

In order to make such cost-sensitive behavioural decisions and engage in antipredator responses, individuals must reliably assess local risk conditions (Bouskila and Blumstein 1992; McNamara and Dall 2010; Schmidt et al. 2010). To do so, prey must acquire, perceive, and process public information regarding local risks (Bouskila and Blumstein 1992). Public information is any cue from the abiotic or biotic environment that is potentially available to any organism (See *Glossary* for information terminology; Schmidt et al. 2010; Wagner and Danchin 2010). However, accurate assessment of information requires that cues are reliable (i.e., consistently correlated with immediate predation risk; Searcy and Nowicki 2005). For example, some reliable indicators of acute predation risk in aquatic systems include damage-released chemical alarm cues, disturbance cues, and learned predator odours (Brown and Godin 1999; Brown, Ferrari, and Chivers 2011). An antipredator response to these reliable, 'known', risk cues is either strongly selected for (i.e., innate) or reinforced by experience through the well-documented process of learning (i.e., learned predator recognition, where prey gain personal knowledge with an unreliable cue such that it becomes a reliable known cue; Brown et al. 2009; Brown, Ferrari, and Chivers 2011; Brown et al. 2015; Feyten and Brown 2018). Conversely, prey may be faced with 'unknown' cues, which are unreliable since they are not consistently correlated with an event (e.g., predation). That is, prey lack an innate response to this cue and have not yet learned whether this unknown cue represents a threat. This absence of learning could be due to a lack of opportunity to do so, or due to the costs associated with learning. Learning can be costly since it requires time and energy to gather information on unknown risks, which might otherwise be invested in other fitness-related activities (Dall et al. 2005; Ferrari et al. 2007; McNamara and Dall 2010). Moreover, when a novel cue corresponds to a novel predator, direct experience requires survival after initial encounter with this predator (Brown et al. 2013). Thus, there are contexts in which prey face unreliable cues, and are unable to accurately assess predation risk.

### *ii. Uncertainty of predation risk & information limitations*

When individuals cannot accurately estimate the probability of the outcome of an event, the literature states that they experience uncertainty (Wagner and Danchin 2010; Johnson et al. 2013), and sometimes this is more specifically termed 'perceived environmental uncertainty' (Milliken 1987). Uncertainty can apply to any type of decision that an animal faces, and it means decision-makers do not always know the ideal action (Dall and Johnstone 2002; Mathot et al. 2012). In the case of prey who cannot precisely predict the probability of the outcome of a predation event, I term this ***uncertainty of risk***. Generally, uncertainty is associated with limitations in a decision-maker's knowledge, and it can arise at any stage of the decision process (Dow 2012). If a decision-maker's personal knowledge is the sum of information possessed by an individual (Wagner and Danchin 2010), then limitations in knowledge must be linked to information limitations experienced by the decision-maker. In the field of economics, uncertainty has been categorized according to some information limitations, including a lack of information, overly abundant or complex information, or conflicting information (Zimmermann 2000). Under the context of predation, these information limitations also likely occur due to cues that are unknown (novel), unreliable, or represent incomplete information (See *Glossary*; Crane et al. *in review*). These information limitations are not necessarily independent, and they may instead be linked to one another. For example, unknown cues can be unreliable (see above), however not all unreliable cues are novel. Similarly, incomplete risk information may occur when individuals detect a single risk cue that is missing specific information about the source, location, and/or severity of risk. Thus, prey face uncertainty of risk when there are limitations in risk information, but this also requires some non-negligible level of predation risk. Uncertainty of risk likely exists on a continuous scale, ranging from low to high, since most information limitations and mean predation risk also exist on a continuous scale.

### iii. *Dealing with uncertainty*

Understanding how animals manage uncertainty is a core issue when assessing decision-making (Trimmer et al. 2011), especially in prey where the costs of making an error are high. Indeed, uncertainty may hinder context-appropriate antipredator responses (Sih 1992; Wagner and Danchin 2010; Johnson et al. 2013). According to error management theory, prey facing uncertainty of risk should have a bias towards exhibiting predator avoidance (i.e., making false positive errors) since it is less costly (Johnson et al. 2013).

However, even in high-predation contexts, it would be detrimental for prey to constantly engage in predator avoidance behaviours at the expense of other activities (Lima and Bednekoff 1999). Successful identification of predators via direct and/or indirect experience (i.e., learning; Mathis et al. 1996; Griffin 2004; Ferrari and Chivers 2013) could mediate uncertainty (Brown, Ferrari, and Chivers 2011; Brown et al. 2013). Learning, the ability to modify behavioural response patterns based on experience (Brown and Chivers 2005), is argued to allow prey to optimize the trade-off between predator avoidance and other fitness-related activities in conditions of variable predation risk (Brown and Chivers 2005; Dall et al. 2005; Ferrari et al. 2007; Brown, Ferrari, and Chivers 2011; Ferrari and Chivers 2013). Prey can learn to respond only to ecologically relevant threats and avoid spending time and energy responding to irrelevant cues (Ferrari and Chivers

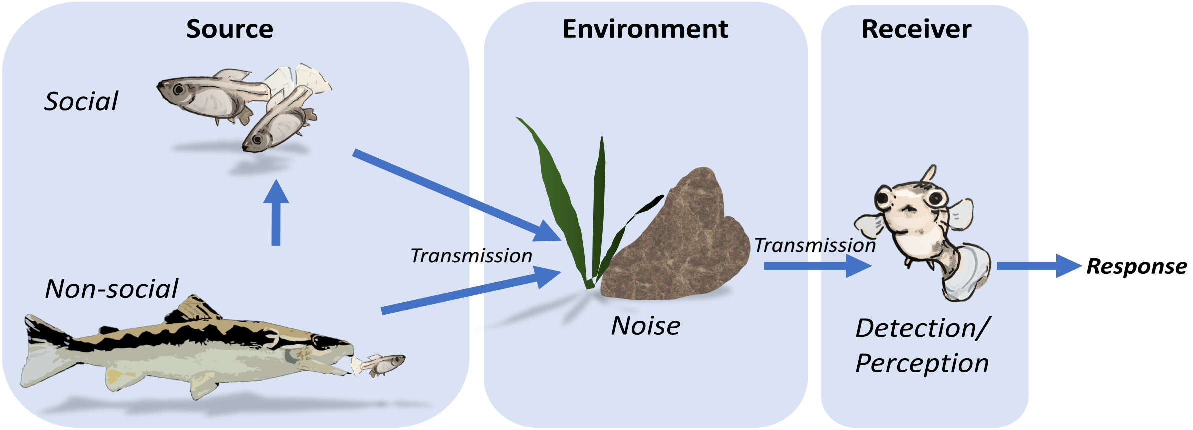
2013). However, learning itself is costly (see above), and one might expect the costs of learning to increase as uncertainty of risk increases (Brown et al. 2013).

An alternative mechanism allowing prey to cope with uncertainty is ***neophobic predator avoidance*** (NPA). This phenotypically-plastic behaviour is defined as the active avoidance of novel stimuli (such as unknown public information) that convey potential predation risk (Brown et al. 2013; Elvidge et al. 2016; Crane and Ferrari 2017). NPA would allow prey to reduce the cost of learning while still allowing behavioural flexibility (Brown et al. 2013). Recent studies suggest that background levels of predation risk (i.e., mean risk) influence NPA. Trinidadian guppies (*Poecilia reticulata*), for example, from high-predation sites exhibit NPA while those from low-predation sites do not (Brown et al. 2013). Additionally, NPA can be induced in Trinidadian guppies and other aquatic prey species (convict cichlids, *Amatitlania nigrofasciata*; woodfrog tadpoles, *Lithobates sylvaticus*) from low-predation sites after exposing them to conditions of elevated mean risk (Brown et al. 2013; Brown et al. 2015). Individuals may gain a survival benefit from NPA, as demonstrated in woodfrog tadpoles conditioned with elevated risk (Ferrari, Crane, et al. 2015). The strength and duration of retention of induced NPA is also (at least in part) determined by mean predation levels. For example, guppies conditioned to respond to an unknown cue can learn when this cue is no longer associated with risk (i.e., extinction), but NPA towards the unknown cue wanes more quickly in guppies from low-predation than high-predation populations (Brown et al. 2015). It has also been shown that NPA in convict cichlids is shaped by mean predation levels, and not concentration of unknown stimuli (Brown, Chivers, et al. 2014). Thus, NPA appears to be context-dependent and induced by elevated mean predation risk. Based on these experiments, Brown et al. (2013; 2015) argued that prey experiencing high mean predation levels also experience fluctuations in risk, and that neophobic responses may be shaped by uncertainty of risk more than just mean risk. More empirically, Ferrari et al. (2018) showed that NPA in woodfrog tadpoles was elicited as a result of uncertain risk (i.e., absence of complete information about predation risk, such as when alarm cues were presented without predator odours, or paired inconsistently with several unknown predator odours), and did not occur when tadpoles were certain (i.e., had complete information regarding risk, when alarm cues and the same predator odour were paired consistently). That is, the presence of limitations in the risk information detected (i.e., lack of complete reliable information regarding risk) resulted in NPA. Thus, NPA is likely shaped by uncertainty of risk, which requires a combination of elevated mean predation risk with information limitations.

### *iv. Goals*

Although it has been suggested that uncertainty of risk impacts NPA, there is a gap in the literature on identifying measurable components of uncertainty that specifically drive neophobia. To address this question, I will use Trinidadian guppies as my model system, as neophobia has been extensively studied in this species (Brown et al. 2013; Elvidge et al. 2016). Trinidadian guppies inhabit pools (separated via riffles and waterfalls) in rivers that have well documented predation risk (Croft, Morrell, et al. 2006; Botham et al. 2008; Deacon et al. 2018). Guppies also respond to conspecific chemical alarm cues (Brown and Godin 1999), allowing a comparison of behavioural response to reliable and unreliable (i.e., unknown) risk cues. It is increasingly important to study what drives neophobia, as prey are more likely to experience novelty and uncertainty due to climate change, anthropogenic factors, invasive species, and other disturbances to habitats.

**Box 1:** The information transfer pathway, adapted from Koops (1998) for information regarding predation risk



In the context of predation, prey individuals (receivers) acquire information regarding risk from their environment, after it is transmitted from a source. This source of information can be non-social or social (See *Glossary* for definitions). It is important to note that social information regarding risk is inevitably linked to the predator. For example, conspecifics serving as a source of social information have themselves gained information regarding risk transmitted from predators (or a continued chain of social information) through their environment. In the absence of a predator (i.e., low risk environments), there should be no information conveying risk stemming from a social source. At the source, uncertainty can arise as a result of diversity in predator guild composition, where prey are less able to predict which predator they may face next, impairing their ability to engage in appropriate behaviours. Predator guild diversity can include measures of species richness and evenness.

Information is transmitted from the source to the receiver through the environment. The environment can prevent transmission/availability of information, such as in large, complex, or heterogeneous habitats. Other considerations in aquatic habitats might include turbidity, pH, and water velocity. This obstruction or obscuring of information transfer can be considered noise (See *Glossary*). Uncertainty of risk can arise due to noise in the environment impairing prey from detecting/acquiring information from the environment. Furthermore, noise may impair the ability of prey to consistently correlate a cue with a predation event, reducing the amount and availability of reliable information. Therefore, noise could potentially affect several information limitations, shaping whether risk information is available, reliable, unknown, and/or complete (See *Glossary* for definitions).

In order to respond to information, receivers (prey) must be able to detect information of a particular modality with the appropriate physiological adaptations and mechanistic processes. Prey then translate the information and integrate this information with their personal knowledge using cognitive processes. Prey receive current information, and once acquired this becomes prior information which contributes to their personal knowledge (See *Glossary* for definitions). Uncertainty of risk results from limitations in knowledge driven by limitations in prior and current risk information. It is possible for individuals to detect more than one cue (current information) at a time. These multiple cues could be multimodal, vary in reliability, be complex in that they are abundant and diverse, be spatially and temporally unpredictable, originate from social and/or non-social sources, convey risk or safety, and/or conflict. Thus, there are many possible ways for prey to experience information limitations while detecting information, and this should shape neophobic responses.

In this thesis, I ask "*How does uncertainty of predation risk shape neophobic responses in prey?*". I identify potential factors contributing to uncertainty of risk and neophobia using information theory as a framework (See *Box 1*). I suggest that uncertainty of risk will increase NPA, since prey should over-estimate risk when uncertain according to the error management theory (Johnson et al. 2013). I have argued above that uncertainty of risk is dependent on elevated mean risk and information limitations regarding risk. Since information regarding predation risk must travel through several stages of information transfer before a receiver responds (Shannon and Weaver 1949; Koops 1998), I will show how uncertainty of predation risk may arise across these stages and shape the neophobic response of the receiver (*Box 1*). Uncertainty arising at different stages of information transfer can interact in potentially unpredictable ways to shape the neophobic behavioural response of prey communities.

In Chapters 1 to 3, I examine how NPA is shaped by prey detection and experience with novel information. These three chapters specifically assess how guppy responses to a novel cue are shaped by i) sensory complementation with reliable or unreliable cues, ii) the diversity as well as spatial predictability of novel cues, and iii) complementation with conflicting safety information from a social source.

Given that prey can use sensory complementation to reduce uncertainty (Lima and Steury 2005; Munoz and Blumstein 2012), I expect that guppies integrate multiple sources of available information when faced with novelty, and that their response to novelty differs based on the reliability of information presented with it. In Chapter 1, I present high-risk guppies with multimodal cues of mixed reliability and test my prediction that neophobia would be greatest when both cues are unreliable.

Having explored guppy responses to simultaneous novel cues in the previous chapter, I next assess how repeated experience with a novel cue over time may shape their retention of neophobic responses. In Chapter 2, I test my prediction that high-risk guppies retain their neophobic responses to a repeatedly experienced novel cue only when this cue is presented with other novel cues (i.e., novelty is diverse). When novelty is not diverse, prey should learn that a particular cue does not convey risk. Conversely, when novelty is diverse, prey should experience more uncertainty of risk and they may also experience some interference in their learning process. I test whether this loss of neophobia is generalized to other novel cues, and I predict that guppies should retain their neophobic responses to a completely novel cue. In this chapter, I also test my predictions that neophobia is retained when a novel cue is repeatedly presented in a spatially unpredictable manner, but not when it is presented in a spatially predicable manner. In the former, uncertainty would be higher when novel risk is less predictable, and guppies should retain their neophobia. In the latter, uncertainty would be lower since novel risk is spatially predictable, and guppies should not retain their neophobic response to the novel cue.

In Chapter 3, I again examine how prey could use multiple risk cues, but this time when presented with conflicting risk and safety information from non-social and social sources, respectively. According to the costly information hypothesis, individuals should favour the acquisition of social information over non-social information when the cost of acquiring non-social information is relatively high, as in a high-predation environment (Boyd and Richerson 1988; Kendal et al. 2004; Webster and Laland 2008). Thus, I predict that high-risk guppies faced with novelty will rely on social safety cues. However, social information can become outdated (Giraldeau et al. 2002; Koops 2004; van Bergen et al. 2004; Webster and Laland 2008; Rieucau and Giraldeau 2011), and prey should favour non-social information in contexts where social information is less reliable than non-social information. Therefore, I also predict that guppies should rely on reliable known risk cues over social safety cues, regardless of background risk conditions.

In Chapter 4, I assess how the composition of predators, the direct cause and source of risk, shapes neophobia. Elevated background risk has already been shown to shape neophobic responses (Brown et al. 2013; Brown et al. 2015), but the specific effects of predator density and diversity on neophobia have yet to be disentangled. According to Shannon (1948), predictability may decrease with increased diversity of information. I therefore predict that predator-naïve guppies conditioned with risk should exhibit neophobic responses when experiencing elevated predator density as well as diversity.

Lastly, in Chapter 5, I examine how the environment and associated noise may affect information limitations by shaping whether information is available, reliable, and/or unknown. If noise in the environment interferes with information transfer (Koops 1998), then I predict that the mean and variance of habitat conditions which contribute to such noise would increase neophobic responses in high-risk guppies. Such microhabitat conditions might include microhabitat complexity, water velocity, microhabitat dimensions, and substrate heterogeneity.

## II. Chapter 1: *How does simultaneous multimodal information of mixed reliability influence NPA?*[1]

### 1.1 Introduction

Prey use public information in order to assess local habitat conditions such as predation risks, competitive interactions, and foraging opportunities (Dall 2005). The availability of reliable information regarding local conditions allows prey to reduce the costs associated with making bad behavioural decisions (Lima and Dill 1990; McNamara and Dall 2010; Schmidt et al. 2010; Brown, Elvidge, et al. 2014). Notably, these costs can be asymmetric (Johnson et al. 2013) since prey can either risk mortality by failing to respond to ecologically relevant threats, or lose opportunities for fitness-related activities (i.e., territory defense, courtship, and/or foraging) by responding to an irrelevant threat (Feyten and Brown 2018). Despite the asymmetry of these costs, prey capable of assessing risks and balancing these decisions should be at a selective advantage (Stephens 1989; Dall et al. 2005; Seppänen et al. 2007).

However, public information can differ widely in reliability. Cues that are consistently correlated with an event (i.e., predation) can be thought of as being reliable indicators of local conditions. Conversely, when information is not consistently correlated with an event, these cues are unreliable (Searcy and Nowicki 2005). Information reliability is expected to decrease with increased uncertainty of environmental conditions (Koops 2004; Dall et al. 2005; Schmidt et al. 2010), where ecological uncertainty[2] is the ambiguity about the state of the environment due to imperfect or incomplete information (Dall et al. 2005; Munoz and Blumstein 2012; Feyten and Brown 2018). For example, predation pressure can be highly spatially and temporally variable, leading to unpredictability in local risks (Sih 1992; Lima and Bednekoff 1999; Dall et al. 2005; Schmidt et al. 2010; Ferrari et al. 2018). As a result, prey may not have reliable information regarding predation risk, and consequently need mechanisms to respond to this uncertainty.

One mechanism with which prey can cope with uncertainty is a well-documented mechanism of learning via predator recognition, where prey learn to recognize novel cues as threats via the pairing of this ‘unknown’ cue with a ‘known’ cue that is a reliable indicator of risk, such as damage-released chemical alarm cues (Ferrari et al. 2010; Chivers et al. 2012). Therefore, prey can gain experience with unknown cues, and learn appropriate responses to these cues that have meanwhile become known. However, learning to recognize new threats is inherently costly, as prey must survive their initial encounter in order to gain relevant experience (Ferrari et al. 2007).

Although prey response to known cues is well studied, recent studies demonstrate that prey may also benefit from exhibiting neophobic responses to unknown or novel cues. Neophobia, defined as the avoidance of novel stimuli, has recently been proposed as a phenotypically plastic response to uncertain background risks (Brown et al. 2013; Ferrari et al. 2018). For example, Trinidadian guppies (*Poecilia reticulata*) from high-, but not low-predation sites exhibit

---

[1] A shortened version of Chapter 1 has been published. Feyten, LEA, Demers, EEM, Ramnarine, IW, and Brown, GE (2018) Predation risk assessment based on uncertain information: interacting effects of known and unknown cues. Current Zoology 65(1): 75-76. DOI: 10.1093/cz/zoy083.

[2] In this chapter, I use the term “ecological uncertainty”, rather than “uncertainty of predation risk” as described in my thesis introduction. Functionally, I am referring to the same definition of the inability to predict the probability of the outcome of an event (e.g., predation), due to limitations in risk information.

neophobic predator avoidance (NPA; Brown et al. 2013). Additionally, NPA can be induced in Trinidadian guppies and other aquatic prey species (convict cichlids, *Amatitlania nigrofasciata*; woodfrog tadpoles, *Lithobates sylvaticus;* fathead minnows*, Pimephales promelas*) from low-predation sites after exposing them to conditions of elevated and unpredictable risk (Brown et al. 2013; Brown et al. 2014; Brown et al. 2015; Crane et al. 2015). Thus, NPA is thought to be a response to ecological uncertainty (Brown et al. 2005; Elvidge et al. 2016; Ferrari et al. 2018; Feyten and Brown 2018), which enables prey to reduce the cost of learning while still allowing sufficient behavioural flexibility to respond to variable threats (Brown et al. 2013). Consequently, prey rely on a variety of sources of information that range from known or reliable (i.e., conspecific alarm cues; learned predator recognition) to unknown (i.e., novel).

The effect of ecological uncertainty, and the resulting reliability of information, becomes increasingly complex when we consider that prey integrate information from multiple sources in order to make behavioural decisions. Sensory complementarity improves decision making of prey, where prey effectively reduce uncertainty by gathering and integrating different sources of information, across sensory systems, in order to assess and respond to acute predation threats (Lima and Steury 2005; Munoz and Blumstein 2012; Brown et al. 2016). Notably, multimodal information provides an independent estimate of events from each modality, granted that each has a unique set of limitations (Munoz and Blumstein 2012). For example, strawberry poison frog tadpoles (*Oophaga pumilio*) integrate visual, chemical, and tactile cues produced by visitors in order to alter their swimming behaviours and avoid predation (Stynoski and Noble 2012). Similarly, past experiments show that Trinidadian guppies integrate 'known' visual and chemosensory cues in order to assess predation risk (Brown et al. 2010; Brown et al. 2013). Recently, Stephenson (2016) showed that, under laboratory conditions, guppies exposed to a known chemosensory cue subsequently reduced their activity but increased their response to an ambiguous visual cue. However, no study has shown how prey integrate and respond to simultaneous multimodal sources of information of mixed reliability under natural conditions.

We hypothesize that prey should integrate and respond differently to paired risk-assessment cues, when these cues are either both known, one known and one unknown, or both unknown. Sensory complementarity (Lima and Steury 2005) should allow prey to integrate multiple sources of public information and enhance the accurate perception of acute threats. Indeed, when prey detect multiple sources of information known to be risky, they should exhibit strong, context-dependent responses (Lima and Steury 2005; Leduc et al. 2010). Prey exposed to known risk cues can mediate their response patterns to avoid predators and retain some time and energy to forage, court, or defend territories. Likewise, when prey perceive cues of mixed reliability, we predict they will show greater reliance on the 'known' vs. 'unknown' information (Brown et al. 2013). However, it is unclear how prey from a high-predation site will respond when both cues are unknown (i.e., unreliable). Error management theory (Johnson et al. 2013), as well as the predation hazard assessment model (Bouskila and Blumstein 1992), predict that as risks become more uncertain (i.e., unknown), prey are expected to shift to a more risk-averse (i.e., cautious) tactic. Therefore, we predict that when prey face multiple unreliable (i.e., unknown) cues, they should 'overestimate' risk.

To test this question, we conducted *in situ* predator inspection trials, which are a well-established estimate of perceived predation risk (Brown et al. 2013). We paired 'known' and 'unknown' visual risk-assessment cues (predator models) with a known, unknown, and control chemosensory risk-assessment cues (alarm cue, lemon odour, and stream water, respectively). We used Trinidadian guppies as our model system, since their neophobic responses have been well studied (Brown et al. 2010; Brown et al. 2013).

## 1.2 Methods

### *1.2.1 Experimental site*

We conducted *in situ* observations in a series of discrete pools, with a distance of at least 5 m between test pools, along a ~ 1 km reach of the Lopinot River in April 2016 (Grid Ref. UTM 20P 683760, 1183881; Figure 1.1). A discrete pool is defined as a pool separated from others by fast-moving ripples, waterfalls, and/or rock boundaries, which limit the movement of guppies. The Lopinot River is a known high-predation site, with abundant populations of pike cichlids (*Crenicichla* sp.), blue acara cichlids (*Aquadiens pulcher*), Hart's rivulus (*Anablepsoides hartii*), and wolf fish (*Hoplias malabaricus*; Croft, Morrell, et al. 2006; Botham et al. 2008; Deacon et al. 2018). Guppies from this river are known to exhibit neophobic predator avoidance (Brown et al. 2018).

### *1.2.2 Chemosensory cue preparation*

We collected damage-released chemical alarm cues from non-gravid female guppies of the Lopinot River. Damage-released chemical alarm cues are widespread among aquatic prey species (Chivers and Smith 1998; Ferrari et al. 2010) and are honest and reliable indicators of risk (Chivers et al. 2012; Brown, Chivers, et al. 2014). Previous studies have shown that alarm cues elicit increased antipredator responses under laboratory and field conditions (Brown and Godin 1999; Brown, Chivers, et al. 2014; Elvidge and Brown 2015). We generated alarm cue from 27 donor guppies (mean ± SD standard length = 23.90 ± 2.02 mm). Cue donors were euthanized via cervical dislocation (in accordance with Concordia University Animal Research Ethics Protocol #AREC-30000255). Due to their small body size, we used whole body extracts. After removing the head and tail, we immediately placed the remaining tissue in 100 mL of chilled dechlorinated water. We then homogenized the tissue samples, filtered through polyester floss, and diluted with dechlorinated water to the desired final concentration (~0.10 $cm^2$ $mL^{-1}$; Brown et al. 2010; Brown et al. 2013; Brown et al. 2015). For our novel chemosensory cue, we diluted Herrera's® lemon essence in dechlorinated water (6 mL lemon extract in 300 mL dechlorinated water; Brown et al. 2013; Brown et al. 2015). Alarm cue and novel chemosensory cues were frozen in 20 mL aliquots at -20°C until needed.

### *1.2.3 Visual cue preparation*

For visual predator models, we 3D printed two translucent polycarbonate models, sprayed them with Rust-Oleum® Painter's Touch® Ultra Cover Primer, hand-painted them with Pebeo® High Viscosity Studio Acrylic paint, and sealed them with Rust-Oleum® Painter's Touch® Ultra Cover Clear Gloss. The first model represented a known visual cue to the guppies by emulating a pike cichlid (Figure 1.2A), thought to be the most dangerous guppy predator (Botham et al. 2008). The other model represented a novel predator, resembling a carp or goldfish painted in bright colours (Figure 1.2A). We attached each model to a wooden dowel (~1 m in length) using transparent fishing line, which allowed controlled placement of the models.

### *1.2.4 Experimental Methods*

We conducted *in situ* observations of guppies exposed to paired visual and chemosensory cues. We paired a model of a known or novel predator with 10 mL of either stream water (control), alarm cue (known risk cue), or lemon essence (unknown odour). We assigned these treatments randomly.

We made observations from the shoreline (n = 12 per treatment combination) of slow-moving pools of the Lopinot river, with observation sites located at least 5 m apart. Prior to each observation, we positioned 2 m of clear acrylic airline tubing (which was used to administer chemosensory cues) anchored to a rock (~3 cm in diameter) at least 75 cm from the edge of the water. We then positioned one of the model predators directly above the terminal end of the tube (Figure 1.2B). Once at least 5 guppies were within a 50 cm radius of the predator model, we presented the chemosensory cue (10 mL slowly flushed through the tube with a 60 mL syringe containing stream water) and began observations for 5 min. In order to minimize contamination, we flushed out the tubing with stream water after each trial to ensure no residual cues remained. Additionally, we conducted trials moving upstream in order to reduce the likelihood of repeated exposures to chemosensory cues.

We recorded latency to inspect (the time it took from the beginning of a trial for a guppy or group of guppies to make a directed, salutatory approach towards the model; Brown et al. 2010), the number of guppies within a 25 cm radius of the model at 15 s intervals, and the total number of inspections. We calculated per capita inspection rate by dividing the total number of inspections by the mean number of guppies present in a trial. We used a rate in order to control for variation in the number of guppies present among trials. Risk-averse behaviour is indicated by an increased latency to inspect, and a reduced inspection rate (Brown et al. 2010; Brown et al. 2013).

### *1.2.5 Statistics*

We conducted a 2-way ANOVA on each observed behaviour (latency to inspect, and per capita inspection rate), with predator model (known or unknown visual cue) and chemosensory cue (known, unknown, or control) as the independent factors. This allowed us to examine the effect of reliability of visual risk-assessment cues, chemosensory risk-assessment cues, and their interaction on prey behaviour. In the case of an interaction, we conducted *t* tests for each chemosensory cue subset of the data. This allowed us to compare the effects of the two predator models on behavioural responses for each chemosensory cue. Statistical analyses for all experiments were conducted using SPSS v24.0.

## **1.3 Results**

Initially, we found that the number of guppies present did not differ for predator models ($F_{(1, 72)} = 3.36$, $p = 0.07$), chemosensory cues ($F_{(2, 72)} = 1.80$, $p = 0.17$), or the interaction of predator models and chemosensory cues ($F_{(2, 72)} = 0.21$, $p = 0.81$, Figure 1.3). We did find that the mean latency to inspect was affected by both the predator model and chemosensory cue (interaction: $F_{(2, 72)} = 9.44$, $p < 0.001$), as well as by the chemosensory cue alone ($F_{(2, 72)} = 30.37$, $p < 0.001$), but not by predator model ($F_{(1, 72)} = 2.49$, $p = 0.12$, Figure 1.4). *Post hoc t* tests suggest that the latency to inspect was significantly longer when a novel chemosensory cue was paired with a novel predator model, compared to when it was paired with a known predator model ($t = -4.12$,

$df = 22$, $p < 0.001$, Figure 1.4). However, the latency to inspect did not significantly differ when a known chemosensory cue was paired with either a novel or a known predator model ($t = 1.42$, $df = 22$, $p = 0.17$). Additionally, there was no significant difference in latency to inspect when the control chemosensory cue was paired with either a known or novel predator model ($t = -0.63$, $df = 22$, $p = 0.54$).

We found the per capita inspection rate was significantly affected by the predator model and chemosensory cue (interaction: $F_{(2, 72)} = 5.59$, $p < 0.01$), as well as by the chemosensory cue alone ($F_{(2, 72)} = 16.32$, $p < 0.001$), but not by the predator model alone ($F_{(1, 72)} = 0.01$, $p = 0.92$, Figure 1.5). *Post hoc t* tests suggest that per capita inspection rate was significantly lower when a novel chemosensory cue was paired with a novel predator model, compared to a novel chemosensory cue paired with a known predator model ($t = 3.75$, $df = 22$, $p = 0.001$). Additionally, there was no significant difference in per capita inspection rate when a known chemosensory cue was paired with either known or novel predator models ($t = -1.50$, $df = 22$, $p = 0.15$), nor when the control chemosensory cue was paired with either predator model ($t = -0.97$, $df = 22$, $p = 0.35$, Figure 1.5).

## 1.4 Discussion

Our results provide support for the hypothesis that the reliability of public risk-assessment cues (i.e., known vs. unknown cues) can shape the perceived predation risk of prey individuals. When exposed to a known predator model, guppies increased their latencies to inspect and reduced inspection rate when the model was paired with a known (alarm cue) or unknown (lemon odour) chemosensory cue, relative to the stream water control. When exposed to an unknown predator model, guppies had the greatest latencies to inspect and lowest inspection rate when the model was paired with an unknown chemosensory cue. Interestingly, the response to an unknown predator model paired with a known chemosensory cue was similar to when a known predator model was paired with an unknown chemosensory cue. Taken together, we suggest that in the absence of at least one source of known (i.e., reliable) information, guppies ‘overestimate’ the level of perceived predation risk. Recently, Brown et al. (2014) demonstrated that the response of Trinidadian guppies to conspecific alarm cues (known cues) was dependent upon individual risk-taking tactics (i.e., ‘personality’; Brown et al. 2005; Réale et al. 2007; Jones and Godin 2010). Shyer guppies exhibited stronger responses than did bolder guppies. However, when exposed to a novel (unknown) cue, all guppies exhibited a strong response, regardless of individual risk-taking tactic. As in the current experiment, guppies faced with unknown public information appear to ‘overestimate’ risk. Combined, our results support the hypothesis that the reliability of public information (i.e., known vs. unknown cues) can shape the perceived predation risk of prey individuals.

Sensory complementarity, or the integration of multiple sources of information across sensory systems, is argued to allow prey to more accurately assess local predation threats (Ferrari et al. 2008) and potentially reduce uncertainty (Munoz and Blumstein 2012; Weissburg et al. 2014). Potentially, when facing a solitary unreliable visual or chemical cue, information regarding risk is “incomplete” in that only part of the key risk information has been obtained (i.e., lacks more information about the associated risk or information source, respectively). Complementing an unreliable cue with additional reliable information offers prey a way to “complete” the risk information, effectively reducing uncertainty. This should improve prey ability to make context-appropriate behavioural decisions. For example, an experiment on the effects of stream acidification on juvenile Atlantic salmon (*Salmo salar*) showed that salmon from neutral streams can exhibit threat-sensitive responses by using sensory complementarity to integrate visual and chemosensory cues about predation, unlike salmon from weakly acidic streams which are hypersensitive to visual cues and have a reduced ability to detect chemosensory cues due to the acidity (Elvidge et al. 2013). Similarly, young-of-the-year Atlantic salmon exhibit more intense chemosensory risk assessment at night compared to day, perhaps because fish integrate both visual and chemosensory cues during the day using sensory complementarity in order to respond with the context-appropriate intensity (Leduc et al. 2010). Our work highlights the importance of information reliability to sensory complementation, having demonstrated that prey may integrate information via sensory complementarity in a disparate manner when exposed to two unknown cues, one known and one unknown cue, or two known cues. Our results suggest that when prey are faced with cues of mixed reliability, they rely on the known cue to assess the level of acute threat (the known cue is dominant; Munoz and Blumstein 2012). Thus, regardless of the modality in which it is received, individual antipredator behaviour is based on reliable information. When both cues are known, there are no additive effects of information when they are integrated via sensory complementarity (i.e., equivalence of redundant information; Munoz and Blumstein 2012). However, our results are consistent with the hypothesis that when both sources of risk-assessment information are unknown, there are additive effects of sensory complementarity (i.e., enhancement of redundant information; Munoz and Blumstein 2012), resulting in the over-estimation of risk.

Moreover, we have demonstrated that both reliable and unreliable sources of information are valuable. If prey can respond to both, they can enhance their behavioural decision-making. This imparts a selective advantage to the organism, thereby making these sources of information valuable in that they change the function of an organism in an evolutionarily relevant manner (Dall et al. 2005; Seppänen et al. 2007). Genetically fixed (i.e., alarm cues; Chivers et al. 2012) or learned cues are likely reliable indicators of local risk, and prey respond to these cues since they are associated with risk. Conversely, while novel (unknown) cues may or may not represent an actual risk, prey effectively over-estimate risk by exhibiting neophobia. As prey gain experience with a novel cue, this risk-averse response will be retained as long as it is advantageous (i.e., the cue represents an actual risk). A reliable cue conveying risk paired with an unreliable cue likely represents the predator recognition learning mechanism, where prey will eventually be able to respond to the initially unknown cue if the two cues are paired enough (Ferrari et al. 2010; Chivers et al. 2012). In the case of two unreliable cues which convey risk, predator avoidance response would be reinforced, similar to the predator recognition mechanism (Brown et al. 2015).

Having a combination of genetically fixed, learned, and neophobic responses may allow prey to minimize costs while make optimal decisions in the face of predation risk. For example, learning can be costly since prey must survive initial predator encounters (Ferrari et al. 2007; Brown et al. 2013), use energy and time which might otherwise be invested in other fitness activities (Dall et al. 2005; McNamara and Dall 2010), and potentially face 'unknown' (i.e., unreliable) cues before they can gain experience and learn how to respond. Meanwhile, neophobic responses can be costly when the missed opportunities of engaging in other fitness related activities accrue. Indeed, if neither the reliable nor unreliable cue conveys an actual threat, the response to these cues will diminish in a process akin to latent inhibition (Acquistapace et al. 2003; Mitchell et al. 2011; Brown et al. 2015). Similarly, if prey are faced with two unknown cues which do not entail risk, we expect neophobic predator avoidance responses to fade (Brown et al. 2015). However, background levels of risk influence how quickly the response is inhibited (Brown et al. 2015). Taken together, we propose that prey can respond to a combination of unreliable 'unknown' and reliable 'known' sources of information by using genetically-fixed responses, learning, and neophobia.

As climate change, disturbances, invasive species, and other anthropogenic factors contribute to fluctuating, and therefore uncertain, environments, prey are increasingly faced with information of mixed reliability. It is accordingly essential to delineate how species may respond to this ecological uncertainty. We have shown how prey can use sensory complementarity to respond to cues of mixed reliability, and how uncertainty of risk shapes prey responses. Furthermore, we have highlighted the complex nature to how the reliability of information, and uncertainty, impact neophobic responses. Future studies should examine what biotic or abiotic factors might contribute to ecological uncertainty, and how these factors could shape neophobic responses in prey.

## 1.5 Figures

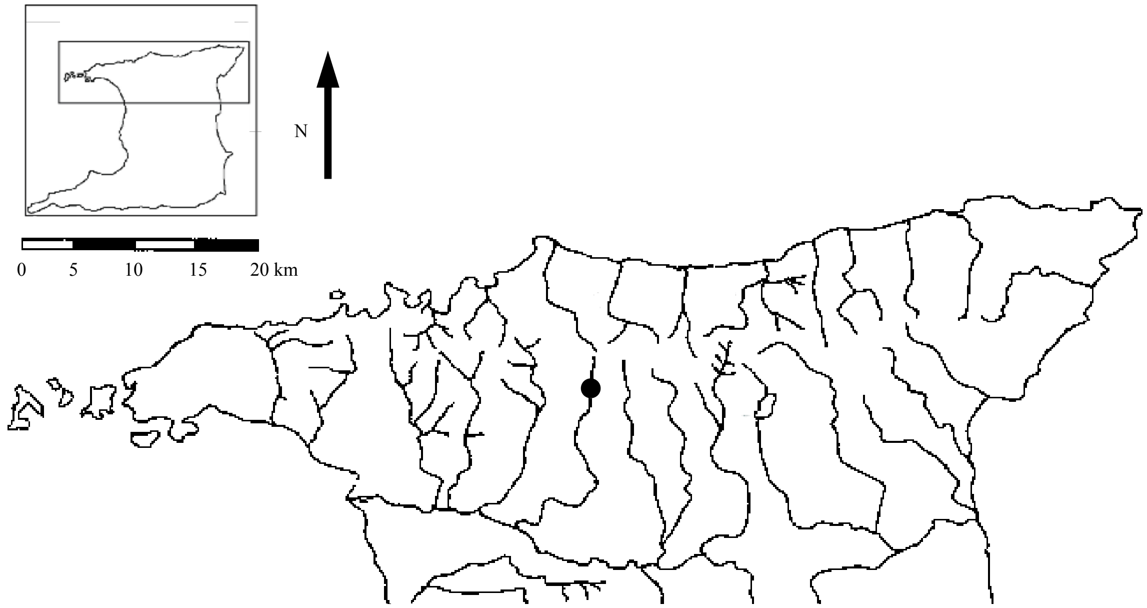


**Figure 1.1:** Map showing the location of our study site on the Lopinot River in the Northern Mountain Range, Republic of Trinidad and Tobago.

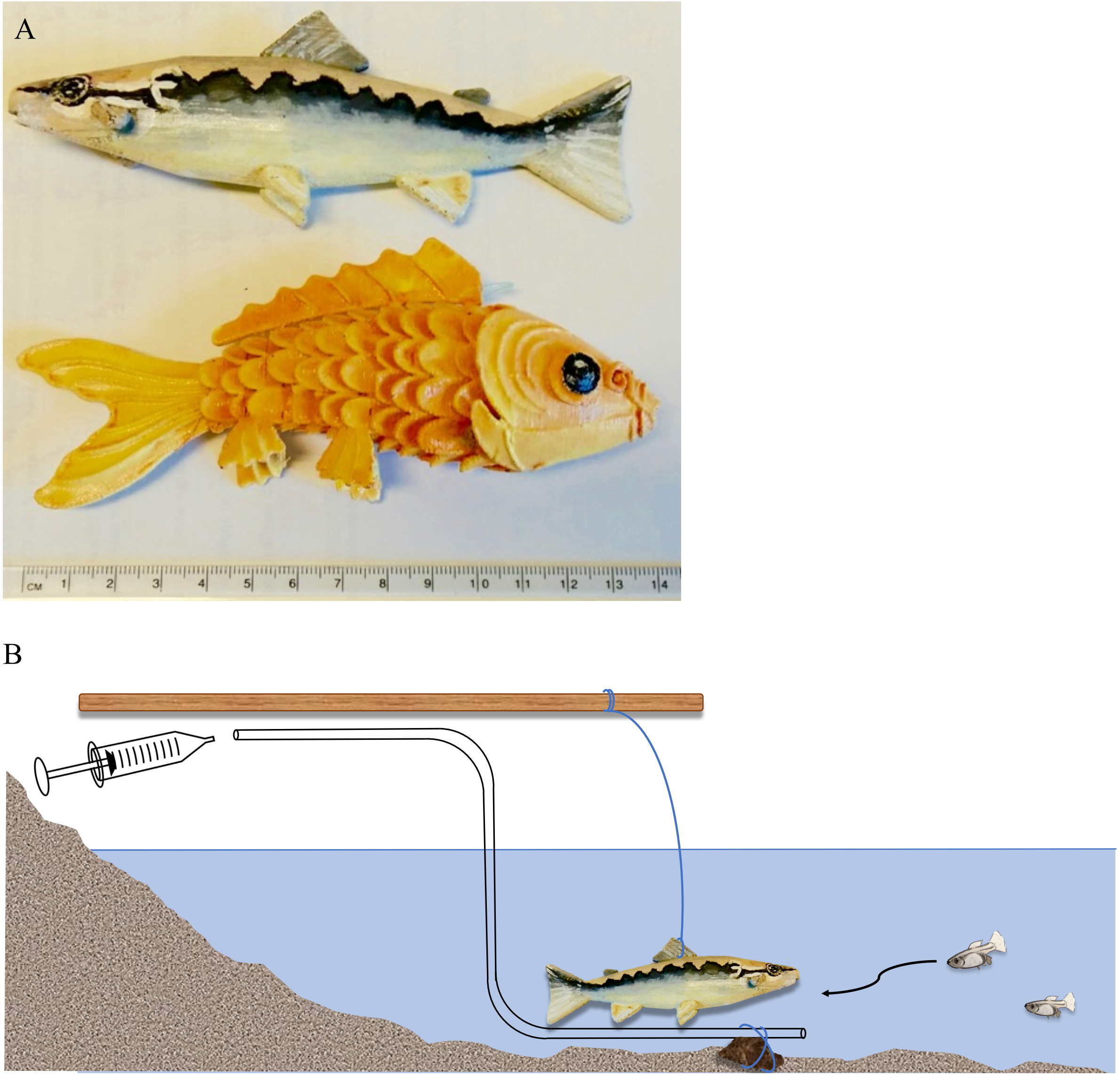


**Figure 1.2:** A) 3-D printed polycarbonate predator models, 14 cm in length. The models resemble a common predator (the pike cichlid, *Crenicichla* sp., top), or a novel heterospecific (bottom). B) Schematic diagram of the cue delivery system, showing a 1 m dowel with a predator model suspended above the terminal end of 2 m of acrylic airline, which was anchored to a rock. 10 mL of a chemosensory cue was administered via a syringe on the opposite end of the acrylic airline, and flushed through the tube with 60 mL of stream water.

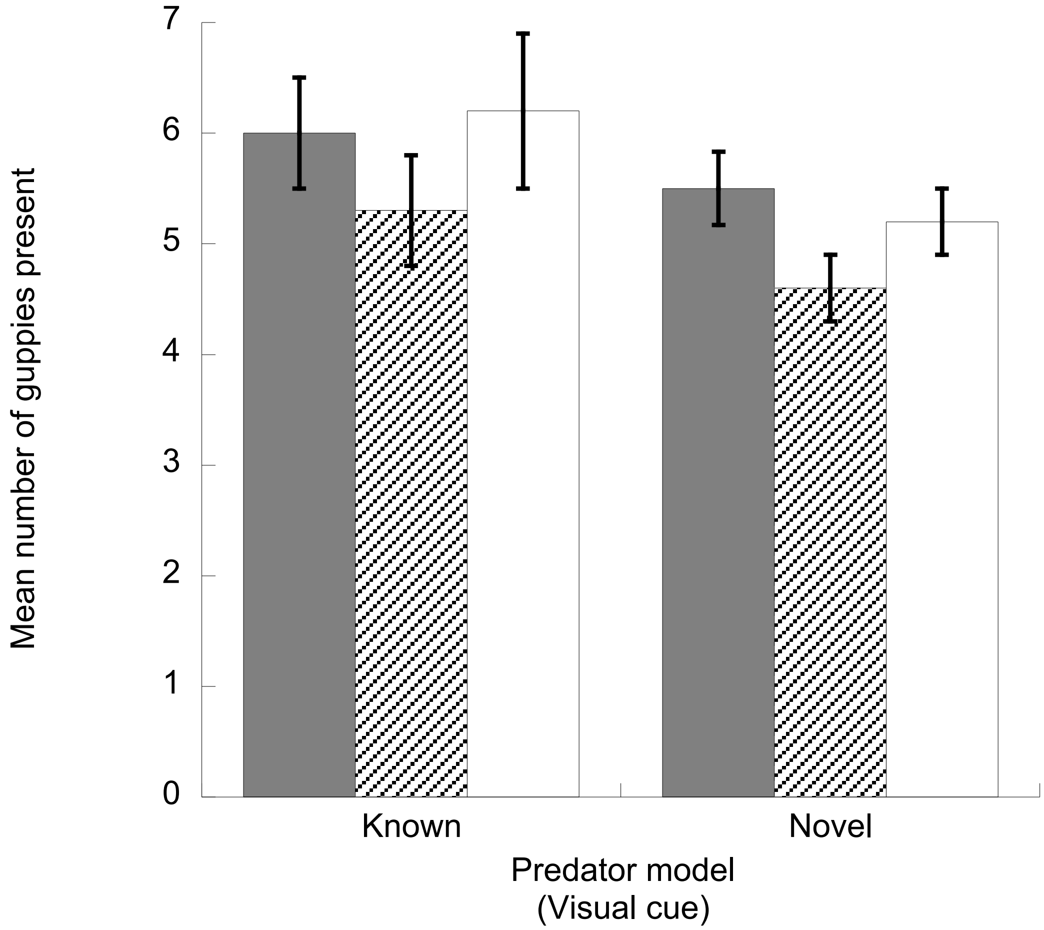


**Figure 1.3:** Mean (± SE) number of guppies present for the treatments of known or novel visual cues (predator models) paired with known (alarm cue; grey bars), unknown (lemon odour; striped bars), or control (stream water; white bars) chemosensory cues. n = 12 per treatment combination.

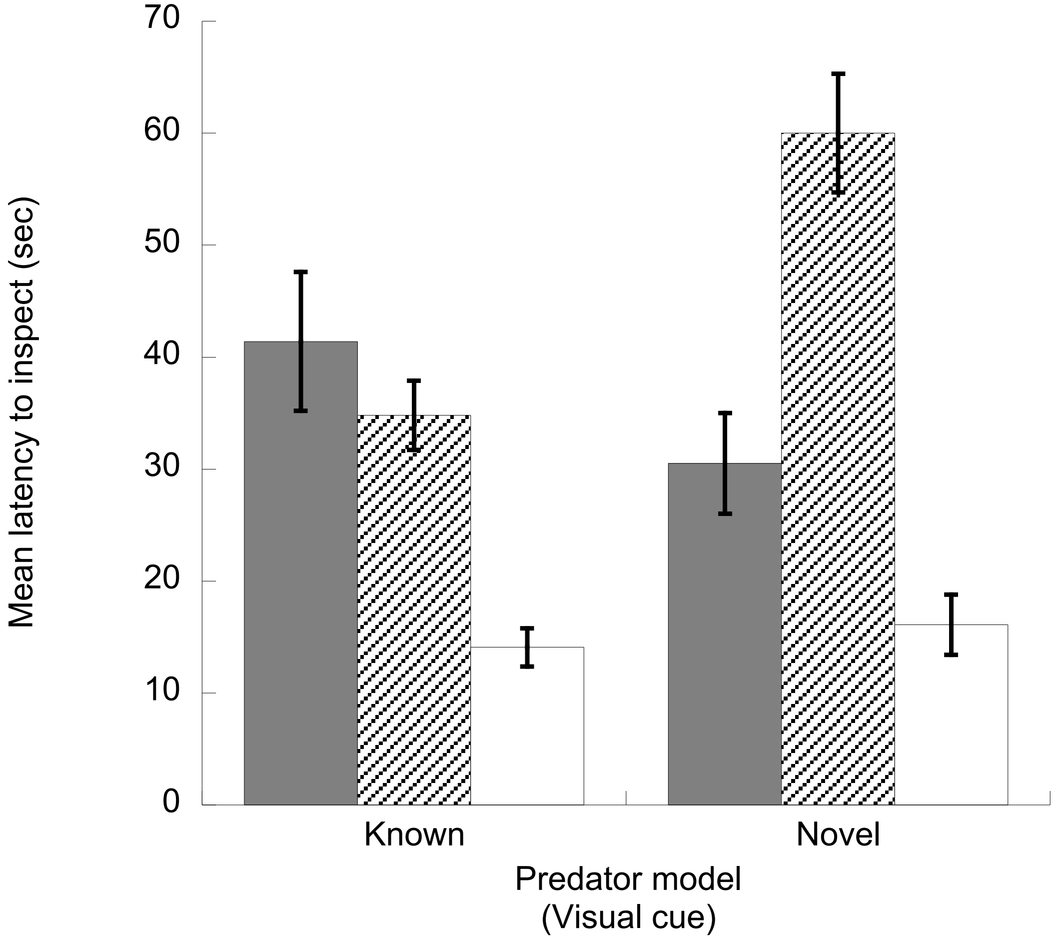


**Figure 1.4:** Mean (± SE) latency to inspect the known or novel visual cues (predator models) paired with known (alarm cue; grey bars), unknown (lemon odour; striped bars), or control (stream water; white bars) chemosensory cues. An increase in latency to inspect is consistent with increased perceived predation risk (Brown et al. 2013). n = 12 per treatment combination.

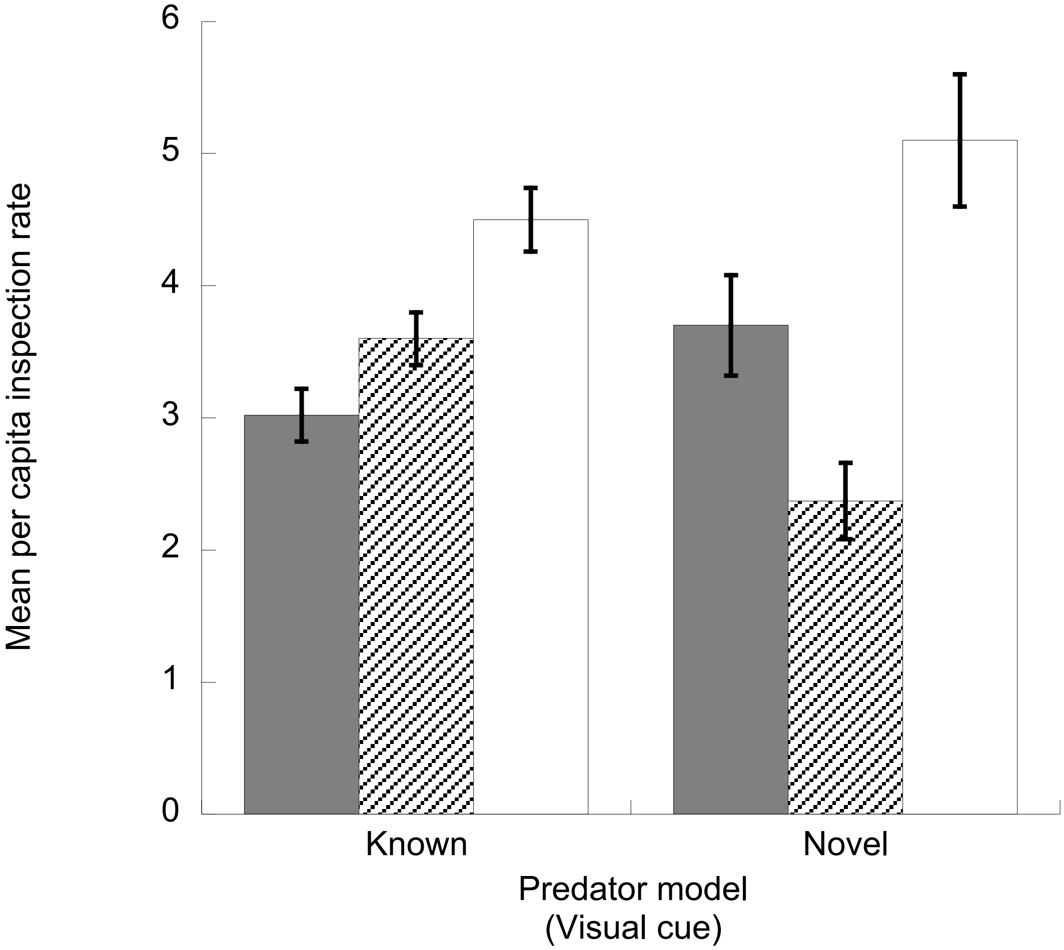


**Figure 1.5:** Mean (± SE) per capita inspection rate of the known or novel visual cues (predator models) paired with known (alarm cue; grey bars), unknown (lemon odour; striped bars), or control (stream water; white bars) chemosensory cues. A decrease in per capita inspection rate is consistent with increased perceived predation risk (Brown et al. 2013). n = 12 per treatment combination.

## III. Chapter 2: *Prey reception and personal knowledge of cues influence NPA retention*[3]

### Preface

In the previous chapter, I examine how NPA is influenced by the availability of simultaneous multimodal cues of mixed reliability. In Chapter 2, I further determine how the reception of multiple cues (that may differ in the reliability of those cues based on prey personal knowledge; See *Glossary*) can influence uncertainty and the resulting neophobic responses (*Box 1*). In this chapter, I conduct a set of experiments to examine how the retention of NPA is shaped by the diversity and spatial predictability of successively detected unknown cues.

### 2.1 Introduction

As a result of the unforgiving nature of predation pressure, prey animals often trade-off short-term gains in activities like foraging and courtship, in favour of predator avoidance (Lima and Dill 1990; Preisser et al. 2005). Not surprisingly, prey that can reliably assess the risk posed by predators should be better able to optimize this cost-benefit trade-off and display adaptive behavioural decisions (Lima and Dill 1990; Ferrari et al. 2010). As the spatial and/or temporal variability of risk increases, either in the nature or intensity of the threat, so does the level of uncertainty associated with an individual's decision making (Sih et al. 2000; Preisser et al. 2005; Stankowich and Blumstein 2005). A lack of information concerning local risk (i.e., ecological uncertainty[4]; Schmidt et al. 2010; Mettke-Hofmann 2014) can increase the costs of risk assessment among prey. Failing to respond to an unknown threat can lead to death, while responding to an irrelevant cue leads to lost foraging or courtship opportunities. Thus, prey are expected to increase their reliance on experientially acquired information (i.e., learning) to fine tune their behavioural decision making (Dall et al. 2005; McNamara and Dall 2010).

Recent studies have begun to examine the role background predation risk plays in shaping the cognitive ecology and decision making of prey organisms. Several studies suggest that neophobic predator avoidance (NPA), that is, responding to a novel cue with an antipredator response, may function as a phenotypically plastic trait in response to variable or unpredictable predation pressures (Brown et al. 2013; Chivers et al. 2014; Crane et al. 2015; Meuthen et al. 2016). A high-risk environment represents a setting in which an individual's vulnerability to threat is high due to the combination of some intrinsic traits of the prey (small size, slow escape, defenceless, exposed, etc.) and some extrinsic traits from the habitat (high predator density, high predator diversity, etc.). Thus, if a prey encounters a novel cue in high-risk environment, it might treat it as potentially risky, while the same cue encountered in a low-risk environment might be ignored. Indeed, the cost-to-benefit ratio should favour prey responding in an anti-predatory fashion to a novel cue in high-risk environments only, simply based on the probability of the cues mediating a threat vs a non-threatening stimulus. Empirical evidence has supported this view, showing prey from a variety of taxa displaying fear towards novel stimuli when they naturally experience high predation, or when they are experimentally exposed to elevated

---

[3] A version of Chapter 2 has been published. Feyten, LEA, Demers, EEM, Ramnarine, IW, Chivers, DP, Ferrari, MCO, and Brown, GE (2019) Who's where? Ecological uncertainty shapes neophobic predator avoidance in Trinidadian guppies. Behavioral Ecology and Sociobiology 73 (5): 1-10. DOI: 10.1007/s00265-019-2687-7.

[4] In this chapter, I again use the term "ecological uncertainty", rather than "uncertainty of predation risk" as described in my thesis introduction. As in Chapter 1, functionally I am referring to the same definition of the inability to predict the probability of the outcome of an event (e.g., predation), due to limitations in risk information.

background predation risk over several days. This includes juvenile convict cichlids (*Amatitlania nigrofasciata*; Brown et al. 2013; Joyce et al. 2016), woodfrog tadpoles (*Lithobates sylvaticus*; Brown et al. 2013), Trinidadian guppies (*Poecilia reticulata*; Brown et al. 2015), damselfish (*Pomacentrus chrysurus*; Chivers et al. 2014; Ferrari 2014), and fathead minnows (*Pimephales promelas*; Crane et al. 2015).

While numerous studies demonstrate that exposure to conditions of elevated predation risk is sufficient to induce an increased level of NPA, Ferrari et al. (2018) have argued that uncertainty of risk, rather than the level of risk *per se*, may function as a key driver for the expression and maintenance of this neophobic phenotype. Uncertainty in local risk is likely influenced by a variety of non-mutually exclusive ecological factors. For example, Ferrari et al. (2018) demonstrated that woodfrog tadpoles exposed to conspecific alarm cues paired with the same novel predator odour over a three-day period were less likely to exhibit induced NPA compared to conspecifics exposed alarm cue paired with different novel predator cues daily over the same three-day period. Both groups received the same amount of risk (i.e., exposure to alarm cues to the same schedule), but the groups with higher uncertainty (a new predator everyday) showed greater NPA.

Here, we conducted a pair of laboratory studies, using wild caught Trinidadian guppies, to test the hypothesis that 'uncertainty' in the predation environment shapes the maintenance of neophobic responses. First, we exposed shoals of guppies from a high-predation and neophobic (Brown et al. 2018) population to 1, 2, or 3 novel chemosensory cues for 3 days and tested for the maintenance of neophobic predator avoidance to one of the cues (Experiment 1a) or to a new novel odour (Experiment 1b). It has been shown that NPA to a novel cue in our focal population wanes over 5 consecutive exposures, therefore any presence of NPA after conditioning was considered 'retention' (Brown et al. 2015). Our logic in this experiment follows that of Ferrari et al. (2018), whereby exposure to a wider diversity of novel cues should be indicative of increasing uncertainty in the nature of the threats in the environment, resulting in the maintenance of NPA. In a second study, we tested the effect of spatial uncertainty on the maintenance of neophobic responses. We repeatedly exposed shoals of guppies to a novel cue in a predictable vs. unpredictable location in the conditioning environment. Spatial unpredictability should also increase the uncertainty of risk experienced by prey, since prey able to predict the spatial distribution of risk should be more certain about risk in their environment, and as such, should not maintain NPA (Campos and Fedigan 2014).

## 2.2 Methods

Six hundred twenty adult female guppies (mean ± SD standard length = 23.90 ± 2.1 mm) were collected between 19 April and 1 May 2016 (Experiment 1a, 2) and between 21 and 29 April 2017 (Experiment 1b) from the Lopinot River, Northern Range Mountains, Trinidad, using a 3-mm mesh seine net. Female guppies are morphologically and behaviourally sexually dimorphic from males (Magurran 2005), so only females were selected for experimentation. The Lopinot River is a high-predation stream, with a diverse predator guild including pike cichlids (*Crenicichla* sp.), blue acara (*Andinocara pulcher*), brown coscarob (*Cichlasoma taenia*) and wolf fish (*Hoplias malabaricus*). Previous research has shown that guppies collected from this population exhibit NPA (Brown et al. 2018). Guppies were transported to the University of the West Indies, Saint Augustine, Trinidad, and housed in one of two 275-L glass aquaria at a density of approximately 200 fish per tank. These holding tanks were kept at ~26°C, under a 12:12-h light:dark cycle and guppies were fed twice daily with commercial flake food and freeze-dried tubifex. The holding tanks were bare, save for two box filters. Guppies were conditioned and tested only once for all our experiments, and then returned to the river system.

We generated four truly novel cues ('odours') using commercially available lemon, almond, aniseed and cherry food additives (Blanche's Essence$^{TM}$). In addition to ensuring that the wild-caught guppies would not have been inadvertently exposed to our 'novel' predator odours in the past, the use of artificial flavorings also allowed us to ensure comparable relative concentrations across treatments (Brown et al. 2013; Brown et al. 2018). For each of the odours, we diluted 1 mL of lemon, almond, aniseed or cherry flavouring into 100 mL of dechlorinated water. At these concentrations, there were no discernable colour differences between the novel odours. Novel odours were prepared fresh, as needed. Artificial cues also allow us to avoid the possibility that our results could be confounded by generalized recognition of cues.

### *2.2.1 Experiment 1*

In Experiment 1A, we tested whether pre-exposure to single vs multiple novel cues would alter the maintenance of NPA. We predicted that environments with a higher diversity of novel odours should represent a more uncertain habitat and as such, guppies should maintain their neophobic phenotype. Within 3 days of capture, we transferred shoals of 24 guppies to each of 3 bare conditioning tanks and allowed a 16-h acclimation period. Conditioning tanks consisted of 47-L glass aquaria, filled with 30 L of dechlorinated tap water. Tanks were continuously filtered and kept with similar conditions as the holding tanks. We conditioned guppies by exposing shoals to novel cues or a water control at ~ 0900, 1200, and 1500 for three consecutive days. Shoals were exposed to one, two or three novel chemosensory cues per day. The 'one-cue' group received one injection of 10 mL of lemon odour at one time, and two additional injections of 10 mL of distilled water at the other two exposure times. The 'two-cue' group received 10 mL of lemon odour at one time, 10 mL of almond odour at another time, and 10 mL of distilled water at another time. The 'three-cue' group received an injection of 10 mL of lemon, almond, or aniseed odour at each of the exposure times. The order of exposure to stimuli was randomized each day, with the proviso that the order could not be the same as the previous day. Our experimental design ensured that if a novel cue was experienced, it was experienced 3 times. Each conditioning tank yielded 8 test shoals (4 experimental and 4 control; see below). We replicated the conditioning phase a total of three times, never reusing guppies, yielding a final sample size of n = 12 shoals per treatment combination, totalling 72 shoals (See Appendix A Table S2.1, Figure S2.1).

Following conditioning, shoals of three guppies (haphazardly selected from the conditioning tanks) were transferred to one of 20 individual testing tanks and allowed to acclimate for at least 1 h. Test tanks consisted of 20-L glass aquaria, filled with 18 L of dechlorinated water (~26°C), aerated, but not filtered, containing a single airstone anchored to the back wall of the tank. We attached an additional length of airline tubing (1.5 m), terminating immediately above the airstone to allow for the introduction of stimuli. After an experimental trial, the test tanks were drained and refilled, and water was flushed through the tubing to remove traces of any stimulus.

Behavioural observations consisted of a 5 min pre-stimulus and a 5 min post-stimulus observation period, separated by a 30-s stimulus injection period, during which we injected 10 mL of lemon odour (experimental cue) or distilled water (control). We used lemon odour as our primary novel cue since previous studies have shown that guppies from high-predation (but not low-predation) populations perceive and respond to this cue (Brown et al. 2013; Brown et al. 2015). Moreover, Brown et al. (2018) have shown that guppies from the Lopinot River do show consistent NPA responses to lemon odour. During both the pre- and post-stimulus observation periods, we recorded an index of shoaling and vertical area use every 15 s. Shoaling index scores ranged between 1 (no fish within one body length of another) to 3 (all fish within one body length of each other). Area use scores were recorded as the position of each guppy within the tank (1 = bottom third; 3 = top third), aided by horizontal lines drawn on the exterior of the tank. Area use scores ranged from 3 (all fish near the bottom) to 9 (all fish near the water surface). The observer was blind to the conditioning treatment and the order of testing was randomized throughout the experiment.

In a second series of trials (Exp. 1B), we repeated the conditioning treatments as described above on newly collected guppies, and tested for their response to a novel cue to which the focal guppies had no experience (i.e., a new novel odour). We used cherry odour, prepared as described above for lemon, almond and aniseed odours. Guppies conditioned with one of three treatments were tested for the response to either cherry odour or a water control. Aside from changing the novel test cue from lemon odour to cherry odour, the experimental set up and design was identical to Experiment 1A. We conducted a total of 10 - 12 focal shoals per treatment combination (total of 68 shoals; See Appendix A Table S2.1, Figure S2.1). Four shoals had to be deleted from the final analysis due to one or more guppies jumping from test tanks.

### *2.2.1.1 Statistical Analysis*

For each experiment, we calculated the mean pre- and post-stimulus shoaling index and area use scores for each replicate and used the change in each (post – pre) as dependent variables in all subsequent analyses. We tested for the effects of conditioning treatments (1, 2, or 3 novel cues) and stimulus (1A: lemon odour vs. water; 1B: cherry odour vs. water) on the change in shoaling index and area use using a nested ANOVAs for each behavioural measure. Data met the assumptions of normality and heteroscedasticity. We used ‘conditioning tanks’ as a nested factor to account of the dependency of fish housed in the same tank during the exposure phase, making ‘tank’ not ‘test shoal’ our level of replication for the conditioning treatment. In the event of significant main effects (or an interaction), we tested for the effect of stimulus at each level of the conditioning treatment using independent sample *t* tests (assuming unequal variance). We employed a Bonferroni correction to account for inflated alphas. Statistical analyses for all experiments were conducted using SPSS v24.0.

### *2.2.3 Experiment 2*

Here, we tested whether spatial predictability of a novel cue would affect the maintenance of NPA. Within 3 days of capture, we transferred shoals of 30 female guppies into one of three 1.5 m diameter opaque fiberglass conditioning tanks, filled to a depth of 30 cm. We placed artificial vegetation in eight, evenly spaced positions 15 cm from the outer rim of the tank, creating visual landmarks for stimulus delivery and shelter for guppies. Conditioning tanks were exposed to a 12:12-h light:dark cycle and maintained at 26 – 28°C. Tanks were aerated but not filtered. After allowing the fish to acclimate ~ 16 h, we introduced 20 mL of lemon odour (in two 10-mL injections at 30 s intervals) four times over the course of an 8-h period (~ 0800, 1000, 1200, and 1400). In order to minimize disturbance created by experimenters, cues were injected from the sides of the tank using a 10-mL syringe connected to ~ 1 m of tubing. The 'predictable' conditioning treatment received the stimulus in a fixed location. The 'unpredictable' conditioning treatment received 2, 10-mL injections of lemon odour at randomly assigned locations. Each subsequent exposure was also randomized. A final conditioning treatment received no exposures (control). We opted for a 'no treatment' control, as opposed to a procedural control such as predictable and unpredictable water, in order to keep the number of guppies tested low. The three conditioning procedures were replicated to generate a final sample size of 10 shoals per conditioning treatment and test stimulus combination (total of 60 shoals; See Appendix A Table S2.2, Figure S2.1).

On the following morning, guppies were placed in shoals of three, in testing tanks and given one h to acclimate. Testing tanks and procedures were identical to the ones described for Experiment 1A. Testing tanks were located in a different room than the conditioning tanks, preventing guppies from using external landmarks to influence their behavioural responses during testing. That is, a specific location where a novel cue may have been introduced in the conditioning tanks did not correspond to any location within the test tanks. Data met the assumptions of normality and heteroscedasticity. Thus the data was analyzed as described for Experiment 1, testing the effects of conditioning treatments (predictable, unpredictable, or control) and stimulus (lemon odour vs. water) on the change in shoaling index and area use. *Post hoc* comparisons were also conducted as described for Experiment 1.

## 2.3 Results

### *2.3.1 Experiment 1A*

Our nested ANOVA revealed no effect of conditioning tank on either change in shoaling index ($F_{6,60} = 0.38$, $p = 0.89$) or change in area use ($F_{6,60} = 1.43$, $p = 0.22$), indicating that response patterns did not differ among rounds of conditioning. For the observed change in shoaling index, we found significant effects of test stimulus (increased shoaling when exposed to lemon odour vs. water control; $F_{1,60} = 34.71$, $p < 0.001$) and conditioning treatment ($F_{2,6} = 9.71$, $p = 0.013$; Figure 2.1A). There was a marginally non-significant treatment x stimulus interaction (F2,60 = 2.53, P = 0.09). For the change in area use, we found a significant effect of test stimulus (decrease in area use when exposed to lemon odour vs. water control; $F_{1,60} = 19.49$, $p < 0.001$; Figure 2.1B), but no significant effect of conditioning treatment ($F_{2,6} = 2.52$, $p = 0.16$), nor a conditioning treatment × stimulus interaction ($F_{2,60} = 0.81$, $p = 0.45$). *Post hoc* comparisons revealed that guppies pre-exposed to a single novel odour did not differ in their response to lemon odour vs. distilled water for either change in shoaling index (Figure 2.1A) or area use (Figure 2.1B; Table 2.1). However, guppies pre-exposed to the 'two-cue' or 'three-cue' novel odour treatments did exhibit significant neophobic responses to lemon odour vs. distilled water (Figure 2.1, Table 2.1).

### *2.3.2 Experiment 1B*

In the second set of trials, we found no significant effect of conditioning tanks for either change in shoaling index ($F_{6,56} = 1.31$, $p = 0.27$) or area use ($F_{6,56} = 1.11$, $p = 0.37$). We did find significant effects of test stimulus (cherry odour vs. water control), where the response was greater to cherry odour than the water control for both the change in shoaling index ($F_{1,56.23} = 25.92$, $p < 0.001$) and area use ($F_{1,56.23} = 18.39$, $p < 0.001$; Figure 2.2A and 2.2B). However, we found no effects of conditioning treatment (shoaling index: $F_{2,5.91} = 2.57$, $p = 0.16$; area use: $F_{2,5.89} = 1.11$, $p = 0.39$), nor a conditioning treatment × stimulus interaction for either behavioural measure (shoaling index: $F_{2,56.24} = 0.27$, $p = 0.76$; area use: $F_{2,56.20} = 0.82$, $p = 0.45$; Figures 2.2A and 2B). Regardless of the conditioning treatment, guppies exhibited significant increases in shoaling index towards a previously undetected novel odour, compared to a water control (Table 2.2). While the reductions in area use for guppies pre-exposed to the 'one-cue' or 'two-cue' treatments were not statistically significant, they were in the predicted direction. Combined, these results suggest strong neophobic responses to a previously undetected novel odour, regardless of pre-exposure treatment.

### *2.3.3 Experiment 2*

We found no significant effect of conditioning tank for either behavioural measure (shoaling index: $F_{3,51} = 1.67$, $p = 0.19$; area use: $F_{3,51} = 0.65$, $p = 0.59$). We found a significant effect of stimulus type (lemon odour vs. water control) on the change in shoaling index (increase in shoaling index when exposed to lemon odour vs. water control $F_{1,51} = 23.57$, $p < 0.001$; Figure 2.3A). While we found no effect of conditioning treatment on the change in shoaling index ($F_{2,3} = 2.29$, $p = 0.25$), we did find a significant conditioning treatment × stimulus interaction ($F_{2,51} = 4.67$, $p = 0.014$; Figure 2.3A). For the change in area use, we found significant effects of stimulus type (decrease in area use when exposed to lemon odour vs water control; $F_{1,51} = 12.36$, $p = 0.001$) and conditioning treatment (decrease in area use when exposed to unpredictable or control treatments compared to predictable treatment; $F_{2,3} = 9.72$, $p = 0.049$; Figure 3B). There was no significant interaction term ($F_{3,51} = 0.65$, $p = 0.56$). As in Experiment 1A, *post hoc* comparisons revealed that guppies pre-exposed to the predictable risk treatment did not differ in their response to lemon odour vs. distilled water for either change in shoaling index (Figure 2.3A) or area use (Figure 2.3B; Table 2.3). However, guppies pre-exposed to the unpredictable risk or control treatments did exhibit significant neophobic responses to lemon odour vs. distilled water (Figure 2.3, Table 2.3).

## 2.4 Discussion

Combined, our results support the hypothesis that uncertainty of risk associated with the exposure to novel chemosensory cues shapes the maintenance of NPA among wild-caught Trinidadian guppies. Consistent with previous reports (Brown et al. 2015; Brown et al. 2018), exposure to lemon odour alone over a 3-day period resulted in lower neophobic response to lemon odour (Exp. 1A), but not to a cue that they had not experienced (Exp. 1B). This indicates that changes in a neophobic response to a particular cue is not generalized to all novel cues. Thus, guppies maintained their neophobic responses, but could learn that a particular cue (lemon odour) was not associated with risk after repeated exposures to that cue. Guppies exposed to multiple odours, however, maintained their response to lemon odour (Exp. 1A), despite having the same amount of exposures to lemon odour as the previous treatment. Thus, guppies appear unable to learn that a particular cue (lemon odour) does not represent predation risk when it is paired with other novel cues. Our second experiment showed a similar finding; guppies exposed to a novel cue in a spatially unpredictable fashion maintained their response, while the response among guppies exposed in a spatially predictable fashion was extinguished. Combined, our results support the prediction that uncertainty of risk shapes the maintenance of NPA responses in wild-caught Trinidadian guppies.

The persistence of NPA to specific cues among prey populations is dependent upon the presence or absence of reinforcements (i.e., presence vs. absence of an actual threat). Indeed, recent studies have shown that prey will rapidly lose the response to specific novel cues if detected in the absence of an ecologically relevant threat (Kaplan and Lubow 2001; Gonzalo et al. 2010). Previous studies on guppies have shown that when unreinforced, NPA lasts between 3 to 4 exposures before responses begin to wane (Brown et al. 2015; Brown et al. 2018). In effect, prey can learn that the cue is non-risky in a process akin to habituation (Rankin et al. 2009). However, when a neophobic response is paired with an actual predation event, the response is reinforced, akin to the well-documented mechanisms of acquired predator recognition learning (Mathis and Smith 1993; Eiben and Persons 2007; Brown et al. 2015; Mitchell et al. 2016). These observations are consistent with the model that NPA allows prey to reduce their acute risks of predation while still having sufficient behavioural plasticity to respond to variable or unpredictable predation risks (Brown et al. 2013; Mettke-Hofmann 2014).

With our experimental design, we have attempted to disentangle risk from the uncertainty of risk, since the novel cues themselves cannot convey predation risk. The guppies tested here were exposed to novel chemosensory cues, therefore they had no prior opportunity to learn specific information regarding risk using these cues (*sensu* Ferrari et al. 2018). Instead, a novel cue could represent information regarding the environment, such as a foraging opportunity. However, in the presence of consistent high levels of risk (as is present in the Lopinot River), novel cues should act as sources of information conveying uncertainty of risk (Feyten and Brown 2018). Since prey do not have experience with these novel cues, they cannot use these cues to assess specific risk per se, and thus are not gaining information regarding local risks. Therefore, our experiments have manipulated uncertainty of risk rather than risk itself.

Uncertainty in local predation risks is based on the likelihood that the cue (or cues) detected by prey accurately represents an acute threat (Dall and Johnstone 2002; Ferrari et al. 2018). Prey experienced with high and/or unpredictable predation risks may indeed gain increased survival benefits from increased NPA (Ferrari, McCormick, et al. 2015). However, factors such as temporal and spatial variability in risk, social context and habitat variability may alter the reliability of risk assessment information. Given the asymmetry of costs associating with responding to novel potential predator cues (missed foraging/mating opportunity vs. death), higher uncertainty of risk should lead to increased caution among prey. Recently, Ferrari et al. (2018) demonstrated that uncertainty in the exposure to risk shapes the strength of induced NPA. Predator naïve woodfrog tadpoles were exposed to elevated risk (injured conspecifics) daily over a four-day period. When exposed to elevated risk alone or elevated risk paired with a different novel predator each day, tadpoles exhibited strong neophobic responses. However, when the high-risk cues were paired with the same predator each day, tadpoles showed no evidence of induced neophobia. These findings, which suggest that uncertainty in exposure to novel cues following the inducement of neophobia shapes the maintenance of this phenotype, are consistent with our current results. Combined, they support the hypothesis that uncertainty of risk shapes both the strength and retention of phenotypically plastic NPA.

It is important to note that while our research highlights the role of uncertainty in driving NPA, we have only tested this under conditions of high predation risk. That is, when facing novel cues, these cues potentially indicate risk to prey (perceived risk; Bouskila and Blumstein 1992). Thus, we cannot say that uncertainty drives neophobic responses in other low-risk contexts. An interesting direction of future research would be to determine if uncertainty (i.e., unpredictability) of information could potentially result in neophobic responses in both high- and low-risk environments. However, we predict this is not likely since eliciting NPA would be costly to prey in low-risk environments; it is likely an error to engage in antipredator behaviours as opposed to other fitness-enhancing behaviours in low-risk environments (Johnson et al. 2013).

Likewise, the changes in response could be due to differing cognitive processes. For example, in Experiment 1, manipulating the number of novel cues experienced could be indicative of how community composition changes might impact neophobic responses in prey species. Examining this question complements the recent results by Deacon et al. (2018) showing that Trinidadian guppies exist on a continuum of predation risk, with varying community composition. Meanwhile, Experiment 2 might reflect how variation in spatial information impacts neophobia. This complements work on vigilance and antipredator behaviours of capuchin monkeys (*Cebus capucinus*), which demonstrated that the monkeys alter their antipredator behaviours depending on the spatial fluctuations of perceived risk (Campos and Fedigan 2014). These differing contexts of uncertainty (i.e., community composition, spatial predictability of risk) could engage a variety of cognitive processes, which produce neophobic responses in prey species.

One potential mechanism for the retention of NPA in the ‘two-cue’ or ‘three-cue’ treatments of Experiment 1A could be that when experiencing multiple novel cues, prey may be unable to learn that one of those odours does not represent an actual threat. This kind of multi-cue interference has been documented to some extent in tadpoles of the edible frog, *Pelophylax esculentus*, where a diversity of novel predator odours may constrain predator recognition learning (Lucon-Xiccato et al. 2018). If this was the case in our experiment, it is likely that this interference mechanism is also at play in natural settings, where guppies may be unable to learn “safety” when experiencing multiple novel cues. Alternatively, during conditioning, exposure to novel odours may have resulted in guppies experiencing differential levels of stress across the pre-exposure treatments. This could, in turn, further shape their behavioural response (Archard et al. 2012; Clinchy et al. 2013). However, it has been argued that depending on the predictability of risk, changes in anti-predator behaviour (e.g., vigilance) and the physiological correlates of fear (e.g., heart rate, hormone or glucocorticoid levels) are not always coupled (Beauchamp 2017). This decoupling could be related to the uncertainty of the environment, and whether or not a cue is novel (Feyten and Brown 2018). Prey experiencing novel cues in predictable environments may not express vigilance, nor have increased physiological correlates of fear (stress). However, as environments become more unpredictable, it is likely that vigilance and stress increase (Feyten and Brown 2018). Thus, although stress may have differed between treatments, physiological stress is likely linked to the uncertainty of the environment. Regardless of the mechanism, the functional result is that guppies express NPA to a particular cue after experiencing it with other novel cues (in a diverse setting), but not when they have experienced that novel cue repeatedly alone (a low diversity setting). A fruitful area of future research would be to further examine the specific cognitive processes and mechanisms that might be involved with NPA as a result of ecological uncertainty.

The combined effects of climate change, invasive species, and anthropogenic habitat modifications are expected to increase the degree of uncertainty experienced by prey populations. As such, understanding how prey deal with ever increasing uncertainty is becoming a pressing challenge for ecologists. Previous definitions of uncertainty have relied on difficult to quantify concepts such as the degree of information available to prey (Dall et al. 2005) or the asymmetry of costs (Johnson et al. 2013; Ferrari et al. 2018). Our current results point to some potentially ecologically relevant factors that shape the uncertainty of risk experienced by prey. Additional factors such as the temporal predictability, variance in the level of acute risk, and microhabitat complexity are expected to likewise impact uncertainty. Likewise, Lee et al. (2016) suggest that the patterns of correlation between ecologically relevant factors can also influence uncertainty, independent of their individual effects. Alone or in combination, such factors could provide a powerful framework in which researchers could directly quantify ‘uncertainty’.

## 2.5 Figures & Tables

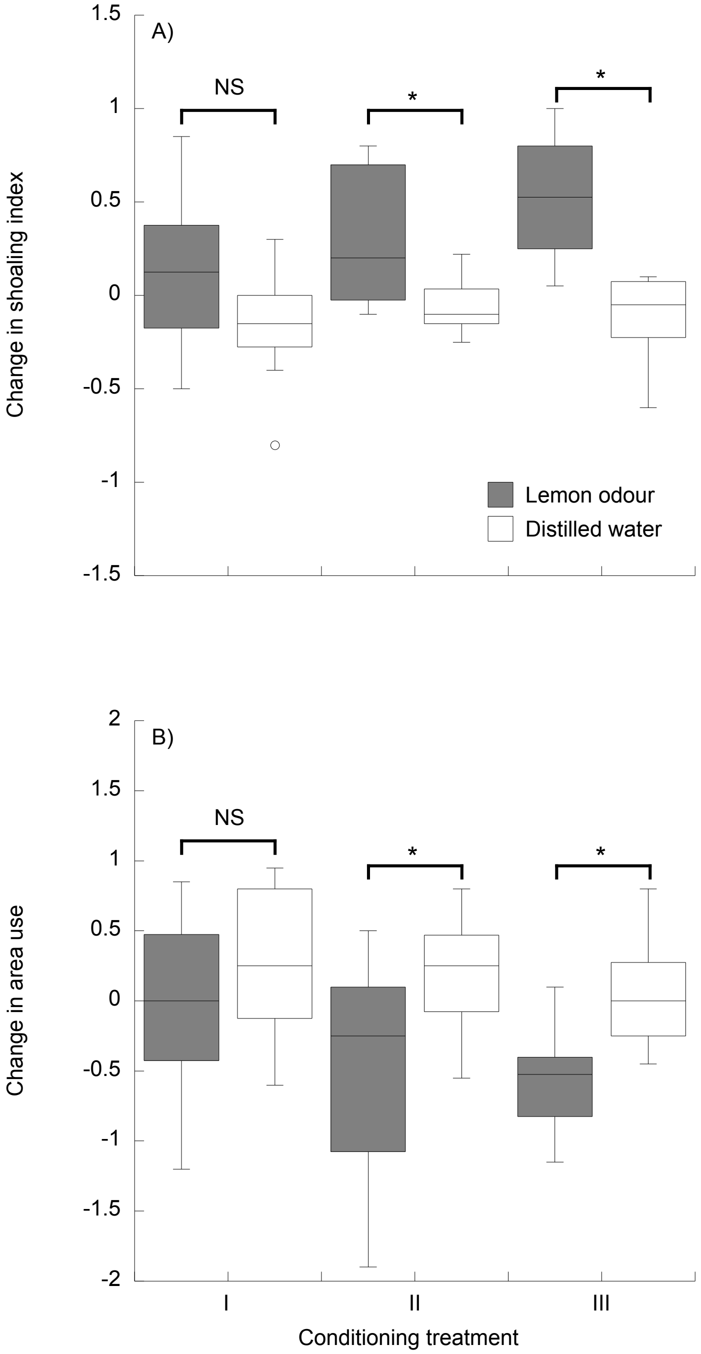


**Figure 2.1:** Change in shoaling index (A) and area use (B) for shoals of guppies conditioned to a single (I), two (II), or three novel odours per day (III) and tested for the response to lemon odour (shaded boxes) or distilled water control (open boxes). n = 12 per treatment combination. Significant *post hoc* comparison-based independent sample *t* tests are denoted as NS (non-significant; $p > 0.05$) or an asterisk ($p < 0.05$). An increase in shoaling index and a decrease in area use are indicative of a predator avoidance response. Box plots show median (line in box), 10th and 90th percentile (whiskers), and outliers (open circles).

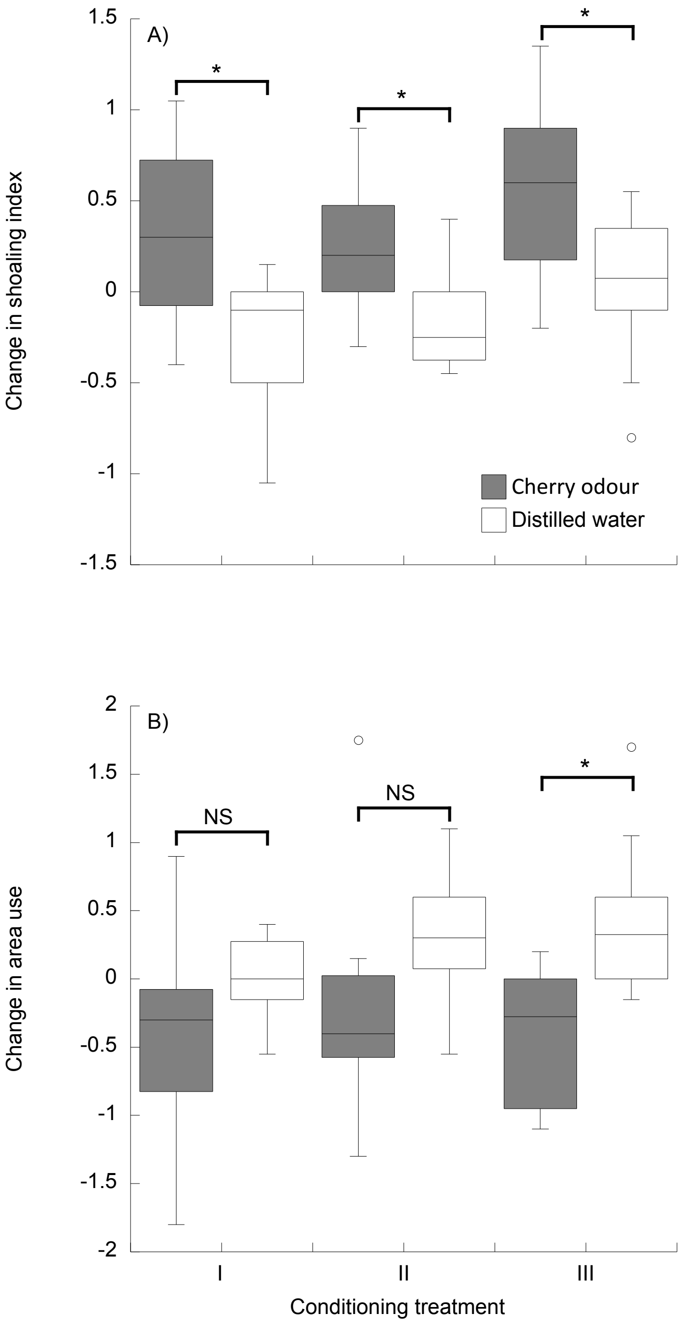


**Figure 2.2:** Change in shoaling index (A) and area use (B) for shoals of guppies conditioned to a single (I), two (II), or three novel odours per day (III) and tested for the response to cherry odour (shaded boxes) or distilled water control (open boxes). n = 10 - 12 per treatment combination. Significant *post hoc* comparison-based independent sample *t* tests are denoted as NS (non-significant; $p > 0.05$) or an asterisk ($p < 0.05$). An increase in shoaling index and a decrease in area use are indicative of a predator avoidance response. Box plots show median (line in box), 10th and 90th percentile (whiskers), and outliers (open circles).

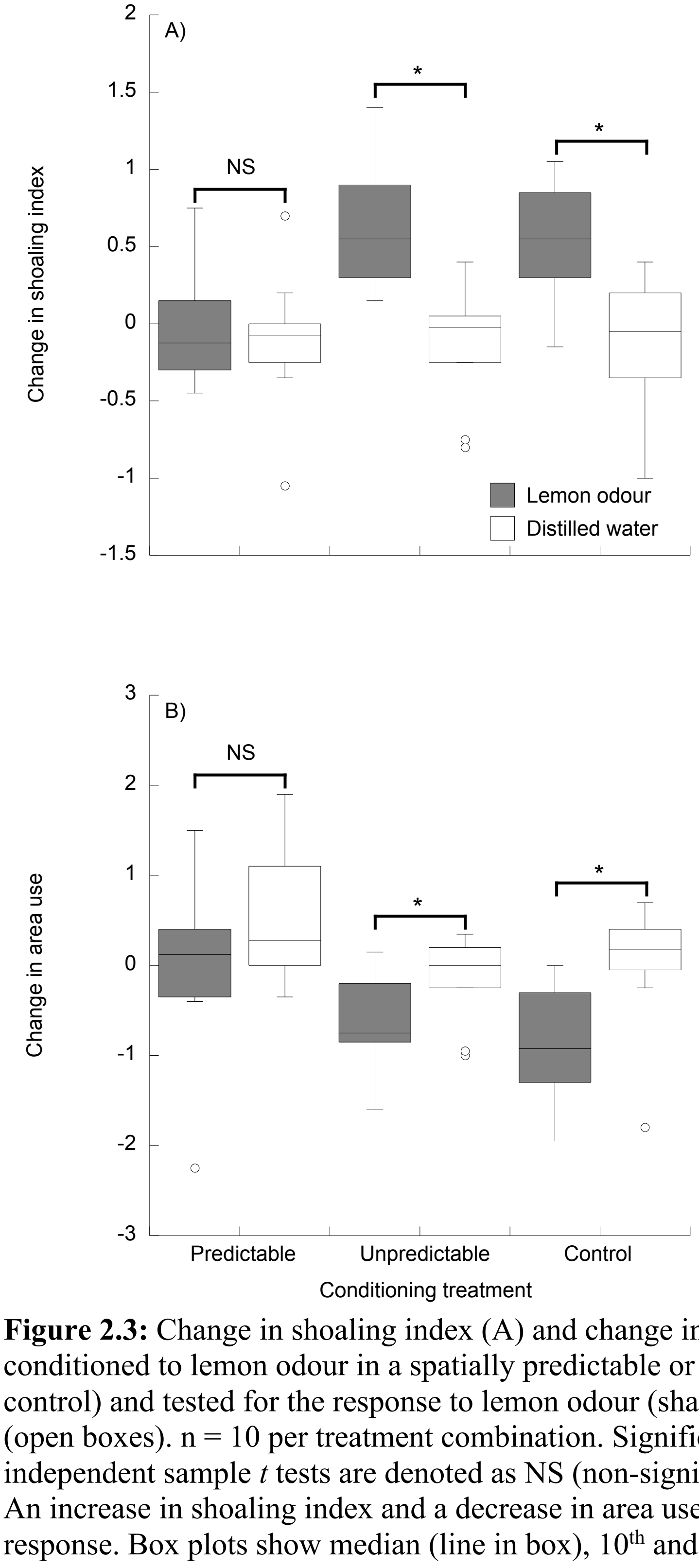


**Figure 2.3:** Change in shoaling index (A) and change in area use (B) for shoals of guppies conditioned to lemon odour in a spatially predictable or unpredictable fashion (vs. an unexposed control) and tested for the response to lemon odour (shaded boxes) or a distilled water control (open boxes). n = 10 per treatment combination. Significant *post hoc* comparison-based independent sample *t* tests are denoted as NS (non-significant; $p > 0.05$) or an asterisk ($p < 0.05$). An increase in shoaling index and a decrease in area use are indicative of a predator avoidance response. Box plots show median (line in box), 10th and 90th percentile (whiskers), and outliers (open circles).

**Table 2.1:** Results of *post hoc* comparisons of the response to lemon odour (experimental) vs. distilled water (control) for guppies pre-exposed to one (I), two (II), or three (III) novel odours in Experiment 1A. Probability calculated based on independent sample *t* tests (unequal variance). NS denotes no significant difference following Bonferroni Correction.

| Treatment | *df* | *Mean difference (± SE)* | *95% CI* | *p* |
|---|---|---|---|---|
| I | | | | |
| Shoaling index | 19.82 | 0.26 (0.14) | -.029, 0.55 | NS |
| Area use | 21.16 | -0.33 (0.24) | -0.83, 0.16 | NS |
| II | | | | |
| Shoaling index | 14.30 | 0.37 (0.11) | 0.12, 0.61 | < 0.05 |
| Area use | 16.50 | -0.69 (0.25) | -1.22, -0.15 | < 0.05 |
| III | | | | |
| Shoaling index | 19.87 | 0.64 (0.11) | 0.42, 0.87 | < 0.05 |
| Area use | 21.65 | -0.65 (0.16) | -0.98, -0.31 | < 0.05 |

**Table 2.2:** Results of *post hoc* comparisons of the response to cherry odour (experimental) vs. distilled water (control) for guppies pre-exposed to one (I), two (II), or three (III) novel odours in Experiment 1B. Probability calculated based on independent sample *t* tests (unequal variance). NS denotes no significant difference following Bonferroni Correction.

| Treatment | *df* | *Mean difference (± SE)* | *95% CI* | *p* |
|---|---|---|---|---|
| I | | | | |
| Shoaling index | 20.71 | -0.57 (0.17) | -0.94, -0.20 | < 0.05 |
| Area use | 15.69 | 0.41 (0.21) | -0.05, 0.87 | NS |
| II | | | | |
| Shoaling index | 18.06 | -0.40 (0.14) | -0.70, -0.09 | < 0.05 |
| Area use | 16.67 | 0.53 (0.27) | -0.03, 1.10 | NS |
| III | | | | |
| Shoaling index | 19.95 | -0.53 (0.19) | -0.93, -0.13 | < 0.05 |
| Area use | 17.87 | 0.83 (0.23) | 0.35, 1.34 | < 0.05 |

**Table 2.3:** Results of *post hoc* comparisons of the response to lemon odour (experimental) vs. distilled water (control) for guppies pre-exposed to predictable or unpredictable presentations of lemon odour or an unexposed control. Probability calculated based on independent sample *t* tests (unequal variance). NS denotes no significant difference following Bonferroni Correction.

| Treatment | *df* | *Mean difference (± SE)* | *95% CI* | *p* |
|---|---|---|---|---|
| Predictable | | | | |
| Shoaling index | 17.41 | -0.60 (09.18) | -0.44, 0.32 | NS |
| Area use | 16.97 | 0.54 (0.39) | -0.28, 1.36 | NS |
| Unpredictable | | | | |
| Shoaling index | 17.95 | -0.77 (0.17) | -1.13, -0.41 | < 0.05 |
| Area use | 17.86 | 0.49 (0.23) | 0.01, 0.96 | < 0.05 |
| Control | | | | |
| Shoaling index | 17.69 | -0.65 (0.18) | -1.03, -0.26 | < 0.05 |
| Area use | 17.56 | 0.88 (0.29) | 0.26, 1.49 | < 0.05 |

## IV. Chapter 3: *How does simultaneous social and non-social information of mixed reliability affect NPA?*[5]

**Preface**: In the previous two chapters, I examined how the reception of multiple cues (that may differ in their reliability based on prey personal knowledge) can influence uncertainty and shape neophobic responses (*Box 1*). I assessed how NPA is shaped by i) the reliability and "completeness" of risk information (i.e., the simultaneous reception of multimodal cues of the same or mixed reliability), and ii) the diversity and spatial predictability of successively detected unknown cues. In this chapter, I assess how prey experience with conflicting information from social and non-social sources may shape neophobia. I examine how the simultaneous reception of social safety cues and non-social risk cues (of varying reliability) shapes NPA.

### 3.1 Introduction

When encountering environmental cues, animals face decisions about how to respond behaviourally (Lima and Dill 1990). Whether they perceive the cue as a reliable indicator of an event such as a foraging opportunity or a predation threat depends on their eco-evolutionary experience with that cue (i.e., personal knowledge; Wagner and Danchin 2010; Carthey and Blumstein 2018). A cue is 'known' when remembered from a previous encounter or when recognized innately based on evolutionary history (Carthey and Blumstein 2018; Feyten and Brown 2018; Crane et al. 2020). Known cues are reliable since they have been consistently correlated with a specific event, and knowledge of such reliability facilitates optimal decisions across varying ecological contexts (Lima and Dill 1990; Jacobs 2002; Koops 2004; Feyten and Brown 2018). In contrast, 'unknown' cues (i.e., novel cues) are, by nature, unreliable, having not yet been paired consistently with an event (Jacobs 2002; Searcy and Nowicki 2005; Feyten and Brown 2018). Thus, animals face uncertain decisions when encountering novel cues, and may need to rely upon their personal experiences with prior novelty. For example, if most novel cues have been non-threatening (e.g., indicated food sources) in the past, a new novel cue is also likely to be non-threatening (i.e., unlikely to represent risk). However, individuals face higher uncertainty of predation risk when prior novel cues have often represented opposing situations. Such a scenario may exist for prey living in high-predation environments (Table 3.1), where they have presumably experienced many novel cues in both non-threatening and risky contexts (e.g., exposure to a diversity of new predator and nonpredator species). In such contexts novel cues convey acute threats more often, and less predictably, than in low-predation contexts where novel cues are typically non-threatening (i.e., have a more predictable and lower probability of indicating risk). As such, novel cues result in greater uncertainty of risk in high-predation sites compared to low-predation sites.

Animals can reduce uncertainty by gathering additional information (Sih 1992; Dall and Johnstone 2002; Dall et al. 2005; Schmidt et al. 2010; Wagner and Danchin 2010). While direct exposure to the cue source may provide the most accurate and up-to-date information, such

---

[5] A version of Chapter 3 has been published. Feyten, LEA, Crane, AL, Ramnarine, IW, and Brown, GE (2021) Predation risk shapes the use of conflicting personal risk and social safety information in guppies. Behavioral Ecology 32 (6): 1296-1305. DOI: 10.1093/beheco/arab096.

‘personal information’[6] (also termed non-social or private in the literature; Danchin et al. 2004; Webster and Laland 2008) can be costly, resulting in injury or death (Dall et al. 2005; Wagner and Danchin 2010; Crane and Ferrari 2013). In contrast, publically-available social information is more prone to copying errors (Giraldeau et al. 2002; Koops 2004; van Bergen et al. 2004; Webster and Laland 2008; Rieucau and Giraldeau 2011), but observing the behaviour of others potentially allows individuals to recognize threats without direct exposure (Boyd and Richerson 1988; Kendal et al. 2004; Dall et al. 2005; Ferrari et al. 2007; Wagner and Danchin 2010; Dubois et al. 2012). Integrating personal and social information allows animals to make optimal foraging, mate-choice, and anti-predator decisions (Dall et al. 2005; Schmidt et al. 2010; McLachlan et al. 2019). However, uncertainty of risk may increase, rather than decrease, when sources of information conflict. Animals must then decide which information source to use or how to integrate them. For example, a decision rule based on the timing of information acquisition (e.g., weighing more recent information more heavily) would be beneficial when older information has become outdated but not when conflicting information sources are simultaneously available. According to the costly information hypothesis, individuals should favour the acquisition of social information over personal information when the cost of acquiring personal information is relatively high, as in a high-predation environment (Boyd and Richerson 1988; Kendal et al. 2004; Webster and Laland 2008). Although several studies have explored conflicting personal and social information in the context of foraging and mate choice decisions (Kendal et al. 2004; van Bergen et al. 2004; Webster and Laland 2008; Grüter et al. 2011; Trompf and Brown 2014; Heinen and Stephens 2016; Smolla et al. 2016; McLachlan et al. 2019), less is known about conflicting sources of predation-related information (i.e., information actively conveying risk or safety), where mistakes are relatively more costly (e.g., injury/mortality vs. missed opportunities). Here, we define safety cues as those which specifically indicate that current levels of risk are low (Luttbeg et al. 2020). On the other hand, risk can be thought of as the probability of a costly outcome, where predation risk cues indicate the likelihood of mortality in the near future due to a predator (Knight 1921; Dall 2010).

Error management theory posits that the cost of being wrong about perceived safety is much greater than the cost of being wrong about perceived danger, and thus animals should err on the side of caution when uncertain (Johnson et al. 2013). Indeed, an abundance of safety information is usually required to out-weigh the effect of a single experience with predation risk (Mineka and Cook 1986; Brown, Ferrari, Malka, et al. 2011; Mitchell et al. 2011; Crane and Ferrari 2016). In these studies, the information about risk and the information about safety were each acquired personally. However, a few studies have found that new personal information about risk does not override an abundance of prior personal information about safety, but new information about risk from a social source is more persuasive (Curio et al. 1978; Vieth et al. 1980; Mineka and Cook 1986; Crane and Ferrari 2015). Whether social information is always more influential when information sources conflict, or whether the social information must indicate risk to be persuasive, remains unclear. Teasing apart these scenarios requires a situation where personal risk information conflicts with social safety information, but we are not aware of any previous studies that have tested such.

---

[6] Although I term this “personal information” in this chapter, I use the term “non-social information” in the rest of the thesis to avoid confusion with other terms such as “personal knowledge” or “personal experience”.

In this study, we conducted *in situ* observations of Trinidadian guppies (*Poecilia reticulata*) in a scenario where personal risk information conflicted with social safety information, and within either a high- or low-predation environment. When guppies entered a foraging arena, they were presented with one of three chemical cues: (1) a known and reliable indicator or risk (damage-released alarm cues; Brown and Godin 1999; Brown, Ferrari, and Chivers 2011), (2) a novel cue (lemon odour), or (3) a control cue (stream water), in either the presence or absence of visual safety cues from conspecifics (Figure 3.1). Without the social safety cues, guppies could decide whether to approach and forage in a patch (the behavioural arena) based upon their personal experience with the chemical cue or their expectation (and error management) due to the level of predation risk in their environment. Guppies from both high- and low-predation sites consistently show innate fear responses toward alarm cues, whereas only guppies from the high-risk sites show fear toward novel cues (i.e., neophobia; Brown et al. 2013; Elvidge et al. 2016; Crane and Ferrari 2017; Feyten, Demers, Ramnarine, Chivers, et al. 2019). We predicted that both high- and low-risk guppies would ignore the social safety information when exposed to alarm cues, relying on their personal knowledge of this cue as a reliable indicator of risk. However, we predicted that the additional uncertainty associated with the novel cue in high-predation settings would cause increased reliance on the social safety information, thus resulting in decreased neophobia for high-predation guppies in the presence of social safety information.

## 3.2 Methods

### *Study sites and cues*

In March 2019, we conducted observations of guppies in the Lopinot and Upper Aripo Rivers in the Northern Range Mountains of Trinidad. The sites on the Lopinot are considered high-predation sites, containing a diversity of predators (e.g., blue acara *Andinocara pulcher*, pike cichlid *Crenicichla* sp., wolfish *Hoplias malabaricus*, and two-spot sardine *Astyanax bimaculatus*), whereas the sites on the Upper Aripo are characterized as low-predation sites, having no aquatic predators of adult guppies (Croft, Morrell, et al. 2006; Botham et al. 2008; Deacon et al. 2018). Previous studies have demonstrated that guppies from both high- and low-predation sites have increased latencies to enter and forage in a novel foraging arena in the presence of alarm cues compared to a stream water control (Elvidge et al. 2016). However, only high-predation site guppies have shown increased latencies in the presence of a novel chemical cue (lemon odour) compared to a stream water control (Elvidge et al. 2016). Despite being transmitted socially, ecologists have traditionally considered alarm cues as personal cues because, unlike true social cues, they cannot be controlled or modified by the emitter (Crane 2017).

We obtained alarm cues using whole body extracts from 62 donor guppies from both high- and low-predation sites (mean ± SD standard length = 22.35 ± 2.37 mm). We used a combined pool of donor guppies to account for population specific differences in alarm cue production (Brown et al. 2010). These individuals were first brought into the laboratory and euthanized via cervical dislocation in accordance with Concordia University Animal Research Ethics Protocol #AREC-3000255. The head and tail were removed and the remaining tissue was placed in 100 mL of chilled dechlorinated water. The tissue samples were then homogenized and filtered (through polyester floss) using dechlorinated water to dilute the product to a final concentration of ~0.10 $cm^2$ $mL^{-1}$ (Brown et al. 2013; Feyten, Demers, Ramnarine, and Brown 2019). We also obtained a novel cue by mixing 12 drops of Blanche's lemon essence into 600 mL of dechlorinated water (Brown et al. 2013; Feyten, Demers, Ramnarine, and Brown 2019).

### *3.2.1 Experiment 1: Conflicting personal risk and social safety*

We conducted field observations by gently placing a novel foraging arena (corrugated plastic; ~30×25×23 cm; See Figure 3.2) into stream pools at locations that visibly contained at least 5 guppies in locations without visible currents or higher velocities. The arena was partially submerged (~10 cm depth), and a rock was placed inside to prevent the arena from moving due to the stream current. We then added a small amount (~0.5 g) of food (OMEGA™ One Freshwater Flakes). For half of the trials, the social safety cue was present, consisting of 3 female guppies inside a clear 1-L chamber (~7×7×24 cm; Figures 3.2 and S3.1) filled with stream water and gravel (~3 cm depth). We collected these female guppies from 1-2 pools each day using seine nets, downstream of subsequent experiments. For shoaling species, like guppies, this presence of a conspecific shoal conveys safety (i.e., lower immediate risk) to isolated individuals via either the calm activity of a shoal, or because it provides a mechanism to reduce immediate predation risk (i.e., dilution effect; Lehtonen and Jaatinen 2016; Luttbeg et al. 2020). We used only female guppies in the stimulus shoals in order to reduce potential confounding effects due to courtship behaviours and intra-sexual aggression likely to occur within a mixed-sex stimulus shoal (Chuard et al. 2016). Additionally, an all-female stimulus shoal should serve as a safety cue for both *in situ* male and female guppies, which may not be the case for a mixed-sex stimulus shoal where the presence of males may impede *in situ* females from entering or foraging (Magurran and Seghers 1994; Croft, Morrell, et al. 2006) in the arena. We used each guppy stimulus shoal for approximately 2-3 replicates before we released them downstream, and replaced them with a new shoal. For the other half of trials, the chamber was filled with water and gravel but did not contain guppies. We began our trials by gently injecting 10 mL of one of the three personal cue treatments (alarm cue, novel cue, or stream water) through airline tubing on the back wall of the arena (See Figure S3.1) before flushing the tubing with 60 mL of stream water and beginning a 5-min observation period. We recorded the latency for guppies to enter the foraging zone (crossing from the approach zone into the foraging zone), as well as the latency to forage inside the foraging zone (Figure 3.2). These two latencies could correspond to two different guppies. After each trial, we moved at least 1 m upstream before initiating the next trial, which ensured we avoided re-testing guppies or testing downstream guppies potentially exposed to the experimental cues from previous trials. The cue treatments were randomly assigned, and we conducted a total of 90 trials per population (180 total), with a sample size of 15 per treatment group (Figure 3.1).

### *3.2.2 Experiment 2: Shoal behavioural assay*

Following Experiment 1, we conducted a behavioural assay to determine whether the movement activity of our experimental shoals differed between the high- and low-predation streams (30 trials from each population). From each stream, we collected 90 female guppies in total, moving 3 individuals at a time into 1-L chambers that were positioned on a flat surface along the stream bank. The chamber again contained stream water (0.75 L) and gravel, was surrounded by opaque walls on 3 sides to minimize external visual stimuli, and was demarcated into 4 quadrants by exterior lines (Figure S3.2). After a 1-min acclimation period, we tallied the total number of lines crossed by all 3 guppies over a 1-min observation period. We also recorded whether the line crosses were dashes (rapid bursts of movement with the entire body crossing the line in under 0.5

s) or were calmer movements that are typical of normal swimming behaviour (> 0.5 s). After each trial, we recorded the standard length (head to caudal peduncle) of each fish and released them at their site of capture.

### *3.2.3 Statistical Analyses*

Since our data from Experiment 1 did not meet parametric testing assumptions of homoscedasticity or normality of residuals, we conducted a 3-way ANOVA on transformed data (Aligned Ranks Transformation ANOVA, R package **ARTool**; Wobbrock et al. 2011; Feys 2016) for each response variable (latency to enter and latency to forage). We tested the effects of all fixed factors [population (high- or low-predation), the personal cue treatment (alarm cue, novel cue, or stream water), and the social treatment (shoal present or absent)] and their interactions. For *post hoc* tests, we first split the data by population, and conducted separate 2-way ANOVAs, and then split the data further by the social cue treatment for 1-way ANOVAs and Tukey *post hoc* comparisons. In order to assess whether guppy responses were affected by any external variables, we also conducted 1-way ANOVAs on the stream water control treatment groups. For data from Experiment 2, the line cross data failed to meet the parametric testing assumption of normality of residuals. We therefore compared the number of line crosses and the proportion of calm line crosses between populations using Mann-Whitney *U* tests. We calculated the average standard length for each testing shoal, and the resulting data met parametric assumptions and were analyzed with an independent sample *t* test. All analyses were conducted in RStudio 3.4.1 using $\alpha = 0.05$.

## **3.3 Results**

### *3.3.1 Experiment 1: Conflicting personal risk and social safety*

For latency to enter, the overall ANOVA revealed a significant 3-way interaction ($F_{2,168} = 5.89$, $p = 0.003$; Table 3.2a), revealing that responses depended on the type of personal cue (alarm cue, novel cue, or stream water control), the population, and the availability of social safety cues (Figure 3.3a,b). For low-predation guppies, we found a significant interaction between the personal cue and the social cue ($F_{2,84} = 4.36$, $p = 0.016$; Table 3.2b, Figure 3.3a), where in the absence of social safety, guppies showed typical alarm cue responses and were not neophobic ($F_{2,42} = 5.76$, $p = 0.006$; alarm cue vs. control: $p = 0.009$; novel cue vs. control: $p = 0.90$; Figure 3.3a, Table 3.3a,c). However, the presence of social safety actually caused these low-predation guppies to ignore the alarm cues ($F_{2,42} = 1.61$, $p = 0.21$; Figure 3.3a, Table 3.3a). For high-predation guppies, we also found a significant interaction between the personal and social cues ($F_{2,84} = 3.28$, $p = 0.043$; Table 3.2c; Figure 3.3b). In the absence of social safety, guppies showed typical responses to alarm cues and were neophobic ($F_{2,42} = 11.85$, $p < 0.0001$; alarm cue vs. control: $p = 0.0001$; novel cue vs. control: $p = 0.003$, Table 3.3b,e), whereas the presence of social safety did not weaken alarm cue responses but eliminated neophobia ($F_{2,42} = 7.70$, $p = 0.001$; alarm cue vs. control: $p = 0.002$; novel cue vs. control: $p = 0.67$; Figure 3.3b, Table 3.3b,d). In the presence of a stream water control groups, neither population nor social cue treatment affected latencies to enter (Table 3.6a).

For latency to forage, we found a pattern similar to that of latency to enter. Again, there was a significant 3-way interaction involving the type of personal cue, the population ambient risk, and the availability of social safety cues ($F_{2,168} = 3.16$, $p = 0.045$; Table 3.4a, Figure 3.3c,d). However, the pattern was slightly weaker than for latency to enter. For low-predation guppies, the interaction between the personal and social cues was marginally non-significant ($F_{2,84} = 2.44$, $p = 0.093$; Table 3.4b). However, markedly different response patterns between the treatment groups (e.g., Figure 3.4c) led us to proceed with *post hoc* analyses. In the absence of social safety, low-predation guppies showed typical alarm cue responses and were not neophobic ($F_{2,42} = 7.2454$, $p = 0.002$; alarm cue vs. control: $p = 0.008$; novel cue vs. control: $p = 0.97$; Figure 3.3c, Table 3.5a,c). However, the presence of social safety actually caused these low-predation guppies to ignore the alarm cues ($F_{2,42} = 2.09$, $p = 0.137$; Figure 3.3c, Table 3.5a). For high-predation guppies, we again found marginal interaction between the personal and social cue ($F_{2,84} = 2.47$, $p = 0.091$, Table 3.4c) and again, proceeded with *post hoc* analyses. In the absence of social safety, guppies showed typical responses to alarm cues and were neophobic ($F_{2,42} = 10.00$, $p < 0.001$; alarm cue vs. control: $p < 0.001$; novel cue vs. control: $p = 0.002$, Table 3.5b,e), whereas the presence of social safety did not weaken alarm cue responses but eliminated neophobia ($F_{2,42} = 3.95$, $p = 0.027$; alarm cue vs. control: $p = 0.029$; novel cue vs. control: $P = 0.84$; Figure 3.3d, Table 3.5b,d). In the presence of a stream water control, neither population nor social cue treatment affected latencies to forage (Table 3.6b).

### *3.3.2 Experiment 2: Shoal behavioural assay*

The populations did not differ in the number of lines crossed ($W = 378.5$, $P = 0.29$; Figure 3.4a). However, low-predation guppies spent a greater proportion of their activity in calmer movements compared to high-predation guppies ($W = 269.5$, $p = 0.007$; Figure 3.4b). Low-predation guppies also trended to have longer body length than high-predation guppies (mean ± SD = 23.27 ± 2.24 mm and 22.08 ± 2.37 mm, respectively), but this was not significant ($p = 0.055$, $df = 57.91$, $t = -1.96$).

## 3.4 Discussion

In this study, guppies faced conflicting information about predation risk and safety from different information sources (personal and social) within their natural high- and low-predation environments. When encountering a personal cue that was novel, high-predation guppies displayed neophobic responses unless social safety cues were available. Presumably, these guppies were highly uncertain about the novel cue due to prior novel cues inconsistently being threats (i.e., less predictable due to past experience indicating that many novel cues were dangerous while many others were safe). Although these guppies would typically err on the side of caution when uncertain about predation risk, the presence of social safety was convincing that this particular novel cue was not a threat. However, social safety was only influential to high-predation guppies when the personal information was unreliable, as these guppies ignored the social safety cue when encountering alarm cues, presumably because they were more certain of the risk that alarm cues posed. In contrast, low-predation guppies, as expected, did not show neophobia, as prior experience would have made novel cues more predictably safe. However, to our surprise, these guppies seemingly ignored alarm cues when social safety cues were also available, which we discuss further below.

It is possible that latencies in each population, as well as each pool, were affected by variation of factors not measured in our experiment (e.g., guppy density, sex-ratios, and background odours). Despite no anecdotally observable differences, an increase in guppy density in a pool may result in shorter latencies due to an increased probability of an individual entering or foraging in the arena by chance, generally lower local predation levels which have contributed to an increase in density, or by actively indicating safety as a result of a) a potentially larger amount of social calm behaviour, or b) the dilution effect. Sex-ratios may also affect latencies due to sex-based differences in risk-taking behaviour (Croft, Morrell, et al. 2006; Piyapong et al. 2010). Lastly, prey likely experience a multitude of chemical cues in their environment which may have occurred simultaneously with release of our experimental cues. However, we have not found differences in baseline responses to stream water controls across populations or across shoal treatments. As such, we feel confident attributing our latency results to differences in population ambient risk (prior experience with risk and safety), personal cues, and shoal treatments.

Our results align with the costly information hypothesis (i.e., copy-when-asocial-learning-is-costly; Boyd and Richerson 1988), as well as a 'copy-when-uncertain' strategy (Laland 2004). High-predation guppies relied on social safety information only when they were uncertain (facing a novel cue), mediating their otherwise neophobic responses. Our results support a study on nine-spined stickleback (*Pungitius pungitius*) that base foraging decisions on social information when the reliability of prior personal information has decreased (van Bergen et al. 2004). However, a study on European minnows (*Phoxinus phoxinus*) showed that individuals experiencing high predation risk relied on current social information about foraging patches even when it conflicted with prior reliable personal information (Webster and Laland 2008). It is possible that these results differed from ours due to their experimental design, since the presentation of social and personal information was not simultaneous, therefore making the social information more current and less likely to be outdated (Rieucau and Giraldeau 2011) compared to the personal information. Another potential explanation for differences in our results is that we manipulated personal and social information whose content was about risk and safety, as opposed to about resources (i.e., foraging opportunities).

Previous experiments on fathead minnows (*Pimephales promelas*) and rhesus monkeys (*Macaca mulatta*) have demonstrated that social risk cues are more persuasive than personal risk cues in overcoming latent inhibition (Mineka and Cook 1986; Crane and Ferrari 2015). However, these experiments did not examine the inverse, whether prey might learn a cue was safe using social and personal safety cues, after they were preconditioned to recognize a cue as dangerous. Recently, optimality modelling has shown that the use of safety cues has been overlooked in the literature, and suggested that safety cues may enhance fitness more than risk cues under contexts of elevated predation (Luttbeg et al. 2020). However, neither theoretical nor empirical experiments have examined the use of simultaneously available risk and safety cues, from both social and personal sources. Our experiments complement previous work by testing whether social safety cues are more persuasive than simultaneously-available personal risk cues, and our results demonstrate that social cues can be persuasive to guppies in overriding personal exposure to risk cues, depending on the ambient predation risk and the reliability of the personal cues.

In our second experiment, we assayed the activity of guppies in the experimental social chamber, finding that the social cues may have been a slightly stronger indicator of safety for low-predation guppies compared to the high-risk population (~17% difference in mean proportion of calm crosses; Figure 3.4b). Clearly, the social cues were persuasive for both populations, eliminating the neophobic responses of high-predation guppies and the alarm cue responses of low-predation guppies. However, we cannot discount that a slightly weaker safety cue for high-predation guppies may have contributed to their greater caution toward alarm cues (Experiment

1). It is worth noting, however, that Luttbeg et al. (2020) suggest that safety cues are more valuable than risk cues under conditions of elevated risk. As such, the slightly weaker safety cue strength for high-predation guppies arguably had negligible effects.

Aside from potential differences in the safety cues, one could argue that the reliability of alarm cues may be lower in the low-predation population. However, prey responses to alarm cues have been demonstrated to be innate regardless of population, and it is unlikely that the reliability of these cues differ between the sites (Ferrari et al. 2010; Chivers et al. 2012; Brown et al. 2013). Instead, the weakened response of low-predation guppies to alarm cues in the presence of a stimulus shoal may be suggestive of a threshold level of predation risk below which prey will rely on social information over conflicting reliable personal risk. This is likely due to the asymmetric costs of making an incorrect (suboptimal) decision (Johnson et al. 2013). In high-risk environments, responding with caution to reliable personal information is less costly than failing to respond and risking mortality. In low-risk environments, responding with caution may be costly over time in terms of missed opportunities for other fitness-enhancing activities (i.e., foraging, courtship, territory defense; Ferrari et al. 2018; Feyten and Brown 2018). Moreover, high-predation fish exhibit graded decreasing responses to increasingly diluted alarm cue concentrations while low-predation fish responses remain constant, presumably as a result of differences in long-term predation pressure (Brown et al. 2009). These differences in threat-sensitivity towards alarm cues may contribute to the increased influence of social information in the low-predation population, and thus, a lower concentration of alarm cues in our study may have led to similar population responses.

The generalizability of our results may depend on several factors. Foremost, our study species is a social species that coordinates group defences and competes agonistically for mates, making information from conspecifics particularly important (Giraldeau et al. 2002). Such information would likely be less persuasive in species that are less social. The social cues in our experiment were presumably perceived as reliable given the high conspecific-to-observer ratio (3:1) and the persuasiveness of the social cues in overriding the personal risk cues. A less reliable social cue (e.g., 1 conspecific instead of 3) or a stronger threat (e.g., a higher concentration of alarm cues or adding visual risk cues) would have likely altered our experimental outcomes. Moreover, our testing methodology likely involved guppies that were potentially bolder individuals (relative to others within the populations) because shyer individuals may have been less likely to approach the testing arena as a result of decreased exploratory behaviour (Trompf and Brown 2014). Perhaps, these shyer individuals would have been more persuaded by social safety cues (Kurvers et al. 2010; Trompf and Brown 2014). Although we did not record the sex of individuals entering or foraging, sex-specific differences in risk-taking behaviour (a personality trait) have been documented (Croft, Morrell, et al. 2006; Piyapong et al. 2010; Trompf and Brown 2014), and it could be worthwhile to examine how sex and personality may impact the integration of risk and safety information.

Another promising avenue for future research would be to repeat our experiments but to manipulate the reliability of the social information in addition to the personal information. If social and personal information conflict, their respective reliabilities should influence behavioural decisions (van Bergen et al. 2004; Rieucau and Giraldeau 2011). As discussed above, social cue reliability could be manipulated by altering the size of the stimulus shoal.

Researchers could also manipulate the familiarity between stimulus shoals and the individuals detecting social information. Familiarity has been shown to shape many guppy behaviours such as shoaling preferences, predator inspection behaviours, and the latency to approach novel foraging locations (Magurran 2005; Croft, James, et al. 2006; Morrell et al. 2008), playing a particularly important role in high-predation populations (Granroth-Wilding and Magurran 2013; Hasenjager and Dugatkin 2017; Crane, Feyten, et al. 2020a).

Our experiments contribute to the growing body of literature describing prey behavioural decisions in response to uncertain predation risk (Lima and Dill 1990; Sih 1992; Brown et al. 2013; Ferrari et al. 2018; Feyten, Demers, Ramnarine, Chivers, et al. 2019; Feyten, Demers, Ramnarine, and Brown 2019; Crane et al. 2020). We posit that such uncertainty is shaped by high ambient predation levels and the reliability of personal information (Table 3.1). We further suggest that historic encounters with novel cues (whether novel cues are predictably safe) may be tightly linked to ambient predation levels, affecting uncertainty of risk. Indeed, recent laboratory studies have demonstrated that prior experience with novelty (spatial and temporal predictability of a novel cue) affects the retention of neophobic responses in guppies (Feyten, Demers, Ramnarine, Chivers, et al. 2019). Uncertainty may hinder context-appropriate antipredator responses (Sih 1992; Wagner and Danchin 2010; Johnson et al. 2013), but prey can reduce uncertainty by acquiring multiple sources of information (Dall and Johnstone 2002; Dall et al. 2005; Schmidt et al. 2010; Wagner and Danchin 2010). When available information is unreliable, prey can cope with uncertainty by being cautious and neophobic (Ferrari et al. 2018; Feyten and Brown 2018; Feyten, Demers, Ramnarine, Chivers, et al. 2019). Here, guppies under high predation risk used social safety information to reduce their uncertainty when facing a novel cue. Understanding such uncertainty of risk is increasingly relevant, as it will likely increase for many species that are facing environmental changes (Sih 2013).

## 3.5 Figures & Tables

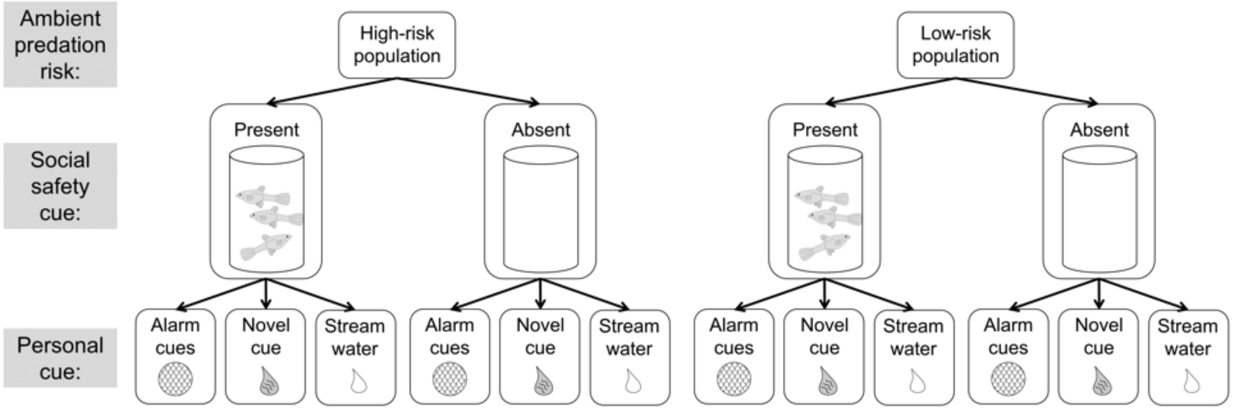


**Figure 3.1:** Experimental design (Experiment 1) for guppies in their natural populations with either high or low ambient predation risk. Trials consisted of the presence or absence of a shoal of 3 conspecifics combined with personal exposure to a chemical cue (alarm cue, novel cue, or a stream water control).

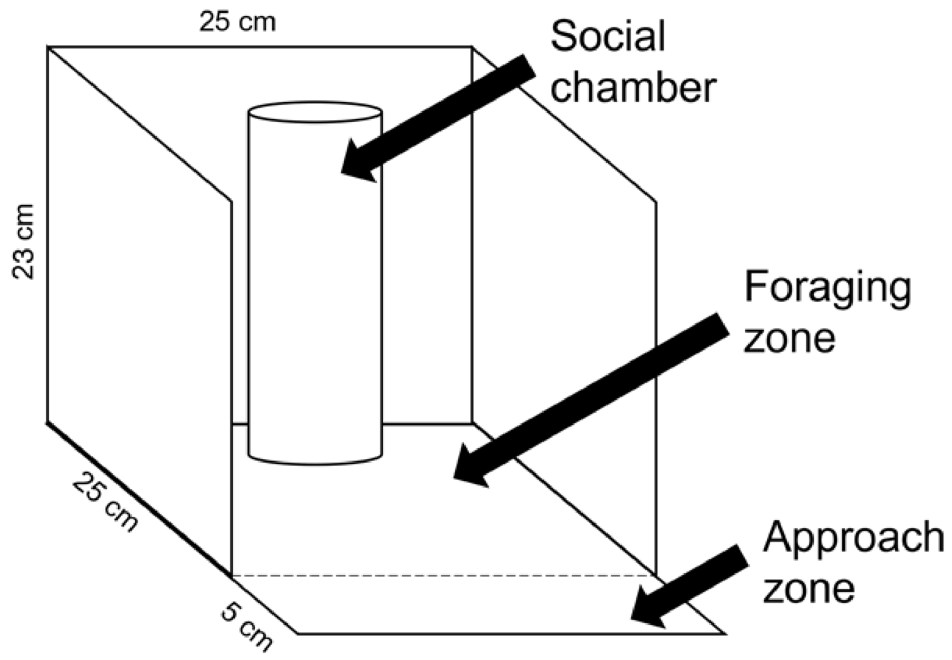


**Figure 3.2:** Testing arena used for *in situ* observations of guppies in natural stream populations.

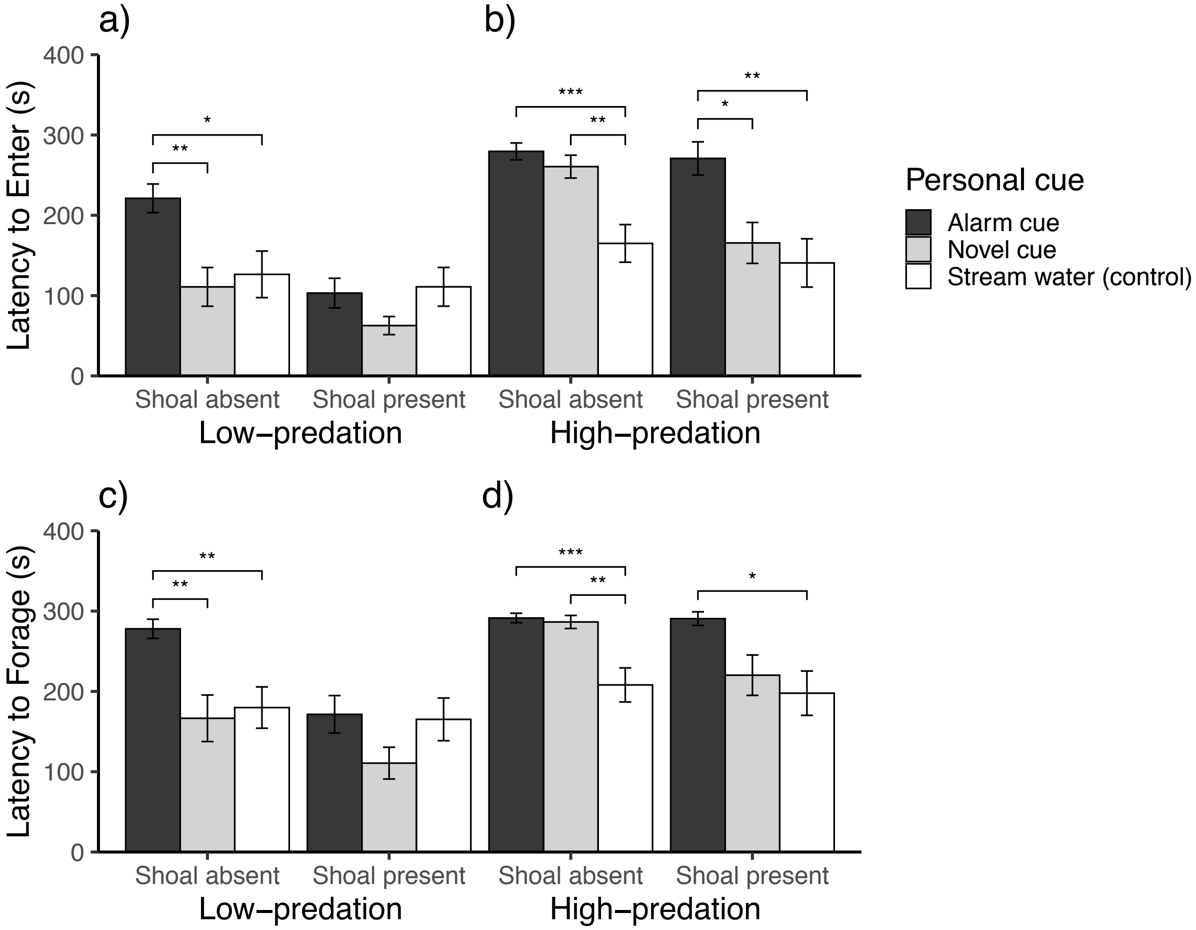


**Figure 3.3:** Mean ± SE latencies to enter the foraging arena (a and b) and forage (c and d) for guppies in low-predation or high-predation populations, after the introduction of a personal cue (alarm cue, novel cue, or stream water), with the presence or absence of a social safety cue. Significant Tukey *post hoc* comparison effects denoted by horizontal bars (* = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$).

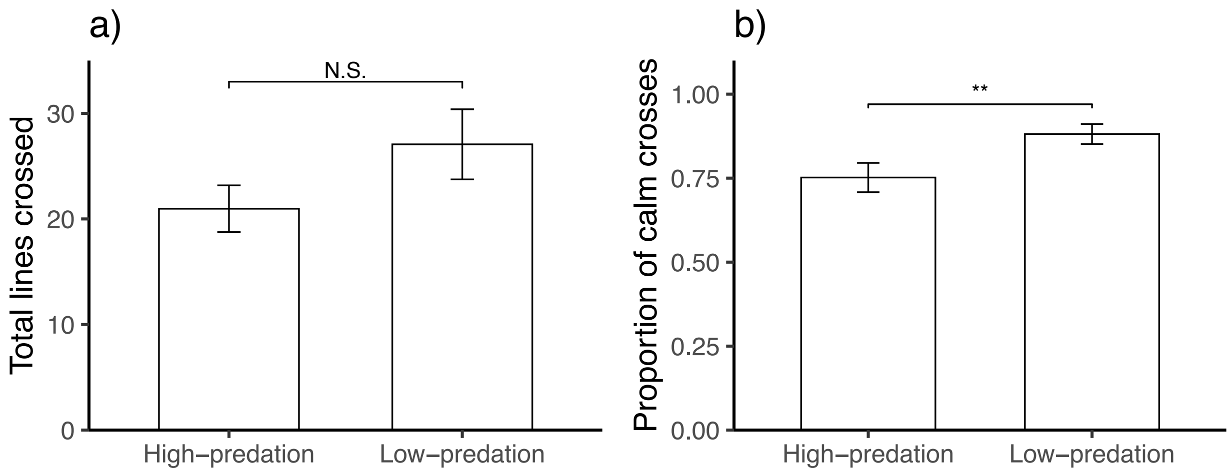


**Figure 3.4:** Mean ± SE number of lines crossed (a) and proportion of line crosses that were calm (b) for experimental shoals from high-predation or low-predation populations. Significant effects of population are denoted by horizontal bars (** = $p < 0.01$, N.S. = $p > 0.05$).

**Table 3.1:** Summary of theoretical framework regarding uncertainty of predation risk, as dictated by ambient predation risk and reliability of information.

| | Ambient predation risk | |
|---|---|---|
| Reliability of information | High | Low |
| Reliable (known) | Low uncertainty | Low uncertainty |
| Unreliable (unknown) | High uncertainty | Low uncertainty |

*Note:* Table is based on Feyten et al. (Feyten and Brown 2018; Feyten, Demers, Ramnarine, Chivers, et al. 2019). Uncertainty of risk is broadly defined as the inability to predict predation events, influencing perceived likelihoods of predation risk, and hindering context-appropriate antipredator responses (Sih 1992; Dall 2010; Wagner and Danchin 2010; Johnson et al. 2013).

**Table 3.2:** Overall ANOVA output for latency to enter (a), as well as separated by population (i.e., ambient predation level) (b and c). Personal cues were alarm cues, novel cue, or stream water, whereas shoal treatment was either the presence or absence of a stimulus shoal. Significant terms of interest are in bold type.

| | *F* | *df* | *p* |
|---|---|---|---|
| a) Overall | | | |
| Population | 59.403 | 1, 168 | <0.0001 |
| Personal cue | 17.558 | 2, 168 | <0.0001 |
| Shoal treatment | 16.993 | 1, 168 | <0.0001 |
| Population × personal cue | 5.327 | 2, 168 | 0.006 |
| Population × shoal treatment | 0.243 | 1, 168 | 0.623 |
| Personal cue × shoal treatment | 1.545 | 2, 168 | 0.216 |
| Population × personal cue × shoal treatment | 5.896 | 2, 168 | **0.003** |
| b) Low-predation population | | | |
| Personal cue | 8.041 | 2, 84 | <0.001 |
| Shoal treatment | 11.404 | 1, 84 | 0.001 |
| Personal cue × shoal treatment | 4.358 | 2, 84 | **0.016** |
| c) High-predation population | | | |
| Personal cue | 15.268 | 2, 84 | <0.0001 |
| Shoal treatment | 7.807 | 1, 84 | 0.006 |
| Personal cue × shoal treatment | 3.279 | 2, 84 | **0.043** |

**Table 3.3:** 1-way ANOVAs for latency to enter separated by whether a stimulus shoal was present or absent for low-predation (a) and high-predation (b) populations, as well as Tukey *post hoc* comparisons of personal cue treatments (c, d, and e) including alarm cue (AC), novel cue (NC), and stream water control (SW). Significant terms are in bold type.

| | *F* | *df* | *p* |
|---|---|---|---|
| a) Low-predation | | | |
| Shoal present | 1.611 | 2, 42 | 0.212 |
| Shoal absent | 5.760 | 2, 42 | **0.006** |
| b) High-predation | | | |
| Shoal present | 7.697 | 2, 42 | **0.001** |
| Shoal absent | 11.853 | 2, 42 | **<0.0001** |

| | *Mean difference* | *SE* | *df* | *T value* | *p* |
|---|---|---|---|---|---|
| c) Low-predation, shoal absent | | | | | |
| AC-NC | 13.6 | 4.34 | 42 | 3.134 | **0.009** |
| AC-SW | 11.7 | 4.34 | 42 | 2.696 | **0.027** |
| NC-SW | -1.9 | 4.34 | 42 | -0.438 | 0.900 |
| d) High-predation, shoal present | | | | | |
| AC-NC | 11.5 | 3.98 | 42 | 2.889 | **0.016** |
| AC-SW | 14.9 | 3.98 | 42 | 3.743 | **0.002** |
| NC-SW | 3.4 | 3.98 | 42 | 0.854 | 0.672 |
| e) High-predation, shoal absent | | | | | |
| AC-NC | 4.23 | 3.72 | 42 | 1.138 | 0.496 |
| AC-SW | 17.37 | 3.72 | 42 | 4.669 | **0.0001** |
| NC-SW | 13.13 | 3.72 | 42 | 3.531 | **0.003** |

**Table 3.4:** Overall ANOVA output for latency to forage (a), as well as separated by population (i.e., ambient predation level) (b and c). Personal cues were alarm cues, novel cue, or stream water, whereas shoal treatment was either the presence or absence of a stimulus shoal. Significant terms of interest are in bold type.

| | *F* | *df* | *p* |
|---|---|---|---|
| a) Overall | | | |
| Population | 34.133 | 1, 168 | <0.0001 |
| Personal cue | 8.865 | 2, 168 | <0.001 |
| Shoal treatment | 9.229 | 1, 168 | 0.003 |
| Population × personal cue | 4.130 | 2, 168 | 0.018 |
| Population × shoal treatment | 4.672 | 1, 168 | 0.032 |
| Personal cue × shoal treatment | 1.655 | 2, 168 | 0.194 |
| Population × personal cue × shoal treatment | 3.158 | 2, 168 | **0.045** |
| b) Low-predation population | | | |
| Personal cue | 5.319 | 2, 84 | **0.007** |
| Shoal treatment | 9.092 | 1, 84 | **0.003** |
| Personal cue × shoal treatment | 2.441 | 2, 84 | 0.093 |
| c) High-predation population | | | |
| Personal cue | 7.993 | 2, 84 | **<0.001** |
| Shoal treatment | 3.447 | 1, 84 | 0.067 |
| Personal cue × shoal treatment | 2.470 | 2, 84 | 0.091 |

**Table 3.5:** 1-way ANOVAs for latency to forage separated by whether a stimulus shoal was present or absent for low-predation (a) and high-predation (b) populations, as well as Tukey *post hoc* comparisons of personal cue treatments (c, d, and e) including alarm cue (AC), novel cue (NC), and stream water control (SW). Significant terms are in bold type.

| | *F* | *df* | *p* |
|---|---|---|---|
| a) Low-predation | | | |
| Shoal present | 2.087 | 2, 42 | 0.137 |
| Shoal absent | 7.245 | 2, 42 | **0.002** |
| b) High-predation | | | |
| Shoal present | 3.946 | 2, 42 | **0.027** |
| Shoal absent | 9.997 | 2, 42 | **<0.001** |

| | *Mean difference* | *SE* | *df* | *T value* | *p* |
|---|---|---|---|---|---|
| c) Low-predation, shoal absent | | | | | |
| AC-NC | 13.667 | 4.01 | 42 | 3.407 | **0.004** |
| AC-SW | 12.733 | 4.01 | 42 | 3.174 | **0.008** |
| NC-SW | -0.933 | 4.01 | 42 | -0.233 | 0.971 |
| d) High-predation, shoal present | | | | | |
| AC-NC | 8.23 | 3.92 | 42 | 2.098 | 0.102 |
| AC-SW | 10.47 | 3.92 | 42 | 2.667 | **0.029** |
| NC-SW | 2.23 | 3.92 | 42 | 0.569 | 0.837 |
| e) High-predation, shoal absent | | | | | |
| AC-NC | 1.63 | 3.39 | 42 | 0.482 | 0.880 |
| AC-SW | 13.87 | 3.39 | 42 | 4.091 | **<0.001** |
| NC-SW | 12.23 | 3.39 | 42 | 3.609 | **0.002** |

**Table 3.6:** 1-way ANOVAs for latency to enter (a) and forage (b) for control treatment groups.

| | *F* | *df* | *p* |
|---|---|---|---|
| a) Latency to enter | | | |
| Population | 2.069 | 1, 56 | 0.155 |
| Shoal treatment | 0.635 | 1, 56 | 0.429 |
| Population × shoal treatment | 0.255 | 1, 56 | 0.615 |
| b) Latency to forage | | | |
| Population | 1.594 | 1, 56 | 0.212 |
| Shoal treatment | 0.489 | 1, 56 | 0.487 |
| Population × shoal treatment | 0.001 | 1, 56 | 0.977 |

# V. Chapter 4: *Contribution of mean predation risk and diversity of predators to uncertainty of risk*[7]

**Preface:** In the previous three chapters, I examined how prey experience during the detection of information shapes uncertainty of risk and prey neophobic responses. Within this theme, I conducted multiple related but independent experiments to examine how NPA is shaped by i) the simultaneous reception of cues across modalities of the same or mixed reliability, ii) the diversity and spatial predictability of successively detected unknown cues, and iii) the simultaneous reception of social safety cues and non-social risk cues of varying reliability. Therefore, information limitations I tested include information reliability, information completeness, and conflicting information. In the previous chapter, I also assessed how "sources" of information (social or non-social) might influence neophobic responses. In my fourth chapter, I address how predators, the cause and direct source of risk information, affect NPA. In this chapter, I disentangle "mean" risk from "diversity" of risk, and ask how predator density and diversity might shape uncertainty of risk and NPA.

## 4.1 Introduction

Prey often exhibit multiple adaptations to counter the effects of predation, including changes in morphology, life-history, and behaviour (Lima and Dill 1990; Chivers et al. 1999; Relyea 2002). However, variable ecological conditions result in uncertainty of risk (Dall et al. 2005). Uncertainty of risk is often described as the inability to predict predation risk, due to the variation of risk in time and space (Sih 1992). Thus, antipredator adaptations are typically plastic (Lima and Dill 1990; Relyea 2002; Brönmark et al. 2011). Although morphological adaptations may be reversible (Chivers et al. 2008), and the timing of life-history traits can be adaptive (Chivers et al. 1999), behavioural plasticity is argued to be the most flexible antipredator adaptation (Lima and Dill 1990; Brown et al. 2013). Therefore, plastic antipredator behavioural adaptations should allow prey to respond optimally to rapid fluctuations in local threats.

Accurate risk assessment enables prey to reliably recognize an ecologically relevant threat, and successfully identify predators (Dall et al. 2005; Brown et al. 2013). Innate predator recognition allows prey to recognize ecologically-relevant predators without previous experience (Brown, Ferrari, and Chivers 2011) and has been observed in a wide range of taxa (Dalesman et al. 2007; Epp and Gabor 2008; Hawkins et al. 2004; Vilhunen and Hirvonen 2003). However, it is likely inflexible under conditions of highly variable predator communities with a high diversity of predators (Wisenden 2003; Brown et al. 2013). Acquired predator recognition occurs through direct (personal) and/or indirect (social) experience (Dall et al. 2005), and unlike innate predator recognition, it allows prey to learn the identity of new or novel potential predators (Mathis and Smith 1993; Kelley and Magurran 2003a; Gonzalo et al. 2007; Brown et al. 2013). For example, wood frog (*Rana sylvatica*) survival after an encounter with a novel predator is affected by prior (embryonic and larval) risk exposure (Ferrari, Crane, et al. 2015). Acquired predator recognition thus reduces a prey's uncertainty of risk via the gathering of more information from their environment (Sih 1992; Dall and Johnstone 2002; Schmidt et al. 2010). However, acquired

---

[7] A version of Chapter 4 has been published. Feyten, LEA, Demers, EEM, Ramnarine, IW, and Brown, GE (2022) Assessing effects of predator density and diversity on neophobia in Trinidadian guppies. Behavioural Processes 201: 104717. DOI: 10.1016/j.beproc.2022.104717

predator recognition may be costly since prey increasingly risk mortality while gathering additional information, as well as upon an initial predator encounter (Brown et al. 2013).

It has been proposed that prey can reduce the costs of learning associated with acquired predator recognition by exhibiting phenotypically-plastic neophobic predator avoidance, or the active avoidance of novel stimuli (Brown et al. 2013). Previous research suggests that neophobia is present in prey after experiencing induced or naturally elevated ambient risk (Brown et al. 2013; Meuthen et al. 2016). When inducing neophobic predator avoidance, these studies typically manipulate ambient risk by increasing the "mean", or average, risk levels by exposing prey to varying concentrations of damage-released alarm cues (an honest indicator of risk; Brown and Godin, 1999; Brown et al., 2011). Recently, Ferrari et al. (2018) argued that uncertainty of risk, rather than mean level of risk per se, may be a key driver of neophobia. For example, when woodfrog tadpoles are exposed to known chemical risk cues (injured conspecific cues) paired with a novel predator cue repeatedly over 4 days, Ferrari et al. (2018) found no evidence of a neophobic response to a second novel predator cue. However when exposed to known risk cues, either in the absence of any predator cue or with a different novel predator cue at each exposure, woodfrog tadpoles exhibited strong neophobic responses to an additional novel predator cue. Ferrari et al. (2018) suggest that in the first scenario, prey can be certain of risk if the known indicator is consistently paired with a specific novel predator cue. In the second scenario, if the known risk is either not associated with a specific predator cue or is associated with multiple (variable) predator cues, prey uncertainty of risk identity is high (Ferrari et al. 2018). Likewise, Trinidadian guppies (*Poecilia reticulata*) from high-risk sites retain neophobic responses to a novel cue when they have been previously presented with that novel cue in conjunction with other novel cues (a diversity of novel chemical information; Feyten, Demers, Ramnarine, Chivers, et al. 2019). However, when a novel cue is repeatedly presented alone, guppies no longer respond to it with neophobia. This suggests uncertainty increases with a diversity of novel chemical information, potentially due to an interference effect in learning (i.e., inability to predict what cue will come next). Similarly, an *in situ* experiment demonstrated high-risk guppies facing multiple, multimodal, novel sources of information respond neophobically, and may even over-estimate risk compared to their baseline response to reliable risk cues (Feyten, Demers, Ramnarine, and Brown 2019). Thus, we may expect uncertainty to be linked with the diversity of information cues/sources within local habitats.

Although gathering more information can reduce uncertainty (Schmidt et al. 2010), the availability of multiple, diverse, sources of information may actually increase uncertainty of risk. For example, Shannon information theory posits that prey facing diverse information should be less able to predict what they might encounter next (Shannon 1948). Thus, an increase in predator diversity (i.e., species richness) may hinder the ability of prey to predict future risk (i.e., they face greater uncertainty of risk). Since predator species differ in tactics (i.e., sit-and-wait vs. pursuit predators) and time of activity (i.e., diurnal vs. nocturnal), learning or predicting predator activity would likely entail large initial learning costs. Prey may therefore be more likely to use alternative tactics to cope with diverse predation, such as neophobia. A link between novel predator diversity and neophobia was demonstrated using chemical cues in woodfrog tadpoles (Ferrari et al. 2018) and guppies (Feyten, Demers, Ramnarine, Chivers, et al. 2019), but as far as we know remains unexplored using visual predator cues. Moreover, researchers have yet to explore this link over a range of mean risk levels. In addition to predator diversity, predator density (i.e., mean risk) may be an important aspect of predation contributing to uncertainty of risk. An increase in a number of predators may result in less predictable (more uncertain) risk due to spatial and temporal variation in predation (Sih 1992), and therefore increased neophobia. Furthermore, an increase in mean risk results in an increased likelihood of predator encounter, and therefore increases the likelihood of prey mortality (Lima and Dill 1990). Such contexts should favor neophobic behaviours. Although neophobia has been linked to elevated, diverse, and unpredictable risk, to our knowledge, no research has specifically examined how the density and diversity of predators may interact to affect neophobia.

We use the Trinidadian guppy as our model system. The Northern Range in Trinidad has an abundance of rivers that contain isolated populations of guppies with varying predator diversities and densities, and many have been classified on a continuum of predation risk to guppies (Botham et al. 2008; Deacon et al. 2018). Previous studies have shown that guppies from high-predation, but not low-predation sites show neophobic responses, and that neophobia can be induced in low-predation guppies following short-term exposure to elevated predation risk (Brown et al. 2013; Brown et al. 2015). We hypothesize that a high diversity of predators would lead to greater uncertainty of risk in prey compared to a low diversity of predators, and thus neophobic predator avoidance. However, neophobia is only elicited when mean risk is high enough (Brown et al. 2013). Thus, we predict that an increased diversity of predators will result in higher neophobic responses, but that predator diversity may interact with mean risk levels to shape neophobic responses. We address this question in a series of laboratory trials, in which we presented guppies with high or low predator diversity (i.e., species richness) using visual predator models, paired with one of three predator density treatments (i.e., high, intermediate, or low mean risk) using different concentrations of damage-released alarm cues (an honest indicator of risk; Brown and Godin, 1999; Brown et al., 2011).

## 4.2 Methods

### *4.2.1 Fish Collection and Maintenance*

Using a seine net, we collected non-gravid adult female guppies from the Upper Aripo river in the Northern Range of the Republic of Trinidad and Tobago. This is considered a low-predation site due to an absence of aquatic predators of adult guppies (Deacon et al. 2018), and guppies here do not naturally exhibit neophobic predator avoidance (Brown et al. 2013). Guppies were collected in April 2017 and moved to a laboratory at the University of West Indies, St. Augustine. We held guppies in 250-L glass aquaria containing 180 L of dechlorinated tap water, which was aerated and filtered (23 °C and a 12:12 L:D cycle). We allowed guppies to acclimate to laboratory settings for at least 12 h before moving them to conditioning aquaria (see below). We fed all guppies twice each day with commercial flake food throughout the experiment. We returned guppies to their site of origin after completing our experiments, as required by collection permits.

### *4.2.2 Predator Models*

We 3D printed three predator models (14 cm in length) from translucent polycarbonate, sprayed them with Rust-Oleum® Painter's Touch® Ultra Cover Primer, hand-painted them with Pebeo® High Viscosity Studio Acrylic paint, and sealed them with Rust-Oleum® Painter's Touch® Ultra Cover Clear Gloss (Figure 4.1). We prepared these models 2 months prior to use, in addition to soaking them in water, in order to reduce potential off-gassing of volatile compounds. These models were attached to a metal dowel (~1 m in length) using transparent fishing line, via small punctures made in the dorsal fins if the models. This allowed controlled movement of the models through the conditioning aquaria. All three models represented novel visual predator cues to guppies from the low-risk site.

### *4.2.3 Stimulus preparation*

We generated stock alarm cue from 61 Upper Aripo donor guppies (mean ± SD standard length = 23.44 ± 3.54 mm). We used a whole body extract, which is a standard method for alarm cue generation in guppies (Brown and Godin 1999; Brown et al. 2013). We euthanized cue donors via cervical dislocation (in accordance with Concordia University Animal Research Ethics Protocol #AREC-30000255). After removing the head, tail, and viscera, we immediately placed the remaining tissue in 100 mL of chilled dechlorinated water. We then homogenized the tissue samples, filtered through polyester floss, and diluted with dechlorinated water to a final concentration of ~0.10 $cm^2$ $mL^{-1}$. For our novel test cue, we diluted Blanche's® lemon essence in dechlorinated water (6 mL lemon extract in 300 mL dechlorinated water; Brown et al., 2013). We froze both chemical cues in 20 mL aliquots at -20°C until needed.

### *4.2.4 Conditioning phase*

Guppies were placed in shoals of 40 into 250 L glass aquaria filled with ~60 L of dechlorinated tap water, which was aerated and contained artificial plant refuge. We conditioned guppies with a predator model paired with an alarm cue (i.e., mean risk) treatment, 3 times per day (at 10:30 AM, 12:30 PM, and 2:30 PM) for 2 days. During each conditioning event, we placed a predator model within the top third of the vertical water column, added one of three mean risk treatments, and moved the model around the perimeter of the tank at a consistent speed for 12 s before removing the model from the conditioning aquarium. Guppies received one of two predator diversity treatments: a 'low diversity' treatment (we exposed guppies to the same model over the duration of the conditioning), or a 'high diversity' treatment (we exposed guppies to three different predator models in a randomized order over the course of a conditioning day, and then exposed to them again in a different order on the following day). The mean risk treatments were manipulated by diluting the stock concentration of alarm cue to create the 'high-risk' treatment (15 mL of alarm cue), 'intermediate-risk' treatment (5 mL alarm cue + 10 mL distilled water), or 'low-risk' control treatment (15 mL distilled water; Figure 4.2).

### *4.2.5 Testing phase*

The day after the last conditioning event, we placed guppies (mean ± SD standard length = 22.45 ± 4.98 mm) into test aquaria in shoals of 3 and allowed them to acclimate for at least 45 min. Trials consisted of a 5-minute pre-stimulus observation period, followed by the injection of 8 mL of chemical stimulus into the tank, followed by a 5-minute post-stimulus observation period. The chemical stimulus was either novel (described above) or a dechlorinated tap water control. We quantified induced neophobia by observing antipredator behaviours including area use and shoaling index. Area use was recorded at 15 s intervals as the position of each fish in the water column. Scores ranged from 3 (all fish near the substrate) to 9 (all fish near the surface). Shoaling index was also recorded every 15 s, with scores ranging from 1 (no fish within one body length of each other) to 3 (all fish within one body length of each other). We also recorded the presence or absence of freezing (cessation of all movement occurring close to substrate, for at least 30 s). Reduced area use, increased shoaling index scores, and occurrence of freezing are consistent indicators of antipredator responses in guppies (Brown et al., 2013). After observations were completed, we measured the standard lengths of guppies. For area use and shoaling index, we calculated an average pre- and post-stimulus value for each trial, and then calculated the proportional change of those averages ((post – pre) ÷ pre). For the presence/absence of freezing, we calculated the difference between pre- and post-stimulus observations. This resulted in ordinal change in freezing data, where freezing increased, decreased, or stayed the same.

### *4.2.6 Statistical Analyses*

We conducted a pair of linear mixed-effects model analyses (LMMs; using *lmer()* function using R package **lme4**; Bates et al. 2015) on proportional change in area use and proportional change in shoaling index. Our area use data met the assumptions of homoscedasticity and normality of residuals, whereas our shoaling index data did not. As such, we transformed the proportional change in shoaling index by adding a constant (the absolute value of the largest negative proportional change in shoaling index) such that all values were $\geq 0$. These values were then square root transformed. These transformed shoaling index data met the assumptions of homoscedasticity and normality of residuals. Our LMMs tested the effects of all fixed factors [density (high, intermediate, or low risk), diversity (high or low), and chemical stimulus (novel cue or water control)] and their interactions, while including conditioning block as a random factor (to account for nesting of observations within conditioning block). Significance was calculated using $F$ tests which applied Satterthwaite's methods to estimate degrees of freedom and generate $p$ values for mixed models (using *anova()* function with the R package **lmerTest**; Kuznetsova et al., 2017). We conducted pairwise *post hoc* comparisons using Tukey or Holm corrections where appropriate (using R package **emmeans**; Lenth, 2019), also applying Satterthwaite's methods to estimate degrees of freedom.

Given the ordinal nature of the change in freezing data (increase, no change, or decrease), we conducted an ordinal regression using a cumulative mixed-effect model (CLMM; *clmm()* function in R package **ordinal**; Christensen, 2019). Again, we tested the effects of all fixed factors [density (high, intermediate, or low risk), diversity (high or low), and chemical stimulus (novel cue or water control)] and their interactions, while including conditioning block as a random factor. Significance for the model was calculated using Likelihood Ratio Tests (using *Anova.clmm()* function with the R package **RVAideMemoire**; Hervé, 2022). Given the 2-way interactions in the overall model, we conducted pairwise contrasts (using Holm correction to account for multiple comparisons; R package **emmeans**, as above) to test differences in response to a novel odour and a water control for each risk × diversity treatments. This allowed us to specifically assess how predator density and diversity may shape neophobia. All analyses were conducted in RStudio 3.4.1 using $\alpha = 0.05$.

## 4.3 Results

### *4.3.1 Proportional Change in Area Use*

For proportional change in area use, our LMM revealed a significant interaction of risk and stimulus ($F_{2,\ 197.456} = 3.5622$, $p = 0.030$; Table 4.1a), indicating that proportional change in area use depended on mean risk (alarm cue concentration during conditioning) and test stimulus (Figure 4.3a,b). Tukey pairwise *post hoc* contrasts (averaged over diversity treatments) demonstrated that none of the risk treatments differed significantly in area use (all $p \geq 0.637$) in response to a water control (Table 4.1c; Figure 4.3a,b). In response to the novel cue (lemon odour), high-risk guppies trended to have lower area use scores (i.e., greater neophobia) than their intermediate-risk counterparts ($t = -2.30$, $df = 28.5$, $p = 0.072$; Table 4.1d). There was no significant difference between high-risk and low-risk conditioned guppies ($p = 0.340$) or intermediate- and low-risk fish ($p = 0.647$) in response to a novel cue. We suspected the lack of significant difference between high- and low-risk guppies was likely due to their similar area use responses to novelty in the low-risk × low-diversity treatment and high-risk × high-diversity treatments (See Figure 4.3a,b). To test this, we conducted specific pairwise contrast tests from our overall model, comparing the effects of novel and control stimuli in the high-risk × high-diversity, high-risk × low-diversity, low-risk × high-diversity, and low-risk × low-diversity treatments. We corrected for multiple comparison using Holm's method. We found that area use response was only significantly different in the high-risk × high-diversity treatments ($t = -2.534$, $df = 197$, $p = 0.045$; Table 4.2). None of the other treatments (high-risk × low-diversity, low-risk × high-diversity, and low-risk × low-diversity treatments) differed in their area use in response to a novel cue compared to a water control (all $p = 1.00$, Table 4.2).

### *4.3.2 Proportional Change in Shoaling Index*

For proportional change in shoaling index, our LMM revealed a significant risk and stimulus interaction ($F_{2,\,197.609} = 4.6676$, $p = 0.010$; Table 4.3a), indicating that proportional change in shoaling index depended on mean risk (alarm cue concentration during conditioning) and test stimulus (Figure 4.4a,b). Tukey pairwise *post hoc* contrasts (averaged over diversity treatments) demonstrated that none of the risk treatments differed significantly in shoaling index in response to a water control (all $p \geq 0.754$; Table 4.3c). In response to the novel cue (lemon odour), high-risk guppies had significantly greater shoaling index scores (i.e., greater neophobia) than their low-risk counterparts ($t = 2.797$, $df = 24.4$, $p = 0.026$; Table 4.3d; Figure 4.4a,b). There was a marginally non-significant trend for a larger shoaling index in high-risk compared to intermediate-risk guppies ($t = 2.316$, $df = 25.7$, $p = 0.072$; Table 4.3d), but no significant difference between intermediate- and low-risk guppies ($p = 0.896$; Table 4.3d).

### *4.3.3 Change in Freezing*

For change in freezing, the overall ordinal regression on differences in freezing resulted in a significant 2-way interaction of risk and diversity ($\chi^2(2) = 6.4799$; $p = 0.039$), as well as risk and stimulus ($\chi^2(2) = 6.9874$; $p = 0.030$; Table 4.4a). *Post hoc* contrast tests demonstrated that only the high-risk × high-diversity treatments increased their freezing response to a novel cue compared to a water control ($z = 2.823$, $df = \text{Inf}$, $p = 0.029$), while no other risk × diversity combination showed significant differences in their response to a novel cue and a water control (all $p = \sim 1$; Table 4.5; Figure 4.5a,b).

## **4.4 Discussion**

As predicted, we found that an increase in mean risk (proxy for predator density) resulted in induced neophobic antipredator behaviour. Interestingly, we did not see a clear graded change in neophobia from high to intermediate to low risk for any of our neophobic metrics (Figure 4.3a,b; Figure 4.4a,b). This differs from previous experiments on juvenile convict cichlids (*Amatitlania nigrofasciata*) demonstrating graded neophobia depending on concentrations of alarm cue used to manipulate background risk (Brown, Chivers, et al. 2014). Moreover, guppies from high-risk sites show varying levels of neophobia after integrating multiple sources of information of varying reliability (Feyten, Demers, Ramnarine, and Brown 2019). It is unlikely that we saw a non-graded response in guppies because they were unable to distinguish between the intermediate- and low-risk concentrations. Previous studies have demonstrated that guppies from our low-predation site show non-graded threat-sensitive responses to varying concentrations of alarm cue (Brown et al. 2009; Elvidge et al. 2014), but these antipredator responses were elicited after experiencing concentrations similar to both our high- and intermediate-risk treatment (Elvidge et al. 2014). Moreover, guppies from a high-predation site within the same river do show graded threat-sensitive responses. Perhaps conditioning guppies to visual predator cues paired with alarm cue result in an all-or-nothing neophobic response, or guppies require a longer duration of elevated ambient risk to develop graded neophobic responses. Conducting the experiments with a smaller difference in alarm cue concentration between intermediate- and high-risk treatments may demonstrate this exact threshold value of mean risk, or demonstrate a graded response at a finer scale of alarm cue concentration.

Visual predator diversity only affected two of our three neophobic metrics, suggesting that although predator diversity may influence uncertainty, it has a weaker effect than predator density. Guppies conditioned with high mean risk increased their shoaling in response to a novel cue (i.e., neophobia) regardless of predator diversity (Figure 4.4a,b). Similarly, neophobic area use trended to increase in the high-risk treatments, compared to intermediate risk-treatments, regardless of predator diversity (Figure 4.3a,b). However, when comparing neophobia in high-risk and low-risk treatments, neophobic area use responses were only present when guppies were conditioned with both high risk and high diversity (Table 4.2, Figure 4.3a,b). Moreover, when exposed to a novel cue, increased freezing occurred only when high mean risk was paired with diverse visual predator cues (Figure 4.5a,b). Our results align with predictions set by Shannon information theory, where a diversity of information results in greater uncertainty of what information will be encountered next. In addition, our results supplement previous work by Ferrari et al. (2018) on woodfrog tadpoles demonstrating uncertainty and neophobia associated with a diversity of novel chemical cues paired with chemical risk cues. Our results add to our understanding of which contexts may result in neophobic responses in prey, with consistent neophobic responses after experiencing elevated risk, and an apparent additive effect of visual diversity of predators. That is, predator diversity elicits neophobia only when mean risk (i.e., predator density) is high enough. This suggests some threshold of mean risk above which predator diversity shapes neophobic antipredator responses in prey, and below which predator diversity should not shape neophobia. This threshold is likely driven by the costs of missed opportunities (e.g., to forage or engage in courtship) when erroneously engaging in neophobia under conditions of low risk.

Potentially, predator diversity had a weaker than expected effect on uncertainty due to our experimental design. Guppies may have needed more time to associate visual cues with risk, which would have further reduced uncertainty of risk (as described in Ferrari et al., 2018) and possibly resulted in an effect of predator diversity on shoaling index and a greater effect on area use. Perhaps consistent presentation of visual cues is not as salient as the consistent presentation of chemical cues used in previous experiments (Ferrari et al. 2018; Feyten, Demers, Ramnarine, Chivers, et al. 2019). This possible difference in salience of visual predator cues vs. chemical cues may be attributed to sensory biases in aquatic organisms. Aquatic animals often preferentially use chemical cues to assess predation risk (Dodson et al. 1994; Kiesecker et al.

1996; Chivers and Smith 1998; Mathis and Vincent 2000; Brown and Magnavacca 2003). However, successful conditioning of visual cues paired with alarm cues have been demonstrated in several fish species (Chivers and Smith 1994; Hall and Suboski 1995; Yunker et al. 1999). A recent study on convict cichlids demonstrated that both visual and chemical cue conditioning can result in similar strengths of neophobia (Brown et al. 2016). Vision and use of visual cues in courtship decisions has been well documented in our model system (discussed in Endler, 1991), and guppies from high-risk sites recognize visual predator models (Kelley and Magurran 2003b). Guppies have been shown to integrate chemical and visual cues presented simultaneously, and respond differently to novel compared to realistic ("known") predator models (Feyten, Demers, Ramnarine, and Brown 2019). Moreover, guppies have been shown to be more attentive to ambiguous visual cues after exposure to chemical alarm cues (Stephenson 2016). Therefore, the salience of visual predator cues is unlikely the cause of the weak effect of predator diversity on neophobia in guppies.

Instead, we suggest predator guild diversity may have had a larger effect on neophobia had our design included predation tactic/timing differences across visual predator diversity treatments. Guppy predators vary in time of activity and type of predation (ambush vs. pursuit; Botham et al., 2006), and manipulation of predator location, movement, tactics, and/or timing could have increased the effect of predator diversity across our neophobia metrics. Indeed, a recent experiment demonstrated that timing and location of novel chemical cues affects neophobic space use and shoaling (Feyten, Demers, Ramnarine, Chivers, et al. 2019). Moreover movement, and not shape, has been suggested as the primary visual cue for threat assessment in fathead minnows (*Pimephales promelas*; Wisenden and Harter 2001), and this may also be true for guppies. Future experiments should assess the effect of diverse predator movement on neophobic responses, to contrast our assessment using controlled movement with diverse predator shape and colouration.

It would also be of interest to explore how individual variation in predation behaviour contributes to uncertainty of risk experienced by prey. Such individual variation in predation behaviour has been observed in a main guppy predator, the pike cichlid (*Crenicichla frenata*; Szopa-Comley et al., 2020). Given that guppies and their predators are confined to the same river pool during the dry season (Magurran 2005), predation is likely more predictable over time and guppies may be more certain of risk due to experiencing consistent predatory behaviour. However, if confined with multiple predators, even of the same species, individual variation in predation behaviour may reduce predictability of risk and increase uncertainty of risk. Understanding how behavioural variation of predators may shape uncertainty and neophobia in their prey would be a valuable avenue of future research.

Our results may provide some insight into why neophobia is observed in certain species, while not in others. For example, the Largespring mosquitofish (*Gambusia geiseri*) and Western mosquitofish (*Gambusia affinis*), two live-bearing species similar to the guppy, fail to show neophobic responses to novel predators (Blake et al. 2015; Blake and Gabor 2016). Potentially, a difference in predator guilds or predation regime dictate whether highly plastic adaptations to predation risk are necessary. Indeed, neophobia is costly in terms of missed opportunities for foraging, courtship, and territory defense. Given that actively invasive species have weaker neophobic responses compared to their established or non-invasive counterparts (Martin and

Fitzgerald 2005; Candler and Bernal 2015; Magory Cohen et al. 2020), understanding what drives neophobia may be useful in controlling invasive species like the Western mosquitofish.

Our experiment contributes to the existing literature on determining prey responses to uncertainty of risk. We have established measurable components of risk which may contribute to uncertainty of risk, and the resulting neophobic responses. As climate change, anthropogenic factors, and invasive species combine to create ecological uncertainty for prey, it is increasingly important to study behavioural adaptations to variability in predation, such as neophobia. Understanding the drivers of neophobia would allow better prediction of short- and long-term effects of changing environments on prey populations, including endangered and socio-economically important species.

## 4.5 Figures & Tables

**Figure 4.1:** 3D printed predator models (14cm in length) used in this experiment, all of which serve as novel visual predator cues to low-predation guppies.

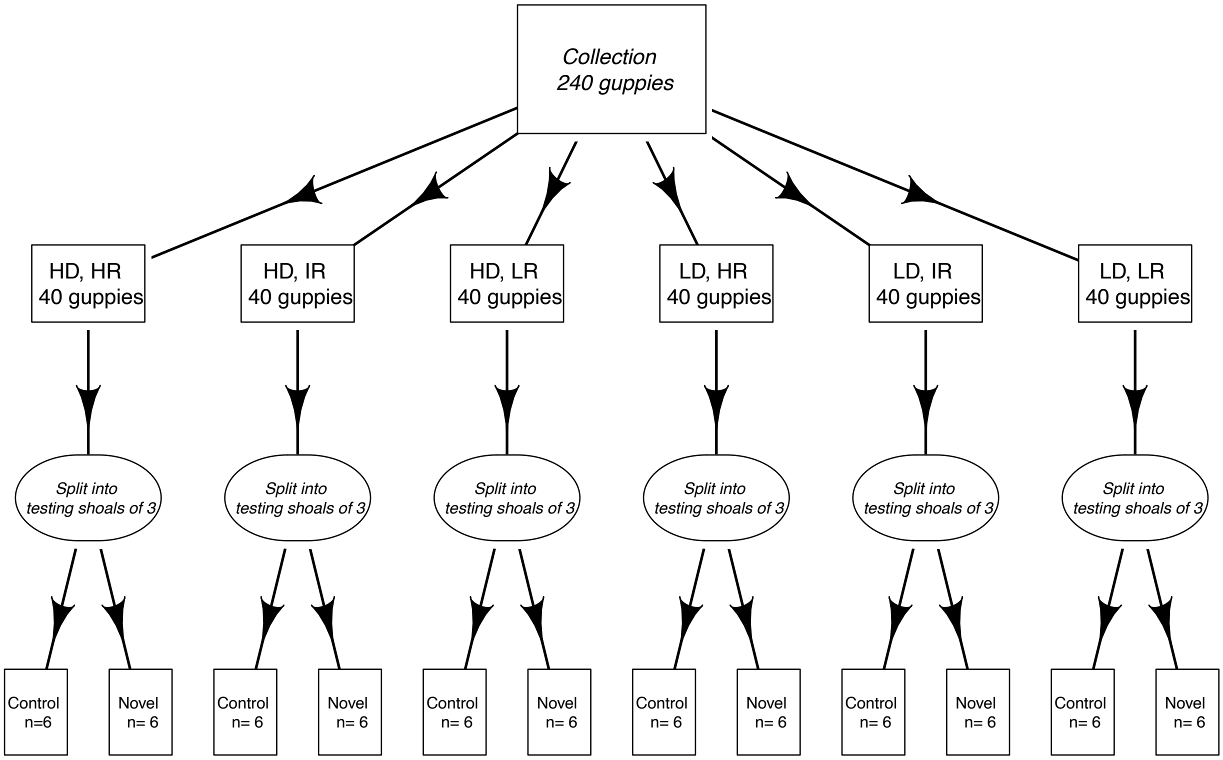


**Figure 4.2:** Experimental flow chart demonstrating a round of conditioning and testing treatments. Conditioning treatments consisted of either high (HD) or low (LD) diversity predator model presentation, combined with either high (HR), intermediate (IR), or low (LR) mean risk treatments. Guppies from conditioned tanks were then split into shoals of 3 and tested for neophobia with either a novel odour or a water control. Each round resulted in a minimum of n = 6 for each conditioning and experimental treatment combination. This set up was repeated 3 times, using a total of 720 guppies, to get a minimum final n = 18.

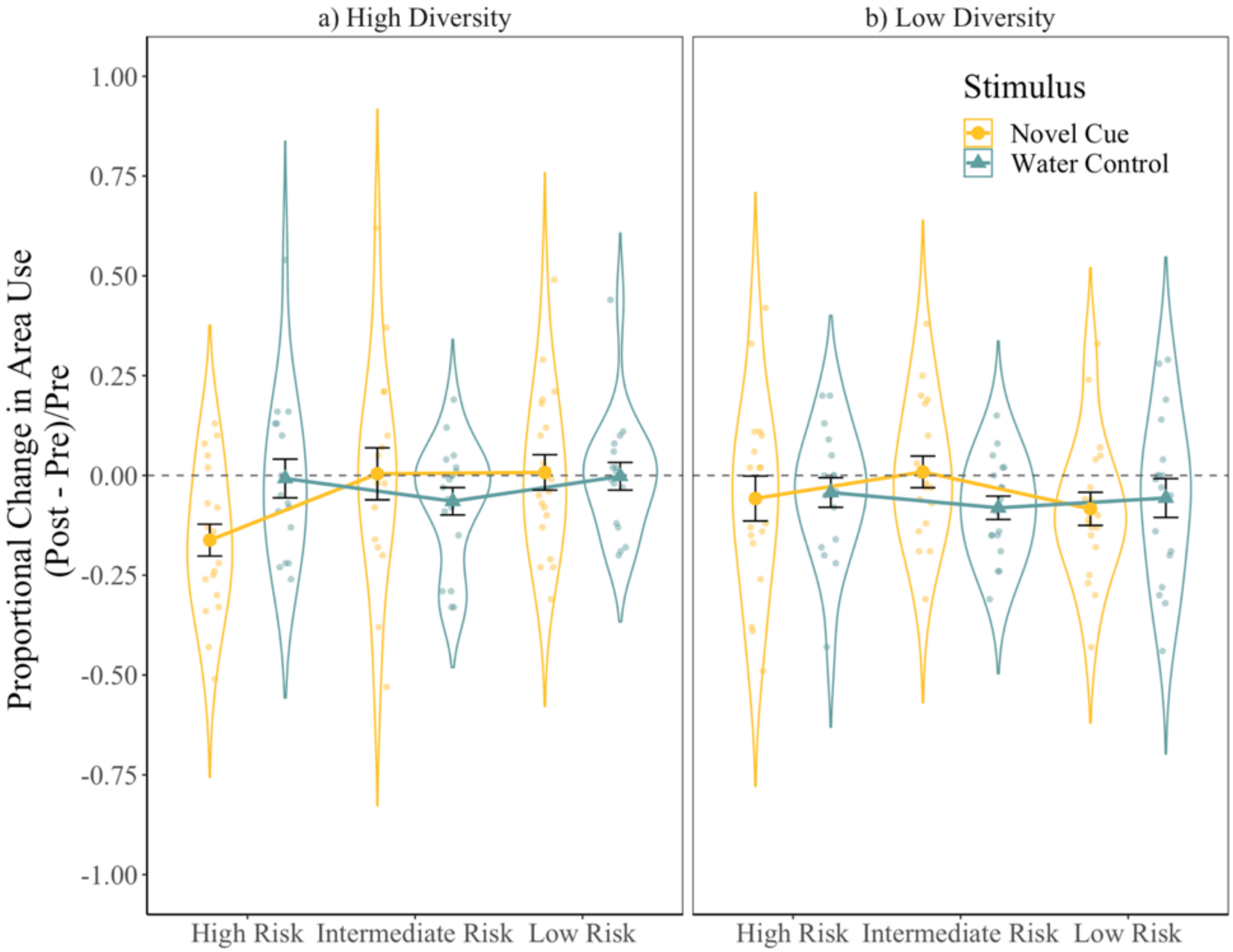


**Figure 4.3:** Mean ± SE of proportional change in area use across high (a) and low (b) predator diversity treatments, for each risk treatment (high, intermediate, or low), in response to each testing stimulus (water control indicated by blue triangles, novel cue indicated by yellow points). Values above the dashed line indicate an increase in area use after the stimulus introduction, below the dashed line indicate a decrease in area use, and values around the dashed line indicate no proportional change. The more negative the area use value, the stronger the antipredator response.

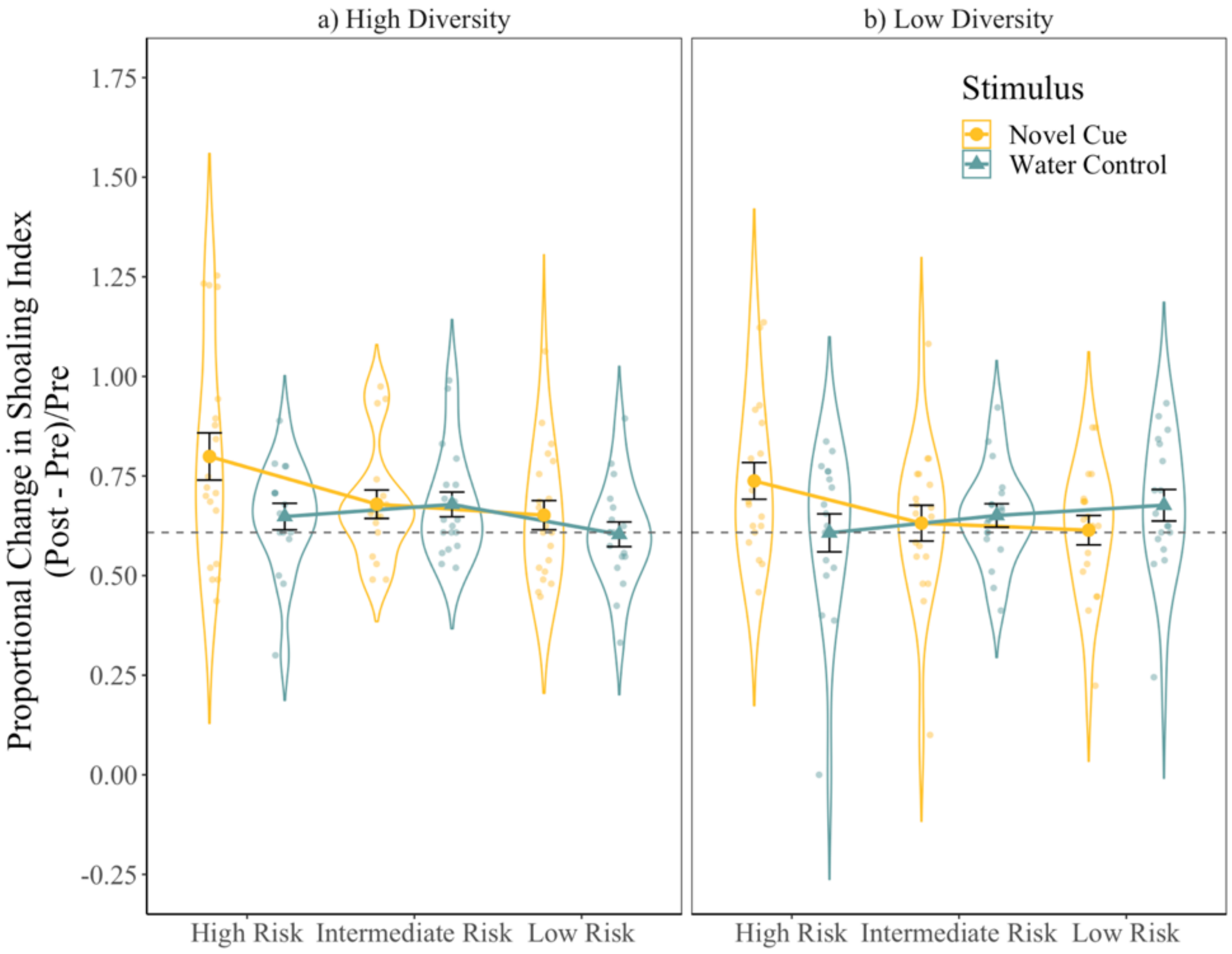


**Figure 4.4:** Mean ± SE of transformed proportional change in shoaling index across high (a) and low (b) predator diversity treatments, for each risk treatment (high, intermediate, or low), in response to each testing stimulus (water control indicated by blue triangles, novel cue indicated by yellow points). Values above the dashed line indicate an increase in shoaling index after the stimulus introduction, below the dashed line indicate a decrease in shoaling index, and values around the dashed line indicate no proportional change. The greater the shoaling index value, the stronger the antipredator response.

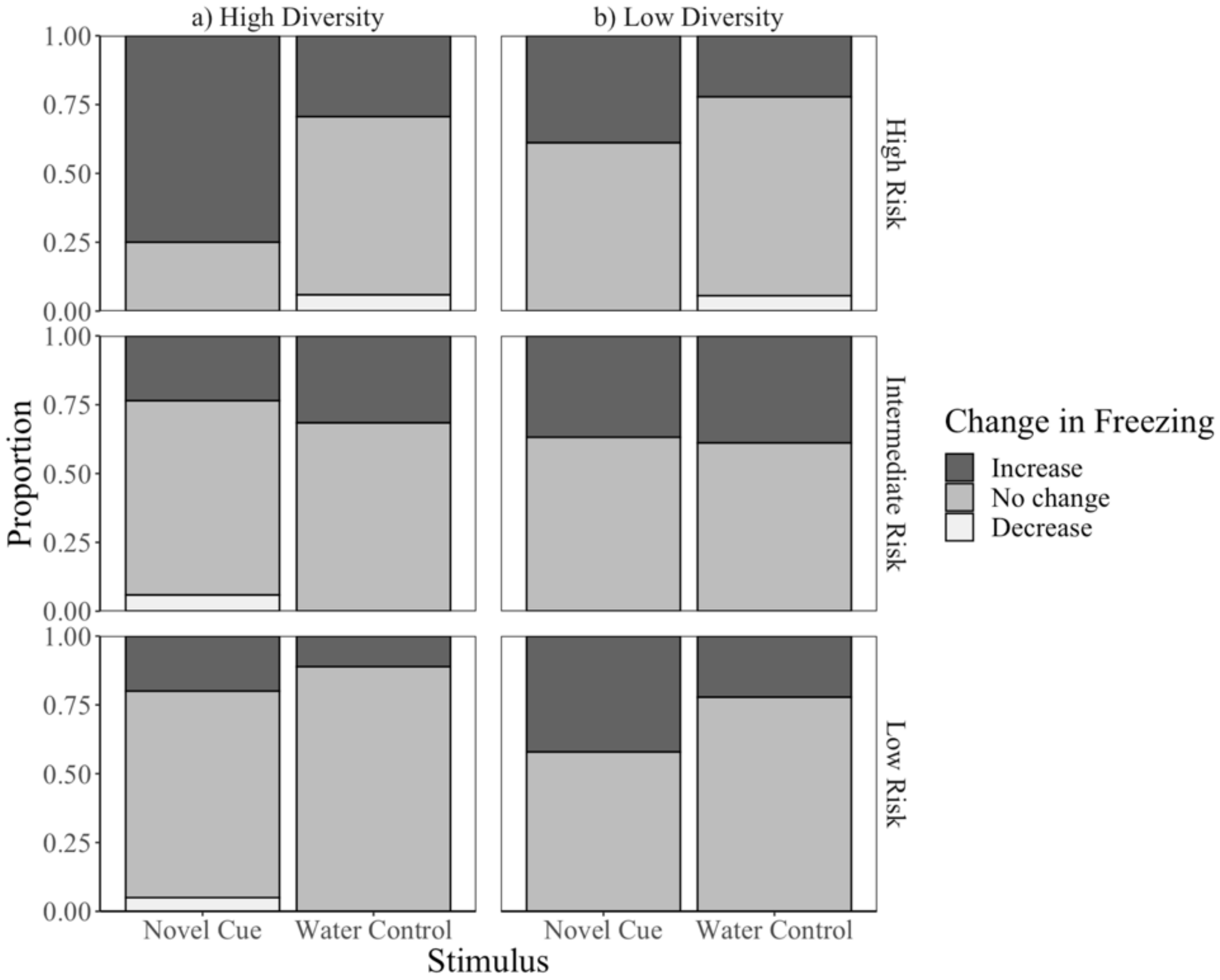


**Figure 4.5:** Stacked bar plot showing the proportion of increase, no change, or decrease in freezing (post – pre stimulus observation periods), across high (a) and low predator diversity (b) treatments, for each risk treatment (high, intermediate, or low), to each stimulus (water control and novel cue).

**Table 4.1:** Linear mixed-effect model output for proportional change in area use, including Satterthwaite *F* tests on fixed effects (a), statistics for random effects (b), and Tukey *post hoc* pairwise comparisons of risk treatments in response to a water control (c), and novel cue (d). Risk treatment included high-risk (HR), intermediate-risk (IR), or low-risk (LR), diversity treatment was either high- or low-diversity, and stimulus was either a novel cue or water control. Significant terms are in bold type, marginally non-significant terms are denoted with " · ". Degrees of freedom and *p* values estimated using Satterthwaite's approximations.

| Condition | *Sum Sq* | *Mean Sq* | *df* | *F* | *p* |
|---|---|---|---|---|---|
| a) Fixed Effects | | | | | |
| Risk | 0.0333 | 0.0167 | 2, 11.917 | 0.4897 | 0.625 |
| Diversity | 0.0074 | 0.0074 | 1, 11.917 | 0.2175 | 0.649 |
| Stimulus | 0.0019 | 0.0019 | 1, 197.455 | 0.0545 | 0.816 |
| Risk × Diversity | 0.0632 | 0.0316 | 2, 11.917 | 0.9290 | 0.422 |
| Risk × Stimulus | 0.2425 | 0.1212 | 2, 197.456 | 3.5622 | **0.030** |
| Diversity × Stimulus | 0.0246 | 0.0246 | 1, 197.455 | 0.7218 | 0.397 |
| Risk × Diversity × Stimulus | 0.0711 | 0.0355 | 2, 197.455 | 1.0440 | 0.354 |

| b) Random Effects | *Variance* | *SD* |
|---|---|---|
| Conditioning Block (Intercept) | 0.002138 | 0.04623 |
| Residual | 0.034035 | 0.18449 |

| | *Mean difference* | *SE* | *df* | *t* | *95% CI* | *p* |
|---|---|---|---|---|---|---|
| c) Water control | | | | | | |
| HR - IR | 0.0466 | 0.0511 | 29.5 | 0.912 | -0.079 – 0.172 | 0.637 |
| HR - LR | 0.0021 | 0.0513 | 30.0 | 0.041 | -0.124 – 0.129 | 0.999 |
| IR - LR | -0.0444 | 0.0508 | 28.9 | -0.875 | -0.170 – 0.081 | 0.660 |
| d) Novel cue | | | | | | |
| HR - IR | 0.1164 | 0.0506 | 28.5 | -2.301 | -0.242 – 0.009 | 0.072 · |
| HR - LR | -0.0713 | 0.0499 | 26.9 | -1.430 | -0.195 – 0.052 | 0.340 |
| IR - LR | 0.0451 | 0.0504 | 28.0 | 0.896 | -0.078 – 0.167 | 0.647 |

*Model equation:*

Proportional Change in Area Use ~ Risk*Diversity*Stimulus + (1 | Conditioning Block)

Model includes 221 observations and 18 conditioning blocks.

**Table 4.2:** *Post hoc* pairwise comparisons on proportional change in area use, for differences in response to a novel cue (NC) and water control (W), for Risk × Diversity treatments including: High Risk × High Diversity, High Risk × Low Diversity, Low Risk × High Diversity, and Low Risk × Low Diversity. Degrees of freedom and *p* values estimated using Satterthwaite's approximations, with Holm corrections. Significant terms are in bold type.

| | *Mean difference* | *SE* | *df* | *t* | *95% CI* | *p* |
|---|---|---|---|---|---|---|
| NC - W | | | | | | |
| High Risk × High Diversity | -0.1542 | 0.0609 | 197 | - 2.534 | -0.308 – 0.001 | **0.048** |
| High Risk × Low Diversity | 0.0051 | 0.0601 | 199 | 0.084 | -0.147 – 0.157 | 1.000 |
| Low Risk × High Diversity | -0.0150 | 0.0615 | 197 | -0.244 | -0.170 – 0.140 | 1.000 |
| Low Risk × Low Diversity | -0.0275 | 0.0607 | 197 | -0.452 | -0.180 – 0.126 | 1.000 |

**Table 4.3:** Linear mixed-effect model output for the transformed proportional change in shoaling index, including Satterthwaite *F* tests on fixed effects (a), statistics for random effects (b), and Tukey *post hoc* pairwise comparisons of risk treatments in response to a water control (c), and novel cue (d). Risk treatment included high-risk (HR), intermediate-risk (IR), or low-risk (LR), diversity treatment was either high- or low-diversity, and stimulus was either a novel cue or water control. Significant terms are in bold type, marginally non-significant terms are denoted with " · ". Degrees of freedom and *p* values estimated using Satterthwaite's approximations.

| Condition | *Sum Sq* | *Mean Sq* | *df* | *F* | *p* |
|---|---|---|---|---|---|
| a) Fixed Effects | | | | | |
| Risk | 0.0704 | 0.0352 | 2, 12.180 | 1.2369 | 0.324 |
| Diversity | 0.0156 | 0.0156 | 1, 12.181 | 0.5486 | 0.473 |
| Stimulus | 0.0974 | 0.0974 | 1, 197.608 | 3.4222 | 0.066 · |
| Risk × Diversity | 0.0235 | 0.0117 | 2, 12.180 | 0.4122 | 0.671 |
| Risk × Stimulus | 0.2657 | 0.1329 | 2, 197.609 | 4.6676 | **0.010** |
| Diversity × Stimulus | 0.0411 | 0.0411 | 1, 197.608 | 1.4450 | 0.230 |
| Risk × Diversity × Stimulus | 0.0308 | 0.0154 | 2, 197.609 | 0.5409 | 0.583 |
| b) Random Effects | | | | *Variance* | *SD* |
| Conditioning Block (Intercept) | | | | 0.002668 | 0.05165 |
| Residual | | | | 0.028462 | 0.16871 |

| | *Mean difference* | *SE* | *df* | *t* | *95% CI* | *p* |
|---|---|---|---|---|---|---|
| c) Water control | | | | | | |
| HR - IR | -0.0358 | 0.0497 | 26.5 | -0.719 | -0.159 – 0.088 | 0.754 |
| HR - LR | -0.0091 | 0.0500 | 26.9 | -0.182 | -0.133 – 0.115 | 0.982 |
| IR - LR | 0.0267 | 0.0495 | 26.0 | 0.539 | -0.096 – 0.150 | 0.853 |
| d) Novel cue | | | | | | |
| HR - IR | 0.1143 | 0.0493 | 25.7 | 2.316 | -0.008 – 0.237 | 0.072 · |
| HR - LR | 0.1362 | 0.0487 | 24.4 | 2.797 | 0.015 – 0.258 | **0.026** |
| IR - LR | 0.0220 | 0.0491 | 25.2 | 0.447 | -0.100 – 0.144 | 0.896 |

*Model equation:*

Proportional Change in Shoaling Index ~ Risk*Diversity*Stimulus + (1 | Conditioning Block)

Model includes 221 observations and 18 conditioning blocks.

**Table 4.4:** Cumulative mixed-effect model output for change in freezing, including Likelihood Ratio $\chi^2$ tests on fixed effects (a), statistics for random effects (b), and thresholds for decreasing to no change in freezing, and no change to increasing freezing. Estimates for thresholds correspond to a latent variable scale produced by the model. Significant terms are in bold type. Degrees of freedom and *p* values estimated using Laplace approximations.

| Condition | *df* | $\chi^2$ | *p* |
|---|---|---|---|
| a) Fixed Effects | | | |
| Risk | 2 | 3.0598 | 0.217 |
| Diversity | 1 | 0.1747 | 0.676 |
| Stimulus | 1 | 4.3296 | **0.037** |
| Risk × Diversity | 2 | 6.4799 | **0.039** |
| Risk × Stimulus | 2 | 6.9874 | **0.030** |
| Diversity × Stimulus | 1 | 0.0000 | 0.995 |
| Risk × Diversity × Stimulus | 2 | 1.8095 | 0.405 |
| b) Random Effects | *Variance* | | *SD* |
| Conditioning Block (Intercept) | 0.04397 | | 0.2097 |
| c) Thresholds | Estimate | SE | z |
| Decreasing \| No change | -6.1211 | 0.7637 | -8.015 |
| No Change \| Increasing | -1.1125 | 0.5341 | -2.083 |

*Model equation:*
Change in Freezing ~ Risk*Diversity*Stimulus + (1 | Conditioning Block)

Link function = “logit”
Model includes 221 observations and 18 conditioning blocks.

**Table 4.5:** Holm-corrected *post hoc* pairwise comparisons of change in freezing responses to a novel cue (NC) and water control (W) for each risk × diversity treatment. Estimates correspond to a latent variable scale produced by the model. Significant terms are in bold type.

| | *Estimate* | *SE* | *df* | *Z ratio* | *95% CI* | *p* |
|---|---|---|---|---|---|---|
| NC - W | | | | | | |
| High Risk × High Diversity | 2.1534 | 0.763 | Inf | 2.823 | -0.141 – 4.17 | **0.029** |
| High Risk × Low Diversity | 0.9804 | 0.748 | Inf | 1.311 | -0.992 – 2.95 | 0.949 |
| Intermediate Risk × High Diversity | -0.6180 | 0.758 | Inf | -0.816 | -2.617 – 1.38 | 1.000 |
| Intermediate Risk × Low Diversity | -0.0837 | 0.670 | Inf | -0.125 | -1.851 – 1.68 | 1.000 |
| Low Risk × High Diversity | 0.2802 | 0.812 | Inf | 0.345 | -1.862 – 2.42 | 1.000 |
| Low Risk × Low Diversity | 0.8699 | 0.705 | Inf | 0.345 | -0.990 – 2.73 | 0.949 |

# VI. Chapter 5: *Microhabitat conditions drive uncertainty of risk and shape neophobic responses*[8]

**Preface:** In the previous four chapters, I examined the influence of prey experience during risk information detection, as well as the source of risk information, on neophobic responses. Having addressed how these components of the information transfer pathway might shape NPA, I next assess how environmental noise shapes NPA. Specifically, I examine how some microhabitat factors, and variability of these factors, are linked to neophobic responses in wild guppies in natural settings.

## 5.1 Introduction

Prey optimize their fitness by integrating and responding to publically available information (i.e., cues) in order to assess predation risk (Lima and Dill 1990; McNamara and Dall 2010). Some of these cues may be unreliable in that they do not consistently correlate with a particular event (Searcy and Nowicki 2005). Such unreliable cues may signify a novel predator or a novel foraging opportunity, and prey lacking innate responses or prior experience with such unreliable novel cues would suffer from an inability to exhibit appropriate behavioural responses. Over time, prey may gain experience with these previously unknown cues, and learn this cue is associated with risk or a foraging opportunity. However, learning novel cues can be costly in terms of the time spent gathering information, and risking mortality when learning about a cue that conveys a predation risk (Dall et al. 2005; Ferrari et al. 2007; McNamara and Dall 2010).

Neophobia, or the fear of novel stimuli, is thought to allow prey to respond to novel potential threats without the initial costs of learning (Brown et al. 2013). Eventually, the antipredator response is lost or retained as prey learn whether the novel cue indicates risk (Brown et al. 2015; Feyten, Demers, Ramnarine, Chivers, et al. 2019; Crane et al. 2020). It has therefore been suggested that neophobia allows prey to deal with uncertainty of predation risk, or the inability to predict risk due to incomplete information (Brown et al. 2013; Feyten and Brown 2018). Recent studies have demonstrated that neophobia is elicited under conditions of elevated predation risk (Brown et al. 2013; Feyten et al. 2022). Predator composition (diversity) also has an effect, albeit a weaker one, on the expression of neophobia among prey (Feyten et al. 2022). Recent studies have also highlighted the importance of information availability and reliability in shaping neophobia (Feyten, Demers, Ramnarine, and Brown 2019; Feyten, Demers, Ramnarine, Chivers, et al. 2019; Feyten et al. 2021).

Information availability may be modified by habitat conditions (Weissburg et al. 2014), therefore we might expect neophobic responses in prey to be shaped by environmental conditions. This interference may be attributed to environmental “noise”, which has often been defined as background patterns or stimuli which interfere with the detection or response to cues of interest and which can occur in several modalities (Koops 1998; McNicol 2004; Brumm 2013). However, noise may also be described as any unwanted or unintended additions to information, including distortions or transmission errors, which generate greater uncertainty (Shannon 1948). Environmental conditions which generate noise may therefore interfere with prey risk assessment, leading to decreased predictability of predation risk and increased uncertainty of risk

---

[8] A version of Chapter 5 is in review as: Feyten, LEA, Ramnarine, IW, and Brown, GE. Microhabitat conditions drive uncertainty of risk and shape neophobic responses in Trinidadian guppies, *Poecilia reticulata*.

and neophobic responses (Figure 5.1). For example, increased stream velocity may contribute to uncertainty via its effect on the transmission (and detection) of chemical information in aquatic systems (Weissburg and Zimmer-Faust 1993). Similarly, the dimensions of a habitat have been suggested to affect the dilution of chemical information (Dickey and McCarthy 2007), and regardless of modality, relevant information likely attenuates over distances (Weissburg et al. 2014) and needs to travel further to reach prey. As a result, prey in larger microhabitats may be left uncertain of risk if they are unable invest sufficient time and energy into gathering information regarding risks. Moreover, larger and/or deeper local habitats may accommodate a larger density and diversity of predators (Harvey and Stewart 1991; Tejerina-Garro et al. 2005), which would likely further decrease predictability of risk (Feyten et al. 2022).

Another likely environmental variable affecting information availability and uncertainty of risk is microhabitat complexity. Although microhabitat complexity may offer more refuge to prey and decrease encounter rates with predators (Gratwicke and Speight 2005; Ross et al. 2007; Kovalenko et al. 2012; Komyakova et al. 2013; Nunes et al. 2015), it may also provide predators with refuge and impair visual or chemical information transmission (Golub et al. 2005; Dolinsek et al. 2007; Rilov et al. 2007). The latter is supported with evidence linking increased microhabitat complexity with increased predation success of ambush predators such as pike (*Esox lucius*; Greenberg et al. 1995) and bluegill sunfish (*Lepomis macrochirus*; Crowder and Cooper 1982). A recent laboratory study on fathead minnows (*Pimephales promelas*) demonstrated that microhabitat complexity reduces baseline fear, but failed to demonstrate a strong effect on neophobic responses (Crane et al. 2020). However, this study used captive-bred fish and did not assess the effect of complexity in a natural setting.

In addition to average values of these environmental variables, we might expect variability of these environmental factors (water velocity, microhabitat dimensions, and microhabitat complexity) within each microhabitat to decrease the predictability of risk (Koops 1998). Indeed, behavioural flexibility has been suggested as beneficial in changing environments (Reader 2003; Sol 2009; Tebbich and Teschke 2014; Dunlap and Stephens 2016). In the same vein, habitat substrate heterogeneity may shape predictability of risk, with heterogeneous habitats being less predictable and more uncertain than homogeneous habitats (Schmidt et al. 2010). Alternatively, heterogenous or diverse habitats may provide prey with more contextual clues in terms of physical landmarks, improving their spatial cognitive abilities when predicting where risk might occur (White and Brown 2015; Schmidt et al. 2022) and resulting in reduced neophobia compared to prey from homogeneous habitats.

Previous studies have attempted to link habitat complexity and habitat variability to a variety of neophobic behaviours including exploratory behaviour in novel environments, neophobic foraging, and object neophobia with mixed results (Mettke-Hofmann et al. 2002; Tebbich and Teschke 2014; Morand-Ferron et al. 2019; De Meester et al. 2021; Jenkins et al. 2021; Aceves-Fonseca et al. 2022). However, we are not aware of any studies linking the average and variance of specific habitat conditions with neophobia under contexts of elevated predation nor under natural conditions. In order to assess which environmental factors contribute to uncertainty and neophobic responses in Trinidadian guppies (*Poecilia reticulata*), we conducted *in situ* field experiments to determine how substrate heterogeneity and diversity, and the average and variance of stream velocity, microhabitat dimensions, and substrate complexity within a pool might influence two measures of neophobic antipredator responses.

## 5.2 Methods

### *5.2.1 Testing sites*

We conducted *in situ* observations in a series of discrete pools, with a distance of at least 10 m between test pools, along a ~ 1 km reach of the Lopinot River in April 2017 and the Acono River in April 2018 (Figure 5.2) in the Trinidadian Northern Range. A discrete pool is defined as a pool separated from others by fast-moving ripples, waterfalls, and/or rock boundaries, which limit the movement of guppies. Both the Lopinot and Acono Rivers are known high-predation sites, with abundant populations of pike cichlids (*Crenicichla* sp.), wolf fish (*Hoplias malabaricus*), blue acara (*Andinocara pulcher*), and brown coscarob (*Cichlasoma taenia*; Deacon et al. 2018). We conducted our experiments in both these sites in order to account for possible population-specific effects. Both these populations experience elevated risk, and therefore should exhibit neophobic predator avoidance (Brown et al. 2013). Indeed, neophobia in guppies originating from this site at the Lopinot has been well documented (Feyten, Demers, Ramnarine, and Brown 2019; Feyten et al. 2021), and we expected this to be the case for the Acono population as well. We sampled 27 discrete pools within the Lopinot River, and 15 discrete pools in the Acono River.

### *5.2.2 Experimental Protocol*

Within each test pool, we assessed neophobic behaviours of guppies and environmental conditions, moving upstream in order to avoid testing a pool after disturbance had occurred upstream. First, we conducted two assessments of neophobic behaviour, including latency to inspect a novel predator model, as well as latency to enter a novel foraging arena. These behavioural measures were based on recent *in situ* experiments testing neophobia and perceived predation risk (Brown et al. 2013; Elvidge et al. 2016). We observed these behaviours 3 times within each testing pool and averaged the response latencies within each pool to avoid pseudo-replication. We had three exceptions to this, where we were only able to sample latency to forage once in one pool, and twice in two pools.

For latency to inspect a novel predator model, we placed our model in an area where at least 5 guppies were observed within a 50 cm radius. The novel predator model was a 3D printed polycarbonate model sprayed with Rust-Oleum® Painter’s Touch® Ultra Cover Primer, hand-painted with Pebeo® High Viscosity Studio Acrylic paint, and sealed with Rust-Oleum® Painter’s Touch® Ultra Cover Clear Gloss. The predator model was designed to represent a novel predator resembling a carp or goldfish painted in bright colours. We attached the model to a wooden dowel (~1 m in length) using transparent fishing line, allowing controlled placement of the model (Figure 5.3). We made observations from the shoreline, with observation sites in each pool located at least 1 m apart. We recorded latencies of guppies to inspect the predator model, for a maximum trial time of 5 min. An inspection was defined as a guppy or group of guppies making a directed, saltatory approach towards the model (Dugatkin and Godin 1992; Brown et al. 2010). Risk-averse behaviour is indicated by an increased latency to inspect (Brown et al. 2010; Brown et al. 2013).

For latency to enter a novel foraging arena, we gently placed a novel foraging arena (white corrugated plastic; ~30×25×23 cm) into stream pools at locations that visibly contained at least 5 guppies in locations without visible currents or higher velocities. The arena was partially submerged (~10 cm depth), and a rock was placed inside to prevent the arena from moving due to the stream current. We then added a small amount (~0.5 g) of food (OMEGA™ One Freshwater Flakes). We recorded the latency for guppies to enter the arena (crossing the dashed line; Figure 5.4), for a maximum trial time of 5 min. Risk-averse behaviour was indicated by an increased latency to enter the novel arena (Elvidge et al. 2016; Feyten et al. 2021). We feel confident attributing increased latencies to enter a novel foraging arena to greater neophobic antipredator responses in higher complexity habitats, especially since the literature suggests complex environments (i.e., those with macrophytes) encourages exploratory behaviour in guppies under risk-free laboratory conditions (Camacho-Cervantes et al. 2015).

### *5.2.3 Environmental Variables*

Immediately after the behavioural assessments, we measured environmental conditions within the test pool. Environmental conditions measured included pool depth and width, pool area, water velocity, substrate complexity (rugosity), and substrate type. We measured pool depth five times: at the upper and lower part of the pool, as well as the middle in the left bank, center, and right bank. We measured pool width thrice at the upper, middle, and lower portion of the pool. We measured water velocity at the surface of the pool, as well as at 40% depth (mid-depth) of the pool. These were measured five times, in similar fashion to pool depth. We measured substrate complexity (rugosity) by systematically placing a chain of 1m up to 5 times in each pool. We then measured the linear distance between the ends of the chain after it was placed on the substrate. We calculated the ratio of this linear distance to the original chain length of 1 m, and obtained a rugosity value by subtracting this ratio from 1 (Aronson and Precht 1995; Leduc et al. 2007; Figure S5.1). This resulted in a rugosity variable that increased with higher microhabitat complexity. We then calculated the average and variance of depth, width, surface velocity, mid-depth velocity, and rugosity for each pool (See Table 5.2 for complete list of environmental variables). There were six exceptions in the Lopinot river, where we failed to collect velocity data. We imputed the missing average and variance velocity values using the respective averages from the Lopinot population. We categorized the substrate type in a pool systematically up to 5 times using a grid frame (Figure S5.2). We categorized substrate type as sand, fine (<1 cm), coarse (1-3 cm), cobble (>3cm), hard (smooth rock/granite), or leaf litter. We calculated the % cover of the grid frame for each substrate type, and from these values, we calculated several diversity measures. We calculated a measure of heterogeneity, Hurlbert's PIE (Probability of Interspecific Encounter; Hurlbert 1971), or the probability that two grid sections selected at random without replacement from a pool belong to different substrate categories. We also calculated alpha diversity (number of substrate types present) for each grid sample, and used the pool average of these alpha diversity values in the data analysis. We calculated our substrate metrics using the *hpie()* function from the **benthos** package (Walvoort 2022), and the *specnumber()* function in the **vegan** package (Oksanen et al. 2022) in R. Larger values in substrate heterogeneity and average alpha diversity indicate potentially greater uncertainty in the environment. Given the nature of these metrics we included these substrate measures in the "variance" category.

### *5.2.4 Statistical Analyses*

Given the large number of potential explanatory variables, we conducted data reduction using Principal Components Analyses (PCA) with the *PCA()* function from the **FactoMineR** package in R (Lê et al. 2008). We conducted PCAs on two environmental variable categories, “average” and “variance”. Before conducting the PCAs, we checked the asymmetry and normality of our explanatory variables. We BoxCox transformed the explanatory variables which did not meet the assumption of normality in order to improve the symmetry of their distribution. We calculated the estimated transformation parameter (lambda) for each variable using *boxcoxfit()* from the **geoR** package in R (Ribiero et al. 2022). We added a small constant (0.0000001) to any variables which included zeros, namely the velocity variables. The environmental data was then standardized to account for the difference in unit measure (to get rid of the magnitude differences between variables), using *decostand()* in the **vegan** package of R (method= “standardize”; Oksanen et al. 2022). From each of the PCAs, we retained 2 principal components to incorporate into further models. We decided to keep an equal number of components for each category in order to ensure average and variance of environmental variables had equal representation in our models, with a threshold of the components explaining at least 45% of the variance of our explanatory variables.

Once we extracted components from the average and variance PCAs, we included these components as explanatory variables in three global models. In order to control for potential population differences, we included population as a random factor in all our models. Given that the populations were not visited during the same year, this population term also served to control for potential effects associated with year. For latency to inspect a novel predator model, we conducted a global linear mixed model (LMM, n = 42). For latency to enter a novel foraging arena, many trials had the maximal trial latency (i.e., guppies did not enter the arena within the 5 min observation period). More specifically, no guppies entered the novel foraging arena in any of the replicated trials in roughly 21% of the tested pools. For this reason, we first conducted GLMMs using a binomial distribution to determine how environmental variables might shape whether guppies entered the novel arena (n = 42). We then followed with a LMM to determine how environmental variables might shape latencies to enter the novel arena for trials in which guppies did enter the novel arena (n = 33). For both our LMMs, our models did not violate the assumption of normality of residuals. However, our latency to inspect model had heteroscedastic residuals. As such, we ran the LMM using BoxCox-transformed latency to inspect. We calculated the estimated transformation parameter (lambda) for this response variable using *boxcoxfit()* from the **geoR** package in R (Ribiero et al. 2022).We also checked multicollinearity of our explanatory components in each model using the *vif()* function from the **car** package (Fox and Weisberg 2019). We used the *lmer()* or *glmer()* functions from the **lme4** package in R (Bates et al. 2015) for our LMM and GLMM models, respectively.

All analyses were conduction using R-Studio version 4.2.2.

## 5.3 Results

### *5.3.1 Environmental Principal Components*

Prior to conducting PCAs, we transformed several of our explanatory variables including pool length, width, width variance, depth, depth variance, Hurlbert’s PIE (heterogeneity), surface velocity, and mid-depth velocity. The transformed mid-depth and surface velocity variables were more symmetrical but still differed significantly from normal ($p < 0.01$ for both). Similarly, the velocity variance metrics were significantly different from normal but were the most symmetrical without transformation, and therefore we used the untransformed variables in the subsequent PCA.

For the variation explained in environmental "average" variables, we retained only the first 2 Principal Components for reasons discussed above. Together, the first 2 PCs represented 57.37% of the total variance in the environmental data, the first representing 37.99 % of the variation, and the second 19.38% (Table 5.1a). The loadings, square loadings, and contribution of each variable for the first 2 PCs were described in Table 5.2a. The variables contributing most to the first component were mid-depth velocity, surface velocity, pool width, rugosity, and depth (which had 73.27%, 62.97%, 32.26%, 25.49%, and 24.71% of their variation explained by the first component, respectively; Table 5.2a), and this component is hereafter referred to as Velocity-Complexity. Mid-depth velocity, surface velocity, and rugosity were all highly positively correlated with Velocity-Complexity (0.8560, 0.7936, and 0.5049, respectively), while width and depth were negatively correlated with Velocity-Complexity (-0.5679 and -0.4971, respectively; Figure S5.3). Thus, positive values in Velocity-Complexity were characterized by high mid-depth velocity, high surface velocity, and high rugosity, while negative values in Velocity-Complexity were characterized by high pool depth and high pool width. The variables that contributed most to the second component were length and depth (which had 49.33% and 32.66% of their variation explained by the second component, respectively Table 5.2a), and this component is hereafter referred to as Pool Dimension. Length and depth were highly positively correlated with Pool Dimension (0.7023 and 0.5715; Figure S5.3). Rugosity and surface velocity were also positively correlated with Pool Dimension (0.3279 and 0.3296, respectively), however these variables were poorly represented with each variable having around 10% of their variation explained in this component. Positive values in Pool dimension were thus characterized by high values of length and depth.

For the variation explained in environmental "variance" variables, we retained only the first 2 Principal Components. Together, the first 2 PCs represented 47.52% of the total variance in the environmental data, the first representing 27.40 % of the variation, and the second 20.12% (Table 5.1b). The loadings, square loadings, and contribution of each variable for the first 2 PCs were described in Table 5.2b. The variables contributing most to the first component were substrate heterogeneity and average alpha diversity, as well as mid-depth velocity variance and pool width variance (which had 60.40%, 59.53%, 31.29%, and 24.77% of their variation explained by the first component, respectively; Table 5.2b). This component is hereafter referred to as Substrate Diversity. Substrate heterogeneity, average alpha diversity, and mid-depth velocity variance were highly positively correlated with Substrate Diversity (0.7772, 0.7715, and 0.5593, respectively), while pool width variance was negatively correlated with Substrate Diversity (-0.4977; Figure S5.4). Thus, positive values in Substrate Diversity were characterized by high substrate heterogeneity, high average alpha diversities, and high mid-depth velocity variation, while negative values in Substrate Diversity were characterized by high pool width variance. The variables that contributed most to the second component were pool depth variance and pool width variance (which had 52.37% and 40.38% of their variation explained by the second component, respectively; Table 5.2b). This component is hereafter referred to as Dimension Variance. Pool depth variance and pool width variance were highly positively correlated with Dimension Variance (0.7237 and 0.6355, respectively; Figure S5.4). Mid-depth velocity variance and substrate heterogeneity also contributed to this component, and were correlated positively with it (0.4356 and 0.3845, respectively), but were not well-represented with 18.97% and 14.78% of their variation explained (Table 5.2b). Positive values in Velocity Variance were thus characterized by high values of pool depth variance and pool width variance.

### *5.3.2 Latency to inspect*

Our GLMM revealed that the Velocity-Complexity component best explained the latency to inspect a novel predator model, where an increase in Velocity-Complexity resulted in increased latencies to inspect ($p = 0.0153$, $df = 37$, $t = 2.544$; Figure 5.5, Table 5.3). In other words, guppies increased their latencies to inspect a novel predator model (i.e., were more neophobic) when their microhabitats had high mid-depth and surface velocities, complex substrate, as well as shallow and narrow dimensions.

### *5.3.3 Latency to enter*

We found that none of our components significantly explained whether guppies entered a novel foraging arena ($p > 0.23$ for all components; Table 5.4).

The LMM on latencies to enter a novel foraging arena, in trials where guppies did enter, revealed that our Substrate Diversity component best explained when guppies entered, where an increase in Substrate Diversity resulted in decreased latencies to enter ($p = 0.001$, $df = 28$, $t = -3.658$; Figure 5.6, Table 5.5). In other words, guppies were quicker to enter a novel foraging arena in microhabitats with larger values in substrate heterogeneity, average substrate alpha diversity, and variance in mid-depth velocity, and with lower values of pool width variance.

## 5.4 Discussion

Our results suggest that several environmental variables, including water velocity, microhabitat complexity, pool width and depth, as well as substrate diversity and heterogeneity, shape uncertainty of risk and neophobic predator avoidance in guppies from high-risk environments. Interestingly, we found that the "average" and "variance" categories differentially affected our two neophobic metrics. One of the "average" category components shaped neophobic predator inspection behaviours, whereas one of the "variance" category components shaped how prey engage with novel foraging opportunities. This highlights the importance of examining the effects of both "average" and "variance" environmental categories, which is generally lacking in the existing literature. Moreover, these results highlight potential differences in drivers of strictly predator-related neophobia and food-related neophobia. Future studies of neophobia should therefore continue to incorporate multiple forms neophobic responses. Although we found no effect of population in any of our analyses, we kept them in our models as a control for genetic differences and observation year. Given the lack of population effect, we feel comfortable extrapolating the effects of environmental habitats on neophobia to guppies generally experiencing "high-risk" contexts.

For latency to inspect a predator model, we found a significant positive effect of the Velocity-Complexity component. In other words, an increase in water velocity and microhabitat complexity, as well as a decrease in pool width and depth, resulted in greater latencies to inspect a novel predator model. The results for water velocity and microhabitat complexity fall in line with our predictions, where habitats with higher water velocity may result in chemosensory information that is often unavailable or quickly diluted (Weissburg and Zimmer-Faust 1993). Additionally, increased velocity potentially increases turbidity, which may interfere with the perception of visual risk information. Although we did not measure turbidity levels in our study, turbidity was uniformly low and did not differ visibly across our testing pools. Thus, visual risk information was unlikely to be affected by water velocity in our study, but this relationship should be noted for future studies. Similarly, in habitats with elevated substrate complexity, prey may be more uncertain of risk given visual obstruction (i.e., noise) in their environment. As a result, when microhabitats contain noise in the form of water velocity or substrate complexity, prey may generally be less likely to predict risk given a lack of experience with novel cues, in addition to having reduced ability to perceive cues or predict risk at any given time. Thus, in such environments under conditions of elevated risk, prey likely experience elevated uncertainty of risk and exhibit neophobic predator avoidance in order to err on the side of caution. Furthermore, given that individuals can use sensory complementarity to integrate cues from multiple modalities to more accurately assess risk and reduce uncertainty (Munoz and Blumstein 2012), microhabitats which interfere with at least one sensory modality should restrict such complementation and likely increase prey uncertainty of risk and neophobic responses.

Unlike our predictions that increased habitat dimensions may lead to greater uncertainty, we found that decreased habitat dimensions (width and depth) resulted in greater latencies to inspect novel predator models. Although deeper and larger pools generally have a greater number and diversity of predators (Harvey and Stewart 1991; Tejerina-Garro et al. 2005), perhaps such pools provide more refugia or cover for prey. Contrastingly, shallower and narrower pools may provide less cover or may decrease prey ability to escape, especially given that guppy movement between discrete pools is seasonally limited. Moreover, shallow pools may increase exposure to other guppy predators such as the semi-aquatic fishing spiders (including *Dolomedes* sp. and *Ancylometes bogotensis),* which situate themselves at the edge of a pool and use water surface disturbances and physical contact with dorsal fins to detect and capture prey (Bleckmann and Lotz 1987; Nyffeler and Pusey 2014; Deacon et al. 2015). Similarly, guppies in shallower pools may be more exposed to aerial predators such as fishing bats (*Noctilio leporinus*), which can rake pool surfaces with their claws or detect ripples at the waters' surface using echolocation (Brooke 1994; Magurran 2005). Increased risk of mortality in such smaller confined habitats may have caused individuals to err on the side of caution by expressing greater neophobia in response to a novel predator model. Another possible explanation is that larger habitats hold greater densities of conspecifics, which may provide a single individual with a greater probability of survival when all are under threat of predation (i.e., safety in numbers; Lehtonen and Jaatinen 2016). Although we ensured a minimum number of guppies were present before starting a trial, we did not measure guppy densities within each pool. Furthermore, increased guppy density may provide individuals with additional social information regarding risk or safety levels. Indeed, guppies use social information such as alarm cues to inform their decisions regarding risk and novelty (Brown et al. 2013). Guppies in high-risk environments also increasingly use disturbance cues (i.e., a cue released after disturbance from a predator or disruption) from familiar conspecifics to inform their antipredator decisions (Crane, Feyten, et al. 2020a). Moreover, guppies can perceive "safety" from calm conspecifics and accordingly reduce their neophobia towards a novel foraging arena (Feyten et al. 2021). Potentially, the novel model may have been perceived as safer in larger habitats given that the model was more likely to be surrounded by calm conspecifics. Therefore, we cannot discount that the observed decrease of neophobic predator inspection behaviours in larger pools may have been shaped by guppies a) being more informed (i.e., less uncertain) about general risk conditions due to increased availability of social cues, or b) having greater perceived safety due to safety in numbers or availability of social safety cues.

For latency to enter a novel foraging arena, although there were no good predictors for whether guppies entered, there was a significant negative effect of the Substrate Diversity component on latencies in trials where guppies did enter. Thus, somewhat surprisingly, increases in substrate heterogeneity and average substrate alpha diversity resulted in decreased neophobia. Perhaps, from a long-term standpoint, more heterogeneous environments promote ecosystem stability (Brown 2003; Schmidt et al. 2022), and consequently create more predictable risk. Schmidt et al. (2022) argue that when habitats have greater heterogeneity, individuals are more able to discriminate between options. In contexts of predation, perhaps greater substrate heterogeneity and diversity promotes prey ability to accurately assess location-specific risk. Potentially, prey in such habitats may be generally more certain of risk, spatially, and less likely to exhibit neophobia. Indeed, guppies exhibit lower neophobia when risk is spatially predictable compared to spatially unpredictable (Feyten, Demers, Ramnarine, Chivers, et al. 2019). This is in line with results from another study, which demonstrated that multiple species of gobies from more homogeneous habitats were slower to learn about reward locations and did not use visual landmarks as much as gobies from stable, more heterogeneous, complex habitats (White and Brown 2015). Furthermore, our results provide evidence of how habitat heterogeneity and complexity, which are often used interchangeably in the literature (Kovalenko et al. 2012), may exert differing effects on behaviours of interest. In our study, although substrate complexity resulted in an increase of one measure of neophobia, substrate heterogeneity resulted in a decrease of another neophobic measure.

Previous studies have attempted to link neophobia and habitat complexity, however often in the absence of risk or without accounting for potential differences in predation between high- and low-complexity sites. For example, a previous study on the closely related porthole livebearer (*Poecilipsis gracilis*) demonstrated no effect of habitat complexity (i.e., macrophyte presence) on latency to locate food in novel habitats, or latency to emerge, in fish that were bred, raised, and tested without predation risk (Aceves-Fonseca et al. 2022). Similarly, a prior study on a 61 parrot species (*Psittacidae*) demonstrated shorter food neophobic latencies in complex habitats, but did not consider the potential effects predation might have on this relationship (Greenberg and Mettke-Hofmann 2001). Another study on woodpecker finches (*Cactospiza pallida*) examined differences in neophobia based on habitat complexity and variability, but could not account for potential differences in predation between sites (Tebbich and Teschke 2014). By assessing how microhabitat variables shape neophobia at several sites within two high-risk populations, our study expands on this prior work while accounting for the overlooked, and potentially confounding, effect of predation risk. We did not assess the effects of microhabitat variables for guppies from low-predation sites, since guppies from these sites have been shown to lack neophobia (Brown et al. 2013; Feyten et al. 2021). To our knowledge, our study is also the first to directly assess the link between neophobia and habitat complexity, in addition to other environmental variables, *in situ*.

It is important to note that the effects of habitat complexity may not be straightforward. For example, habitat complexity has been shown to decrease anxious behaviour in zebrafish (*Danio rerio*; DePasquale et al. 2016), fearful behaviour in fathead minnows (Crane et al. 2020), and flight initiation distances in reef fishes (Nunes et al. 2015; Chan et al. 2019). There is likely an amount of habitat complexity that is the most advantageous based on the size of both prey and predator in that it provides refuge for small prey, without providing refuge for larger predators or obstructing information transfer (Almany 2004; Crane et al. 2020), potentially leading to greater uncertainty. If there is some optimal amount of habitat complexity for prey, the relationship between complexity and neophobia may not be a positive linear one. Although we did not assess a non-linear relationship between habitat complexity and neophobia in our study, it may be worth exploring how the scale of prey and predator compared to refuge size and habitat complexity size shapes this relationship in several study systems. Additionally, open habitats lead to more efficient foraging of pursuit predators and complex habitats lead to greater success of ambush predators (Crowder and Cooper 1982; Greenberg et al. 1995; Beukers and Jones 1998), thus predator foraging tactics may also influence how habitat complexity shapes neophobia. Future studies should therefore account for predator foraging tactics when assessing the effects of microhabitat complexity on prey behaviour.

Although we did not explore the cognitive mechanisms for our demonstrated link between habitat variables and neophobia, the literature suggests that environmental variables such as habitat complexity and heterogeneity shape cognitive spatial abilities. Several taxa of fish have been shown to have lower spatial learning and memory when raised in barren unenriched environments compared to when raised in complex enriched environments (Salvanes et al. 2013; Makino et al. 2015; Bergendahl et al. 2016; Roy and Bhat 2016; Carbia and Brown 2019; Zhang et al. 2021). Even variation in enrichment location and novel object presentation enhanced learning (DePasquale et al. 2016). As discussed above, gobies from stable spatially complex habitats were quicker to learn about reward locations and used both visual landmarks and turn directions, whereas gobies from dynamic homogeneous low-complexity habitats made more errors and relied more on turn direction to orient themselves (White and Brown 2015). This enhanced cognition and behavioural flexibility may be reflected with larger brain sizes. Indeed, guppies raised in laboratory settings have smaller brain sizes than wild-caught guppies, potentially due to differences in complexity (Burns et al. 2009). Larger brains may allow individuals to cope with changing environments (i.e., uncertainty) by allowing for enhanced cognition and behavioural flexibility (Sol 2009; De Meester et al. 2021). If individuals from complex environments have increased cognitive abilities and are more behaviourally flexible, neophobia may be an especially adaptive behavioural response between initial encounter with novelty and learning. Prey from high-risk and high-complexity environments may exhibit increased neophobia upon initial detection of novel information, but should be better able to learn and remember spatial risk compared to individuals in simple environments. Greater cognitive ability and behavioural flexibility may allow prey to quickly learn whether a novel cue is risky, and neophobia can rapidly be extinguished or can lead to rapid learning of the spatial nature of threat. Thus, fish from high-complexity environments may be better equipped to deal with novel risks, such as invasive predators, compared to their low complexity counterparts.

Future studies should also assess how other environmental conditions may contribute to uncertainty of risk and neophobia by interfering with information transmission. In aquatic environments, both turbidity and acidification would interfere with prey reception of visual and chemical cues, respectively, and may result in increased neophobic responses. For example, pH affects freshwater snail (*Physa acuta*) responses to olfactory predator cues (Cothran et al. 2021), and increased acidification increases antipredator responses to visual predator cues in Atlantic salmon (Elvidge et al. 2013). As such, we may expect acidification to play a role in uncertainty of risk, shaping neophobic responses in the absence of complementary visual information. Moreover, turbidity and acidity may both be present and interact to shape antipredator responses and neophobia. We suggest that future studies should carefully consider which neophobic metrics are used to assess these effects, given that turbidity has been demonstrated to decrease social behaviours such as shoaling, and a decrease in activity may be an effective antipredator strategy in turbid conditions (Borner et al. 2015). Future studies should also assess neophobia in terrestrial study systems, determining the effects of environmental variables which impede information transfer. Such variables might include those which impair visual information such as habitat complexity and heterogeneity, chemosensory information such as temperature and atmospheric CO2 (Roggatz et al. 2022), or auditory information such as vegetation and ambient noise (Weissburg et al. 2014) or anthropogenic noise pollution (Corcoran and Moss 2017). These future studies should consider that environmental variables which generate noise in one modality may also interfere with antipredator responses in other modalities (Morris-Drake et al. 2016). Additionally, neophobia may increase due to variability and unpredictability of risk in an environment stemming from seasonality, habitat degradation, and urbanization (Morand-Ferron et al. 2019; Ferreira and Faria 2021; Jenkins et al. 2021).

Our results have demonstrated the importance of studying both the average and variance of environmental variables on neophobic responses in two high-predation populations of the Trinidadian guppy. Our research adds to the theoretical framework of how uncertainty of risk shapes neophobia and arises due to prey experience with information and predator composition (Feyten, Demers, Ramnarine, Chivers, et al. 2019; Feyten, Demers, Ramnarine, and Brown 2019; Crane et al. 2020; Feyten et al. 2022), highlighting how uncertainty arising from environmental variables may interfere with information transfer (i.e., environmental noise) to shape neophobic antipredator responses. It is important to understand in which contexts prey may be neophobic, as it is an adaptive response to the potential introduction of novel, invasive, predators.

### 5.5 Figures & Tables

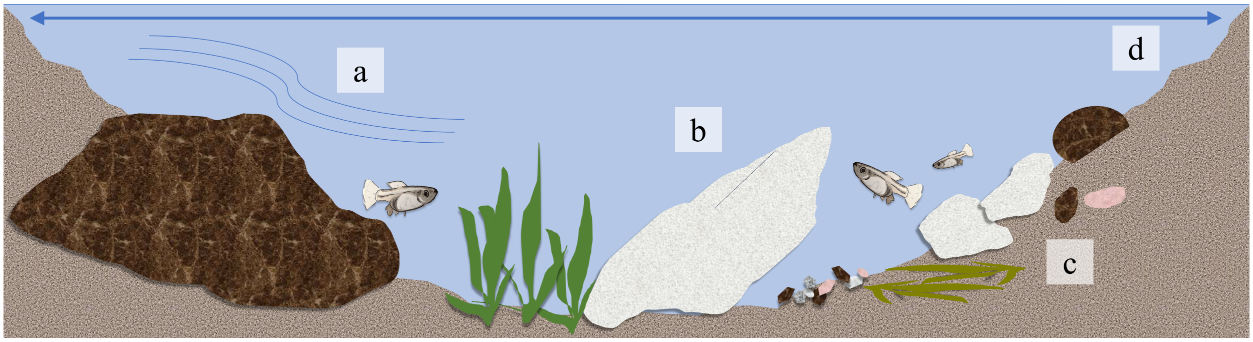


**Figure 5.1:** Variables within each microhabitat which may affect the availability of information to prey and predictability of risk, including a) water velocity, b) habitat complexity (i.e., rugosity), c) substrate diversity and heterogeneity, and d) pool dimensions.

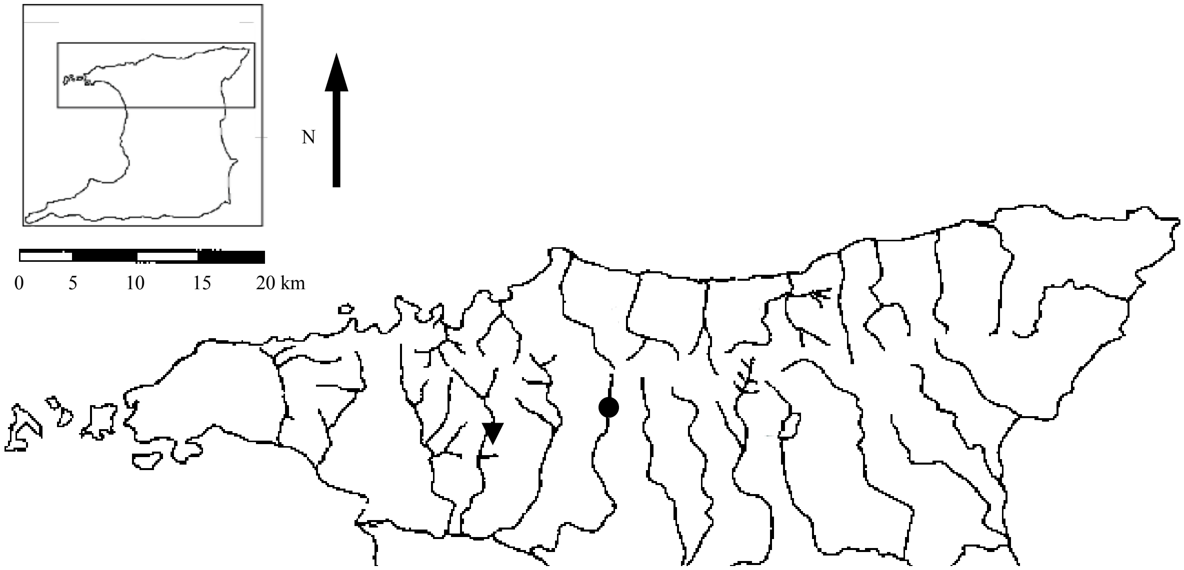


**Figure 5.2:** Map showing the location of our study site on the Acono (triangle) and Lopinot (dot) rivers in the Northern Range, Republic of Trinidad and Tobago.

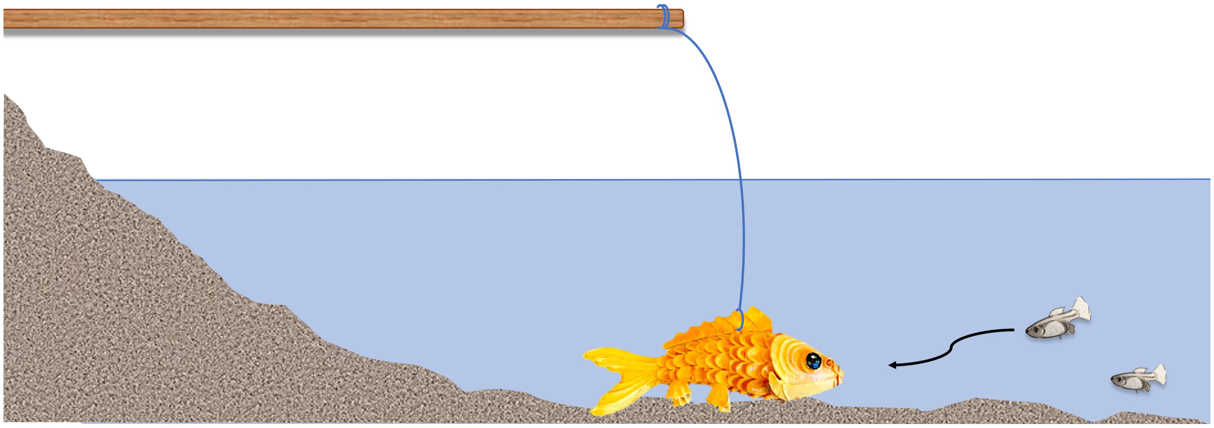


**Figure 5.3:** Schematic diagram of the 3D printed novel heterospecific predator model, 14 cm in length, attached to a dowel and placed gently along the banks of each testing pool.

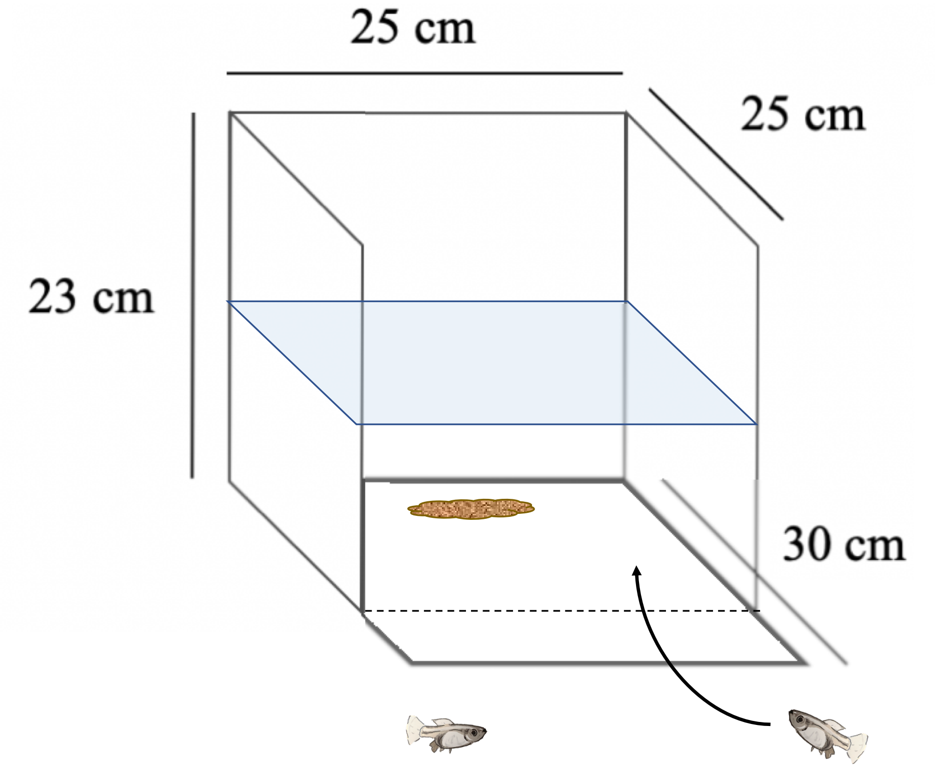


**Figure 5.4:** Schematic of novel foraging arena used for measuring latencies to enter a novel foraging arena, showing the delineation determined as an entry into the arena (dashed line) and the novel food (fish flakes) inside.

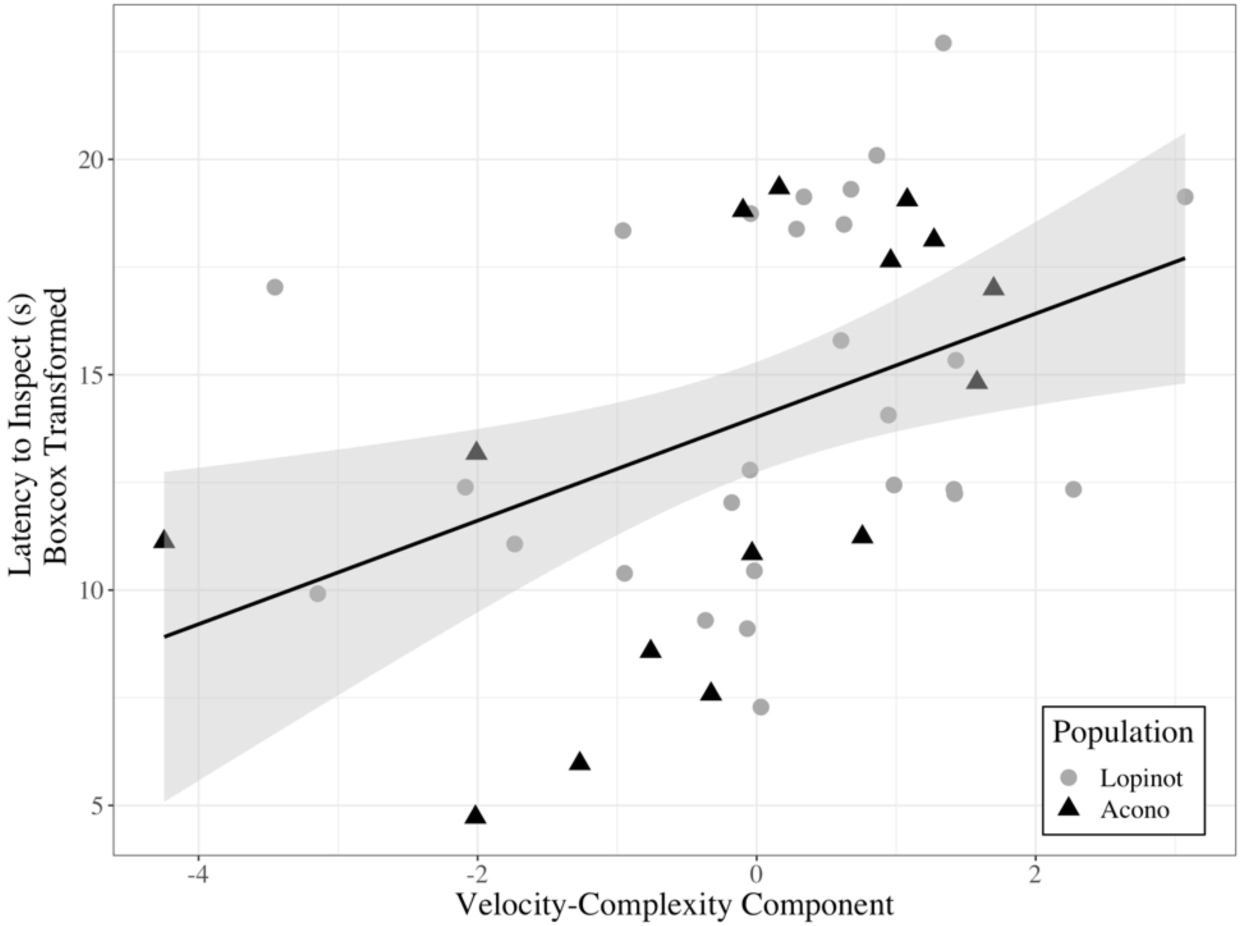


**Figure 5.5:** Relationship (± SE) between the BoxCox-transformed latency to inspect a novel predator model (s) and the Velocity-Complexity Component. The shape and colour of the data points indicate whether the observation came from the Lopinot (grey dot) or the Acono (black triangle) population.

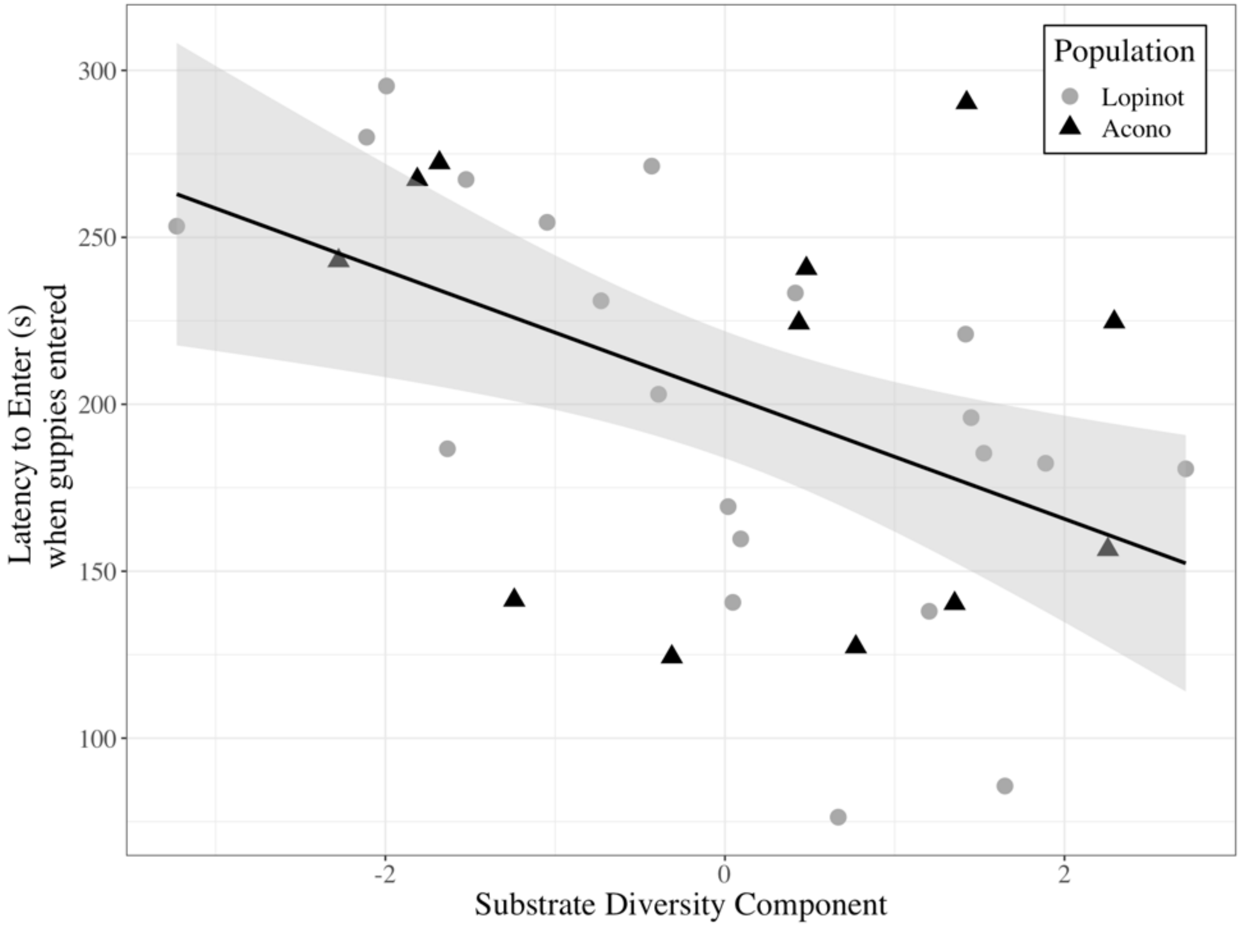


**Figure 5.6:** Relationship (± SE) between the latency to enter, when guppies entered, a novel foraging arena (s) and the Substrate-Diversity Component. The shape and colour of the data points indicate whether the observation came from the Lopinot (grey dot) or the Acono (black triangle) population.

**Table 5.1:** Eigenvalues of the first 3 Principal Components given by PCAs for the a) "average" and b) "variance" environmental variable category.

a)

| | | Components | | |
|---|---|---|---|---|
| | | **1** | **2** | **3** |
| PCA | Eigenvalues | 2.225 | 1.132 | 0.900 |
| | Cumulative Eigenvalues | 2.225 | 3.357 | 4.257 |
| | Cumulative Proportion of total variation | 37.99 | 57.32 | 72.68 |

b)

| | | Components | | |
|---|---|---|---|---|
| | | **1** | **2** | **3** |
| PCA | Eigenvalues | 1.918 | 1.408 | 1.057 |
| | Cumulative Eigenvalues | 1.918 | 3.326 | 4.383 |
| | Cumulative Proportion of total variation | 27.34 | 47.52 | 62.61 |

**Table 5.2:** Summary of the loadings, square loadings (quality representation of variable within PC), and contribution of each response variable in the first 2 PCs for a) "average" and b) "variance" environmental variable categories. In bold are the variable loadings of interest (where the square loadings ≥ 0.24) for the components retained for subsequent models.

a)

| Explanatory variables | | Components<br>1 | <br>2 |
|---|---|---|---|
| Rugosity | Loadings | **0.3344** | 0.3041 |
| | Square Loadings | 0.2549 | 0.1075 |
| | Contribution | 11.1838 | 9.2502 |
| Pool length (transformed) | Loadings | -0.2011 | **0.6514** |
| | Square Loadings | 0.0922 | 0.4933 |
| | Contribution | 4.0445 | 42.4262 |
| Pool width (transformed) | Loadings | **-0.3762** | 0.2348 |
| | Square Loadings | 0.3226 | 0.0641 |
| | Contribution | 14.1540 | 5.5145 |
| Pool depth (transformed) | Loadings | **-0.3292** | **0.5300** |
| | Square Loadings | 0.2471 | 0.3266 |
| | Contribution | 10.8399 | 28.0901 |
| Surface velocity (transformed) | Loadings | **0.5256** | 0.3057 |
| | Square Loadings | 0.6297 | 0.1086 |
| | Contribution | 27.6296 | 9.3446 |
| Mid-depth velocity (transformed) | Loadings | **0.5670** | 0.318 |
| | Square Loadings | 0.7327 | 0.0625 |
| | Contribution | 32.1481 | 5.3744 |

| b) | | Components | |
|---|---|---|---|
| Explanatory variables | | **1** | **2** |
| Rugosity variance | Loadings | -0.1432 | -0.1227 |
| | Square Loadings | 0.0393 | 0.0212 |
| | Contribution | 2.0469 | 1.5055 |
| Pool width variance (transformed) | Loadings | **-0.3594** | **0.5355** |
| | Square Loadings | 0.2477 | 0.4038 |
| | Contribution | 12.9146 | 28.6716 |
| Pool depth variance (transformed) | Loadings | -0.1708 | **0.6098** |
| | Square Loadings | 0.0559 | 0.5237 |
| | Contribution | 2.9163 | 37.1821 |
| Substrate Alpha Diversity | Loadings | **0.5571** | -0.1697 |
| | Square Loadings | 0.5953 | 0.0406 |
| | Contribution | 31.0414 | 2.8814 |
| Substrate Hurlbert's PIE (transformed) | Loadings | **0.5612** | 0.3239 |
| | Square Loadings | 0.6040 | 0.1478 |
| | Contribution | 31.4972 | 10.4941 |
| Mid-depth velocity variance | Loadings | **0.4039** | 0.3670 |
| | Square Loadings | 0.3129 | 0.1897 |
| | Contribution | 16.3138 | 13.4717 |
| Surface velocity variance | Loadings | 0.1808 | 0.2407 |
| | Square Loadings | 0.0627 | 0.0816 |
| | Contribution | 3.2698 | 5.7936 |

**Table 5.3:** Linear mixed-effect model output for the latency to inspect a novel predator model (BoxCox-transformed), including Satterthwaite *t* tests on (a) fixed effects, and (b) statistics for random effects. Velocity-Complexity and Pool Dimension correspond to the first and second components of the "average" environmental variable category, respectively. Substrate Diversity and Dimension Variance correspond to the first and second components of the "variance" environmental variable category, respectively. Significant terms are in bold type. Degrees of freedom and *p* values estimated using Satterthwaite's approximations.

| Condition | *Estimate* | *SE* | *df* | *t* | *p* |
|---|---|---|---|---|---|
| a) Fixed Effects | | | | | |
| Intercept | 14.0151 | 0.6314 | 37 | 22.196 | **<0.0001** |
| Velocity-Complexity | 1.0849 | 0.4264 | 37 | 2.544 | **0.0153** |
| Pool Dimension | 0.9045 | 0.6724 | 37 | 1.345 | 0.1868 |
| Substrate Diversity | 0.7543 | 0.4856 | 37 | 1.553 | 0.1289 |
| Dimension Variance | -0.1512 | 0.5885 | 37 | -0.257 | 0.7986 |
| b) Random Effects | | | | *Variance* | *SD* |
| Population (Intercept) | | | | 0 | 0 |
| Residual | | | | 16.75 | 4.092 |

Marginal $R^2$ / Conditional $R^2$ 0.2210 / 0.2210

*Model equation:*

Latency to Inspect ~ Velocity-Complexity + Pool Dimension + Substrate Diversity + Dimension Variance + (1 | Population)

Model includes 42 observations and 2 populations, fit using REML

**Table 5.4:** Generalized linear mixed-effect model (Binomial distribution) output for whether guppies entered a novel foraging arena, fit by maximum likelihood (Laplace approximation) on (a) fixed effects, and (b) statistics for random effects. Velocity-Complexity and Pool Dimension correspond to the first and second components of the "average" environmental variable category, respectively. Substrate Diversity and Dimension Variance correspond to the first and second components of the "variance" environmental variable category, respectively. Significant terms are in bold type.

| Condition | *Estimate* | *SE* | *z* | *p* |
|---|---|---|---|---|
| a) Fixed Effects | | | | |
| Intercept | 1.4157 | 0.4168 | 3.397 | **0.0007** |
| Velocity-Complexity | -0.3609 | 0.3064 | -1.178 | 0.2389 |
| Pool Dimension | -0.1778 | 0.3995 | -0.445 | 0.6562 |
| Substrate Diversity | 0.1429 | 0.2941 | 0.486 | 0.6271 |
| Dimension Variance | 0.3476 | 0.3672 | 0.946 | 0.3439 |

| b) Random Effects | *Variance* | *SD* |
|---|---|---|
| Population (Intercept) | 0 | 0 |

Theoretical Marginal $R^2$ / Conditional $R^2$ 0.1178 / 0.1178
Delta Marginal $R^2$ / Conditional $R^2$ 0.0689/ 0.0689
*Model equation:*
Binary Enter ~ Velocity-Complexity + Pool Dimension + Substrate Diversity + Dimension Variance + (1 | Population), family= binomial(link = "logit"))

Model includes 42 observations and 2 populations, fit using REML

**Table 5.5:** Linear mixed-effect model output for the latency to enter a novel foraging arena, in trials where guppies entered, including Satterthwaite *t* tests on (a) fixed effects, and (b) statistics for random effects. Velocity-Complexity and Pool Dimension correspond to the first and second components of the "average" environmental variable category, respectively. Substrate Diversity and Velocity Variance correspond to the first and second components of the "variance" environmental variable category, respectively. Significant terms are in bold type. Degrees of freedom and *p* values estimated using Satterthwaite's approximations.

| Condition | *Estimate* | *SE* | *df* | *t* | *p* |
|---|---|---|---|---|---|
| a) Fixed Effects | | | | | |
| Intercept | 202.908 | 8.966 | 28 | 22.632 | **<0.0001** |
| Velocity-Complexity | 8.941 | 5.633 | 28 | 1.587 | 0.1237 |
| Pool Dimension | -14.559 | 9.996 | 28 | -1.456 | 0.1564 |
| Substrate Diversity | -22.956 | 6.276 | 28 | -3.658 | **0.0010** |
| Dimension Variance | 15.428 | 9.150 | 28 | 1.686 | 0.1029 |
| b) Random Effects | | | | *Variance* | *SD* |
| Population (Intercept) | | | | 0 | 0 |
| Residual | | | | 2612 | 51.11 |
| Marginal $R^2$ / Conditional $R^2$ | 0.3350 / 0.3350 | | | | |

*Model equation:*

Latency to Enter ~ Velocity-Complexity + Pool Dimension + Substrate Diversity + Dimension Variance + (1 | Population)

Model includes 33 observations and 2 populations, fit using REML

# VII. Review, Prospectus, & Conclusion

## *i. Review*

In my thesis, I conduct a series of experiments exploring what drives neophobic antipredator responses. To do this, I assess the contexts in which prey experience uncertainty of risk. When uncertainty is high, neophobic predator avoidance (NPA) allows prey to reduce their risk of mortality by immediately responding to novel cues, which may represent potential risks. I argue that within the context of predation, prey face increased uncertainty due to factors that reduce the predictability of predation risks. This can occur due to limitations of predation risk information. Examples of such limitations include when risk information is unavailable, unknown, unreliable (i.e., not consistently correlated with a predation event), or incomplete (Crane et al. *in review*). In addition, information limitations may arise when risk information is abundant or diverse, or when multiple cues conflict (Zimmermann 2000).

First, I consider how uncertainty of risk might arise from an individual's current experience with information, specifically the reliability, amount, sensory modality, and type (i.e., risk vs. safety) of information detected. Initially, the question of how prey integrate multiple risk cues was poorly understood. In particular, it was unknown how they might integrate cues of mixed reliability (e.g., novel and known risk cues). In Chapter 1, I demonstrate that the reliability of public risk-assessment cues can shape the perceived predation risk of guppies, and that both known and unknown cues are valuable sources of information in that they can change the function of an individual in an evolutionarily relevant manner (Dall et al. 2005; Seppänen et al. 2007). When faced with cues of mixed reliability across modalities, guppies seemingly complement unknown cues with simultaneously available known risk cues. I argue this complementation represents more "complete" risk information and lower uncertainty of risk. However, when presented with two unknown cues, risk information remains incomplete, and guppies overestimate risk.

Given that multiple novel cues can lead to increased uncertainty of risk, in Chapter 2 I examine how the diversity and the spatial predictability of successively presented novel chemical cues shape the retention of neophobia. My results suggest that uncertainty of risk associated with a novel cue is greatest when the novel cue is presented with other novel cues compared to alone, supporting my results from Chapter 1. That is, diversity of novelty interferes with learning about a particular novel cue. I also show that when one novel cue is learned not to represent risk, this is not generalized to other novel cues. Additionally, I demonstrate in Chapter 2 that the spatial predictability of novelty also alters uncertainty of risk, where guppies retain greater neophobia after experiencing novel cues in a spatially unpredictable manner compared to in a spatially predictable manner.

Given the results of Chapter 1 and 2 demonstrating that guppies can collect and integrate multiple known and/or unknown risk cues across modalities to inform their antipredator responses, in Chapter 3 I establish how guppies integrate simultaneous conflicting cues of mixed reliability. In addition to "prey experience" with risk information detection, this chapter also incorporates the "source" of risk by assessing how guppies weighed non-social and social information which conveyed risk and safety, respectively. My results in Chapter 3 demonstrate that high-predation guppies rely on social safety information only when they are uncertain (facing a novel cue), mediating their otherwise neophobic responses in the absence of social safety cues. When more certain of risk (facing a non-social known risk cue), high-predation guppies do not rely on social safety cues. Somewhat surprisingly, social safety information is persuasive over non-social known risk cues in low-predation guppies.

The previous chapters established that uncertainty of risk may arise due to prey experience with information and novelty (information limitations during risk information detection), and may be shaped by the source of information. In Chapter 4, I examine how predators (i.e., direct sources of risk information) might shape uncertainty and neophobia. Prior research on neophobia had suggested that it is shaped by elevated and unpredictable levels of risk (Brown et al. 2013), but it had not disentangled the effects of predator density and diversity on neophobia. In this chapter, I demonstrate that neophobia is strongly shaped by "mean" risk (i.e., predator density), but also to some extent by the diversity of predators experienced.

Having demonstrated that neophobia is shaped by prey experience during information detection and due to the source of risk, in Chapter 5 I assess environmental conditions that shape information availability. If some aspect of the environment interferes with information transfer and detection (i.e., creates noise; Koops 1998), prey should be less able to predict predation risk (i.e., be more uncertain of risk) compared to individuals where information can be consistently detected without interference. In Chapter 5, I identify several environmental variables that may interfere with information transfer and detection, shaping neophobia. My results suggest that neophobia in guppies is shaped by water velocity, microhabitat complexity, pool width and depth, as well as substrate diversity and heterogeneity. Furthermore, I find differential effects of "average" and "variance" environmental categories on food- and predator-related neophobia, highlighting how different neophobic behaviours may be driven by different environmental conditions.

Overall, my thesis highlights how uncertainty of risk arises at several parts of the information transfer pathway. Using Trinidadian guppies as my model system, I show that neophobic predator avoidance is shaped by ***prey experience*** with information (i.e., quantity, reliability, and spatio-temporal patterns of information), the ***source*** of risk information (i.e., predators), and the ***environment*** interfering with information transmission and detection (*Box 1*).

### *ii. Further considerations*

My thesis has identified an information theory framework of how predation risk in combination with several information limitations drive uncertainty of risk and neophobia. I discuss how neophobic predator avoidance is an adaptive response to uncertainty through the lens of error management. As a result, I have also highlighted gaps in the literature that are promising avenues for future research.

For example, the cognitive mechanisms by which individuals integrate simultaneous sources of information in order to assess risk remains unexplored. These cognitive mechanisms may be affected by the information type (i.e., risk and safety), reliability (i.e., known and unknown) which engages memory, source (i.e., social or non-social, conspecific or heterospecific), and modality (e.g., visual, chemical). In humans, neuromodulators (including acetylcholine and norepinephrine) have been shown to signal known or unexpected information, interacting to allow inference and learning in uncertain environments (Yu and Dayan 2005). These cognitive mechanisms may be species-specific due to differences in sensory biases, and/or species sociality shaping how individuals weigh risk and safety information from social sources. In addition to species-specific cognitive mechanisms of information integration, there is also likely individual variation in how prey weigh information. For example, guppies have been shown to differ in boldness (Brown et al. 2005), and such individual risk-taking tactics shape responses to alarm cues but not novel cues (Brown, Elvidge, et al. 2014). Additionally, shyer guppies may rely more on social information, and may have reduced exploratory behaviour, compared to bolder guppies (Kurvers et al. 2010; Trompf and Brown 2014). Therefore, individuals likely weigh information differently depending on the type (i.e., risk, safety) and source (i.e., social, non-social) of information available. According to Mathot et al. (2012), personality (consistent differences in behaviour across contexts) may be tightly linked with how individuals manage uncertainty. There are also documented sex-specific differences in risk-taking behaviour (Croft, Morrell, et al. 2006; Piyapong et al. 2010; Trompf and Brown 2014). According to the asset protection principle, individuals with greater reproductive investment (e.g., female guppies) should protect their investment by being more cautious compared to individuals with low reproductive investment (Clark 1994; Katwaroo-Andersen et al. 2016). Thus, sex, age, and/or reproductive investment may shape individual cost-benefit trade-offs of antipredator behaviour. A recent study has shown neophobia is sex-specific in guppies (Brusseau et al. *in review*). Predation may also shift according to age (and size) of prey species. For example, guppies have a variety of predators that differentially predate on guppies based on guppy age, size, and sex (Rodd and Reznick 1997; Magurran 2005). Such switches in predation regime experienced would affect the cost-benefit balance of neophobic responses according to age of prey. Age may also shape the accumulated personal knowledge of prey, and older individuals may have better estimates of probabilities in their environment (Trimmer et al. 2011). It has been theorized that juveniles should have lower neophobia in order to facilitate an optimal expression of neophobia as adults (Greenberg and Mettke-Hofmann 2001), however there is contradicting evidence in a wide variety of taxa including birds, fish, and mammals (Crane and Ferrari 2017; Greggor et al. 2020). To address the generalizability of my results, I suggest replications of my experiments in a variety of study systems given that there are likely species-specific, sex-specific, and age-specific effects on neophobia, as well as consistent individual differences in neophobic responses.

Future studies should also consider how predator behaviour factors into uncertainty of risk. In particular, studies should assess how diversity of predator movement and foraging tactics shape neophobia in prey. Although my results in Chapter 4 show a weak effect of predator morphological diversity on neophobia compared to predator density, diversity in movement or foraging tactic could be more influential on neophobic responses. This diversity of predatory behaviour is worth studying both across and within predator species. Like prey, predators may have individual variation in their predatory behaviour, which may shape uncertainty of risk experienced by prey. Such individual variation in predation behaviour has been observed in a main guppy predator, the pike cichlid (*Crenicichla frenata*; Szopa-Comley et al., 2020). Given that guppies and their predators are confined to the same river pool during the dry season (Magurran 2005), predation is likely more predictable over time and guppies may be more certain of risk due to experiencing consistent predatory behaviour. However, if confined with multiple predators, even of the same species, individual variation in predation behaviour may reduce predictability of risk and increase uncertainty of risk. Understanding how behavioural variation of predators may shape uncertainty and neophobia in their prey would be a valuable avenue of future research. Predators may also alter their foraging patterns if they are uncertain of foraging opportunities (i.e., they cannot predict the outcome of a foraging event). In such cases, predators may face uncertainty of reward. That is, they are unable to predict the probability of a beneficial, rather than costly, outcome of a particular event. Studies have documented how individuals weigh information when facing uncertainty of reward in several contexts. For example, bumblebees with variable rewards (uncertainty of foraging reward) rely on social information over unreliable personal information, and copy conspecific foraging decisions (Smolla et al. 2016). Similarly, house-hunting ants less familiar with new nest sites (uncertainty of reward as it pertains to site quality) copy others more than ants familiar with new nest sites (Stroeymeyt et al. 2017). In other words, uncertainty of reward is also shaped by information limitations. Predators may be uncertain of foraging opportunities when information regarding prey (e.g., quality, quantity, or location) is unreliable, or otherwise limited. This could result from a lack of experience with prey, from environmental variables that generate noise and interfere with predator assessment of foraging conditions, or due to prey themselves contributing noise to interfere with predator assessment of prey quality, quantity, or location. For example, tiger moths (*Bertholdia trigona*) and some species of hawkmoths (family *Sphingidae*) produce clicks to interfere with bat sonar detection (Corcoran et al. 2009; Kawahara and Barber 2015). Contexts in which predators may face uncertainty of foraging opportunities may also include when prey are variable in time and space. This may result from complex trophic interactions, which affect prey density and behaviour as well as predator competition (Preisser et al. 2005). If predator behavioural patterns change in response to uncertainty of reward, this may in turn increase uncertainty of predation risk for prey.

The generalizability of the results presented in my thesis may also depend on several environmental attributes of the study system. As discussed in Chapter 5, additional environmental variables worth exploring when considering the Trinidadian guppy model system include pH, turbidity, and temperature. All of these have the potential to interfere with the availability (and subsequent reliability) of information, depending on the modality. It is worth noting that each of these variables may differentially affect antipredator responses. For example, red shiners (*Cyprinella lutrensis*) and sand shiners (*Notropis stramineus*) decreased collective behavior in response to turbidity (Michael et al. 2021). Therefore, researchers assessing the effects of turbidity on antipredator behaviour should consider additional antipredator metrics in combination with the shoaling and area use indices used in this thesis. In non-aquatic model systems, environmental variables that interfere with information availability and shape uncertainty of risk likely still include microhabitat complexity, dimensions, and heterogeneity. However, additional variables such as canopy cover, vegetation, temperature, and atmospheric $CO_2$ may also be important in interfering with visual, auditory, and chemosensory information (Weissburg et al. 2014; Roggatz et al. 2022). Furthermore, assessing multiple ecological variables through time and considering their variance and covariance is as important as simply assessing the average environmental conditions (Lee et al. 2016). Therefore, future works should consider ecological variables in correlation with one another, over time, to understand potential effects on antipredator behaviour. Habitat conditions may alter information availability in a complex manner, and future studies should examine how environmental variables within several systems shape uncertainty and prey behaviour.

Seasonality also likely influences uncertainty of risk and neophobic predator avoidance. Seasonality may affect how prey weigh information during risk-assessment if they experience fecund periods (e.g., mating seasons where reproductive investment is high). For example, rooks (*Corvus frugilegus*) increase fear of novel people during breeding season, and individual object neophobia is consistent within season, but not between seasons (Greggor et al. 2016). Seasonal migration may also shape how individuals weigh information. For example, blackbirds (*Icteridae*) that are migratory are more neophobic than their resident (non-migratory) counterparts, likely as a result of lower familiarity with their environment (Mettke-Hofmann et al. 2013). It would be worth examining whether this neophobia fluctuates over seasons within a migratory population. Potentially, familiarity with other individuals may change over the migratory season. Since individual responses to novelty are shaped by familiarity (Morrell et al. 2008; Feyten et al. 2021), season likely shapes neophobia in migratory species. Predators may also migrate seasonally. Resident prey may therefore experience select periods of time with increased but potentially unpredictable predation risk. Moreover, areas with high seasonality may experience temporal variation in community diversity and turnover (Tonkin et al. 2017), which likely decreases the ability of resident prey to predict risk. Seasonality may also impact predictability of predation risk due to changes in environmental conditions. For example, loss of cover in the dry season at a site, rather than the site's general microhabitat complexity, increases lizard predation by birds (Ferreira and Faria 2021). In such a scenario, it is unclear whether uncertainty of risk would increase if risk is elevated but not necessarily unpredictable. However, if seasonality results in environmental conditions that are more variable or impede information transfer, then uncertainty of risk and neophobic responses likely increase. For example, the wet season in Trinidad may bring heavy rains, which would result in frequent turbid periods with high water velocity. In such contexts, individuals would be less able to visually or chemically assess risk. It may therefore benefit prey to be cautious and neophobic during these periods. Moreover, heavy rains may cause discrete pools to become larger unified streams. In such cases, prey would suddenly be exposed to a greater quantity and diversity of predators, with each predator potentially expressing individual variability in predation tactics. However, they may also experience greater safety in numbers if unified pools have greater prey densities, and may be better able to escape predators if not confined to a small pool. Thus, seasonality and rainfall likely effect neophobia in aquatic prey. These nuanced effects of the wet-dry seasons on neophobia should be explored in future studies, especially given the opportunity to study it in the existing Trinidadian guppy model system. In addition to comparing prey behaviour between seasons (where one may represent periods of elevated and diverse predation compared to the other), it would be informative to also study the transitional period between seasons. This transition period is where prey face the most changes, and potentially the least predictable risk until it is stabilized later in the season. Thus, neophobia may me greatest during transitions between seasons.

Urbanization also likely shapes uncertainty of risk. For example, we can expect that anthropogenic noise pollution would affect the detection of auditory information (Corcoran and Moss 2017), which may be used for risk assessment. Auditory disturbances may also deflect attention from relevant information, and have other unexpected effects on behaviour. For example, Dusky damselfish (*Stegastes fuscus*) have increased anxiety and reduced memory after exposure to anthropogenic noise via high-intensity music (de Souza et al. 2022). These fish also spend less time foraging and decrease antipredator responses during high sound impact days of Brazilian carnival in reefs near Salvador City, Bahia (Leduc et al. 2021). In addition to interference with auditory information, urbanization and anthropogenic disturbances also likely increase prey exposure to novelty (Candler and Bernal 2015). A recent study has demonstrated that guppies in high-risk sites with high anthropogenic disturbance have reduced neophobic responses compared to guppies from the same population with lower anthropogenic disturbance (Brusseau et al. 2023). Potentially, constant exposure to novelty without risk decreases the adaptive value of neophobia at such sites. In contrast, a study on common voles (*Microtus arvalis*) and striped field mice (*Apodemus agrasrius*) found no difference in neophobia between individuals from urban and rural locations (Mazza et al. 2021). However, this study did not specifically account for predation levels. Potentially, novelty in urbanized areas still represent risks to these individuals, explaining why neophobia was retained in individuals from both rural and urban locations. In the absence of risk, urbanization may mean that individuals facing novelty experience uncertainty of reward. Urbanization may simply increase the quantity of novel and/or unpredictable resources, and selection should favour individuals who exploit these novel resources (Tryjanowski et al. 2016). A study on black-capped chickadees (*Poecile atricapillus*) showed that chickadees in urban habitats explored a new environment faster and seemed to rely less heavily on social information about novel food patches compared to forest chickadees (Morand-Ferron et al. 2019). Neophilic behaviours (attraction to novelty) are suggested to be adaptive in urban environments when those environments are predictable and have increased stability of food availability (Griffin et al. 2017). Studying the effects of urbanization from an informational perspective will provide insight to how individuals deal with uncertainty of risk and reward. This may serve to inform whether populations express neophobic and/or neophilic responses.

A fruitful avenue of future work would be to assess how neophobia is tied to prey vulnerability to invasive predators. In general, low-predation populations may be more vulnerable to newly introduced predators compared to their high-predation counterparts. This is because in a high-predation environment, an unknown cue is likely indicative of risk and prey should err on the side of caution (Johnson et al. 2013) and express neophobia. However, in low-predation environments, prey should engage in other fitness-enhancing activities such as foraging or courtship, given that the likelihood of an unknown cue representing risk is lower. If neophobia imparts individuals with an advantage against invasive predators, then my work serves to identify species vulnerable to invasion based on measurable components of their predation regime and habitat. Vulnerability to invasive predators is likely also shaped by urbanization and anthropogenic disturbance. If prey in highly disturbed sites are less neophobic (Brusseau et al. 2023), they are more vulnerable to invasive predators compared to their undisturbed counterparts. As discussed above, there may be an effect of seasonality on neophobia, and vulnerability to invasion may consequently be seasonal. Additionally, I have discussed a few possible species-, sex-, and age-specific differences in neophobia, and these may also affect vulnerability to invasive predators.

### *Implications & Conclusion*

Overall, my thesis establishes a framework demonstrating that uncertainty of risk and neophobic predator avoidance in prey fish, such as guppies, depends on prey experience during information detection, the source of risk information, and environmental interference in information transmission. My thesis provides empirical evidence that information limitations shape uncertainty of risk and neophobia. Rather than gathering more information from the environment to update their knowledge and reduce uncertainty and reduce informational limitations (Dall and Johnstone 2002; Dall et al. 2005; Valone 2006; Schmidt et al. 2010), I demonstrate contexts in which prey exhibit neophobic predator avoidance. These contexts are shaped by the trade-off between time and energy spent gathering information and engaging in other fitness-enhancing activities (e.g., foraging, territory defense, and/or courtship). They are also shaped by background predation levels which increase the likelihood of mortality when gathering information rather than responding to threats. Moreover, neophobia may serve as a step between uncertainty and learning, where neophobia is initially advantageous in high-predation contexts, but can be extinguished when prey learn a particular cue is not indicative of a risk.

My thesis improves our understanding of how prey respond to variability and changes in their environment, and how they make decisions in response to uncertain predation risk (Lima and Dill 1990; Sih 1992; Brown et al. 2013; Ferrari et al. 2018; Feyten, Demers, Ramnarine, Chivers, et al. 2019; Feyten, Demers, Ramnarine, and Brown 2019; Crane et al. 2020). Previous definitions of uncertainty have relied on difficult to quantify concepts such as the degree of information available to prey (Dall et al. 2005) or the asymmetry of costs (Johnson et al. 2013; Ferrari et al. 2018). I establish some measurable components of risk which contribute to uncertainty of risk and the resulting neophobic responses. Future studies interested in behaviours or responses driven by uncertainty of risk can use the informational framework, as well as measurable components of uncertainty, presented in this thesis as a starting point for experimental design. As climate change, anthropogenic factors, and invasive species combine to create ecological uncertainty for prey, it is increasingly important to study behavioural adaptations to variability in predation, such as neophobic predator avoidance. Understanding such uncertainty of risk is increasingly relevant, as it will likely increase for many species that are facing environmental changes (Sih 2013). My results provide some insight into which prey species may be neophobic, shaping which populations may be invasive and which populations may be vulnerable to newly introduced predators. Understanding the drivers of neophobic predator avoidance would allow better prediction of short- and long-term effects of changing environments on prey populations, including endangered and socio-economically important species.

## VIII. Bibliography

Abrams PA. 1994. Should prey overestimate the risk of predation? Am Nat. 144(2):317–328. doi:10.1086/285677.

Aceves-Fonseca E, Santiago-Arellano A, Camacho-Cervantes M. 2022. Sex, size and habitat complexity effects on emergence latency and latency to locate food of the invasive porthole livebearer (*Poeciliopsis gracilis*). PLOS ONE. 17:e0269384. doi:10.1371/journal.pone.0269384.

Acquistapace P, Hazlett BA, Gherardi F. 2003. Unsuccessful predation and learning of predator cues by crayfish. J Crustac Biol. 23(2):364–370. doi:10.1163/20021975-99990346.

Almany GR. 2004. Does increased habitat complexity reduce predation and competition in coral reef fish assemblages? Oikos. 106(2):275–284. doi:10.1111/j.0030-1299.2004.13193.x.

Archard GA, Earley RL, Hanninen AF, Braithwaite VA. 2012. Correlated behaviour and stress physiology in fish exposed to different levels of predation pressure. Funct Ecol. 26(3):637–645. doi:10.1111/j.1365-2435.2012.01968.x.

Aronson RB, Precht WF. 1995. Landscape patterns of reef coral diversity: a test of the intermediate disturbance hypothesis. J Exp Mar Biol Ecol. 192(1):1–14. doi:10.1016/0022-0981(95)00052-S.

Bates D, Mächler M, Bolker BM, Walker SC. 2015. Fitting linear mixed-effects models using **lme4**. J Stat Softw. 67(1). doi:10.18637/jss.v067.i01.

Beauchamp G. 2017. What can vigilance tell us about fear? Anim Sentience. 2(15). doi:10.51291/2377-7478.1203.

van Bergen Y, Coolen I, Laland KN. 2004. Nine-spined sticklebacks exploit the most reliable source when public and private information conflict. Proc R Soc B Biol Sci. 271(1542):957–962. doi:10.1098/rspb.2004.2684.

Bergendahl IA, Salvanes AGV, Braithwaite VA. 2016. Determining the effects of duration and recency of exposure to environmental enrichment. Appl Anim Behav Sci. 176:163–169. doi:10.1016/j.applanim.2015.11.002.

Beukers JS, Jones GP. 1998. Habitat complexity modifies the impact of piscivores on a coral reef fish population. Oecologia. 114(1):50–59. doi:10.1007/s004420050419.

Blake CA, Alberici da Barbiano L, Guenther JE, Gabor CR. 2015. Recognition and response to native and novel predators in the Largespring mosquitofish, *Gambusia geiseri*. Ethology. 121(3):227–235. doi:10.1111/eth.12331.

Blake CA, Gabor CR. 2016. Exploratory behaviour and novel predator recognition: behavioural correlations across contexts. J Fish Biol. 89(2):1178–1189. doi:10.1111/jfb.12995.

Bleckmann H, Lotz T. 1987. The vertebrate-catching behaviour of the fishing spider *Dolomedes triton* (Araneae, Pisauridae). Anim Behav. 35(3):641–651. doi:10.1016/S0003-3472(87)80100-8.

Borner KK, Krause S, Mehner T, Uusi-Heikkilä S, Ramnarine IW, Krause J. 2015. Turbidity affects social dynamics in Trinidadian guppies. Behav Ecol Sociobiol. 69:645–651. doi:10.1007/s00265-015-1875-3.

Botham MS, Hayward RK, Morrell LJ, Croft DP, Ward JR, Ramnarine I, Krause J. 2008. Risk-sensitive antipredator behavior in the Trinidadian guppy, *Poecilia reticulata*. Ecology. 89(11):3174–3185. doi:10.1890/07-0490.1.

Botham MS, Kerfoot CJ, Louca V, Krause J. 2006. The effects of different predator species on antipredator behavior in the Trinidadian guppy, *Poecilia reticulata*. Naturwissenschaften. 93(9):431–439. doi:10.1007/s00114-006-0131-0.

Bouskila A, Blumstein DT. 1992. Rules of thumb for predation hazard assessment: predictions from a dynamic model. Am Nat. 139(1):161–176. doi:10.1086/285318.

Boyd R, Richerson PJ. 1988. Culture and the Evolutionary Process. Chicago, IL: University of Chicago Press.

Brönmark C, Lakowitz T, Hollander J. 2011. Predator-induced morphological plasticity across local populations of a freshwater snail. PLOS ONE. 6(7):e21773. doi:10.1371/journal.pone.0021773.

Brooke AP. 1994. Diet of the fishing bat, *Noctilio leporinus* (Chiroptera: Noctilionidae). J Mammal. 75(1):212–218. doi:10.2307/1382253.

Brown BL. 2003. Spatial heterogeneity reduces temporal variability in stream insect communities. Ecol Lett. 6(4):316–325. doi:10.1046/j.1461-0248.2003.00431.x.

Brown C, Jones F, Braithwaite V. 2005. In situ examination of boldness–shyness traits in the tropical poeciliid, *Brachyraphis episcopi*. Anim Behav. 70(5):1003–1009. doi:10.1016/j.anbehav.2004.12.022.

Brown GE, Chivers DP. 2005. Learning as an adaptive response to predation. In: Barbosa P, Castellanos I, editors. Ecology of Predator-Prey Interactions. Oxford University Press, USA. p. 34–54.

Brown GE, Chivers DP, Elvidge CK, Jackson CD, Ferrari MCO. 2014. Background level of risk determines the intensity of predator neophobia in juvenile convict cichlids. Behav Ecol Sociobiol. 68(1):127–133. doi:10.1007/s00265-013-1629-z.

Brown GE, Chuard PJC, Demers EEM, Ramnarine IW, Chivers DP, Ferrari MCO. 2018. Personality and the retention of neophobic predator avoidance in wild caught Trinidadian guppies. Behaviour. 155(4):265–278. doi:10.1163/1568539X-00003488.

Brown GE, Elvidge CK, Macnaughton CJ, Ramnarine I, Godin J-GJ. 2010. Cross-population responses to conspecific chemical alarm cues in wild Trinidadian guppies, *Poecilia reticulata*: evidence for local conservation of cue production. Can J Zool. 88(2):139–147. doi:10.1139/Z09-127.

Brown GE, Elvidge CK, Ramnarine I, Chivers DP, Ferrari MCO. 2014. Personality and the response to predation risk: effects of information quantity and quality. Anim Cogn. 17(5):1063–1069. doi:10.1007/s10071-014-0738-z.

Brown GE, Elvidge CK, Ramnarine I, Ferrari MCO, Chivers DP. 2015. Background risk and recent experience influences retention of neophobic responses to predators. Behav Ecol Sociobiol. 69(5):737–745. doi:10.1007/s00265-015-1888-y.

Brown GE, Ferrari MCO, Chivers DP. 2011. Learning about danger: chemical alarm cues and threat-sensitive assessment of predation risk by fishes. In: Brown C, Laland K, Krause J, editors. Fish Cognition and Behavior. 2nd ed. London: Blackwell. p. 59–80.

Brown GE, Ferrari MCO, Elvidge CK, Ramnarine I, Chivers DP. 2013. Phenotypically plastic neophobia: a response to variable predation risk. Proc R Soc B Biol Sci. 280(1756):20122712. doi:10.1098/rspb.2012.2712.

Brown GE, Ferrari MCO, Malka PH, Russo S, Tressider M, Chivers DP. 2011. Generalization of predators and nonpredators by juvenile rainbow trout: learning what is and is not a threat. Anim Behav. 81(6):1249–1256. doi:10.1016/j.anbehav.2011.03.013.

Brown GE, Godin J-GJ. 1999. Chemical alarm signals in wild Trinidadian guppies (*Poecilia reticulata*). Can J Zool. 77(4):562–570. doi:10.1139/z99-035.

Brown GE, Jackson CD, Joyce BJ, Chivers DP, Ferrari MCO. 2016. Risk-induced neophobia: does sensory modality matter? Anim Cogn. 19(6):1143–1150. doi:10.1007/s10071-016-1021-2.

Brown GE, Macnaughton CJ, Elvidge CK, Ramnarine I, Godin J-GJ. 2009. Provenance and threat-sensitive predator avoidance patterns in wild-caught Trinidadian guppies. Behav Ecol Sociobiol. 63(5):699–706. doi:10.1007/s00265-008-0703-4.

Brown GE, Magnavacca G. 2003. Predator inspection behaviour in a characin fish: an interaction between chemical and visual information? Ethology. 109(9):739–750. doi:10.1046/j.1439-0310.2003.00919.x.

Brumm H, editor. 2013. Animal Communication and Noise. Berlin, Heidelberg: Springer Berlin Heidelberg (Animal Signals and Communication).

Brusseau AJP, Feyten LEA, Crane AL, Brown GE. 2023. Exploring the effects of anthropogenic disturbance on predator inspection activity in Trinidadian guppies. Curr Zool. doi:10.1093/cz/zoad002.

Brusseau AJP, Feyten LEA, Groves V, Felismino MEL, Truong DCV, Crane AL, Ramnarine IW, Brown GE. *in review*. Sex and background risk influence responses to acute predation risk in Trinidadian guppies. Behav Ecol. Manuscript ID: BEHECO-2023-0065.

Burns JG, Saravanan A, Rodd FH. 2009. Rearing environment affects the brain size of guppies: Lab-reared guppies have smaller brains than wild-caught guppies. Ethology. 115:122–133. doi:10.1111/j.1439-0310.2008.01585.x.

Camacho-Cervantes M, Ojanguren AF, Magurran AE. 2015. Exploratory behaviour and transmission of information between the invasive guppy and native Mexican topminnows. Anim Behav. 106:115–120. doi:10.1016/j.anbehav.2015.05.012.

Campos FA, Fedigan LM. 2014. Spatial ecology of perceived predation risk and vigilance behavior in white-faced capuchins. Behav Ecol. 25(3):477–486. doi:10.1093/beheco/aru005.

Candler S, Bernal XE. 2015. Differences in neophobia between cane toads from introduced and native populations. Behav Ecol. 26(1):97–104. doi:10.1093/beheco/aru162.

Carbia PS, Brown C. 2019. Environmental enrichment influences spatial learning ability in captive-reared intertidal gobies (*Bathygobius cocosensis*). Anim Cogn. 22(1):89–98. doi:10.1007/s10071-018-1225-8.

Carthey AJR, Blumstein DT. 2018. Predicting predator recognition in a changing world. Trends Ecol Evol. 33(2):106–115. doi:10.1016/j.tree.2017.10.009.

Catano LB, Rojas MC, Malossi RJ, Peters JR, Heithaus MR, Fourqurean JW, Burkepile DE. 2016. Reefscapes of fear: predation risk and reef hetero-geneity interact to shape herbivore foraging behaviour. J Anim Ecol. 85(1):146–156. doi:10.1111/1365-2656.12440.

Chan Y, Lo S, Quan A, Blumstein DT. 2019. Ontogenetic shifts in perceptions of safety along structural complexity gradients in a territorial damselfish. Curr Zool. 65(2):183–188. doi:10.1093/cz/zoy091.

Chivers DP, Brown GE, Ferrari MCO. 2012. The evolution of alarm substances and disturbance cues in aquatic animals. In: Brönmark C, Hansson L-A, editors. Chemical Ecology in Aquatic Systems. Oxford: Oxford University Press.

Chivers DP, Kiesecker JM, Marco A, Wildy EL, Blaustein AR. 1999. Shifts in life history as a response to predation in Western toads (*Bufo boreas*). J Chem Ecol. 25(11):2455–2463. doi:10.1023/A:1020818006898.

Chivers DP, McCormick MI, Mitchell MD, Ramasamy RA, Ferrari MCO. 2014. Background level of risk determines how prey categorize predators and non-predators. Proc R Soc B Biol Sci. 281(1787). doi:10.1098/rspb.2014.0355.

Chivers DP, Smith RJF. 1994. Fathead minnows, *Pimephales promelas*, acquire predator recognition when alarm substance is associated with the sight of unfamiliar fish. Anim Behav. 48(3):597–605. doi:10.1006/anbe.1994.1279.

Chivers DP, Smith RJF. 1998. Chemical alarm signalling in aquatic predator-prey systems: A review and prospectus. Écoscience. 5(3):338–352. doi:10.1080/11956860.1998.11682471.

Chivers DP, Zhao X, Brown GE, Marchant TA, Ferrari MCO. 2008. Predator-induced changes in morphology of a prey fish: the effects of food level and temporal frequency of predation risk. Evol Ecol. 22(4):561–574. doi:10.1007/s10682-007-9182-8.

Christensen RHB. 2019. **ordinal**—Regression Models for Ordinal Data. [accessed 2022 Jul 13]. https://CRAN.R-project.org/package=ordinal.

Chuard PJC, Brown GE, Grant JWA. 2016. The effects of adult sex ratio on mating competition in male and female guppies (*Poecilia reticulata*) in two wild populations. Behav Processes. 129:1–10. doi:10.1016/j.beproc.2016.05.001.

Clark CW. 1994. Antipredator behavior and the asset-protection principle. Behav Ecol. 5(2):159–170. doi:10.1093/beheco/5.2.159.

Clinchy M, Sheriff MJ, Zanette LY. 2013. Predator-induced stress and the ecology of fear. Funct Ecol. 27(1):56–65. doi:10.1111/1365-2435.12007.

Corcoran AJ, Barber JR, Conner WE. 2009. Tiger moth jams bat sonar. Science. 325(5938):325–327. doi:10.1126/science.1174096.

Corcoran AJ, Moss CF. 2017. Sensing in a noisy world: lessons from auditory specialists, echolocating bats. J Exp Biol. 220(24):4554–4566. doi:10.1242/jeb.163063.

Cothran RD, Monahan PJ, Relyea RA. 2021. Antipredator behaviour affected by prey condition, food availability and pH-mediated info-disruption. Anim Behav. 171:111–118. doi:10.1016/j.anbehav.2020.11.007.

Crane AL. 2017. Social learning of predation risk and safety: fish behaviour as a model for fear recovery [PhD Thesis]. University of Saskatchewan. [accessed 2020 Nov 21]. https://harvest.usask.ca/handle/10388/8166.

Crane AL, Brown GE, Chivers DP, Ferrari MCO. 2020. An ecological framework of neophobia: from cells to organisms to populations. Biol Rev. 95(1):218–231. doi:10.1111/brv.12560.

Crane AL, Ferrari MCO. 2013. Social learning of predator recognition: advances and future directions. In: Clark KB, editor. Social Learning Theory: Phylogenetic Considerations Across Animal, Plant and Microbial Taxa. NY, USA: Nova Science Publisher. p. 53–82.

Crane AL, Ferrari MCO. 2015. Minnows trust conspecifics more than themselves when faced with conflicting information about predation risk. Anim Behav. 100:184–190. doi:10.1016/j.anbehav.2014.12.002.

Crane AL, Ferrari MCO. 2016. Uncertainty in risky environments: a high-risk phenotype interferes with social learning about risk and safety. Anim Behav. 119:49–57. doi:10.1016/j.anbehav.2016.06.005.

Crane AL, Ferrari MCO. 2017. Patterns of predator neophobia: a meta-analytic review. Proc R Soc B Biol Sci. 284(1861):20170583. doi:10.1098/rspb.2017.0583.

Crane AL, Ferrari MCO, Rivera-Hernández IAE, Brown GE. 2020. Microhabitat complexity influences fear acquisition in fathead minnows. Behav Ecol. 31(1):261–266. doi:10.1093/beheco/arz187.

Crane AL, Feyten LEA, Pregola AA, Ferrari MCO, Brown GE. *in review*. Uncertainty in antipredator decisions: a conceptual review.

Crane AL, Feyten LEA, Ramnarine IW, Brown GE. 2020a. High-risk environments promote chemical disturbance signalling among socially familiar Trinidadian guppies. Oecologia. 193(1):89–95. doi:10.1007/s00442-020-04652-6.

Crane AL, Feyten LEA, Ramnarine IW, Brown GE. 2020b. Temporally variable predation risk and fear retention in Trinidadian guppies. Behav Ecol. 31(4):1084–1090. doi:10.1093/beheco/araa055.

Crane AL, Mathiron AGE, Ferrari MCO. 2015. Social learning in a high-risk environment: incomplete disregard for the 'minnow that cried pike' results in culturally transmitted neophobia. Proc R Soc B Biol Sci. 282(1812):20150934. doi:10.1098/rspb.2015.0934.

Croft DP, James R, Thomas POR, Hathaway C, Mawdsley D, Laland KN, Krause J. 2006. Social structure and co-operative interactions in a wild population of guppies (*Poecilia reticulata*). Behav Ecol Sociobiol. 59(5):644–650. doi:10.1007/s00265-005-0091-y.

Croft DP, Morrell LJ, Wade AS, Piyapong C, Ioannou CC, Dyer JRG, Chapman BB, Wong Y, Krause J. 2006. Predation risk as a driving force for sexual segregation: A cross-population comparison. Am Nat. 167(6):867–878. doi:10.1086/504853.

Crowder LB, Cooper WE. 1982. Habitat Structural Complexity and the Interaction Between Bluegills and Their Prey. Ecology. 63(6):1802–1813. doi:10.2307/1940122.

Curio E, Ernst U, Vieth W. 1978. The adaptive significance of avian mobbing. Z Für Tierpsychol. 48(2):184–202. doi:10.1111/j.1439-0310.1978.tb00255.x.

Dalesman S, Rundle SD, Cotton PA. 2007. Predator regime influences innate anti-predator behaviour in the freshwater gastropod *Lymnaea stagnalis*. Freshw Biol. 52:2134–2140. doi:10.1111/j.1365-2427.2007.01843.x.

Dall SRX. 2005. Defining the concept of public information. Science. 308(5720):353c–356c. doi:10.1126/science.308.5720.353c.

Dall SRX. 2010. Managing risk: The perils of uncertainty. In: Westneat DF, Fox CW, editors. Evolutionary Behavioral Ecology. Oxford; New York: Oxford University Press.

Dall SRX, Giraldeau L-A, Olsson O, Mcnamara JM, Stephens DW. 2005. Information and its use by animals in evolutionary ecology. Trends Ecol Evol. 20(4):187–193. doi:10.1016/j.tree.2005.01.010.

Dall SRX, Johnstone RA. 2002. Managing uncertainty: information and insurance under the risk of starvation. Philos Trans R Soc B Biol Sci. 357(1427):1519–1526. doi:10.1098/rstb.2002.1061.

Danchin É, Giraldeau L-A, Valone TJ, Wagner RH. 2004. Public information: from nosy neighbors to cultural evolution. Science. 305(5683):487–491. doi:10.1126/science.1098254.

De Meester G, Sfendouraki-Basakarou A, Pafilis P, Van Damme R. 2021. Contribution to the special issue on reptile cognition: Dealing with the unexpected: the effect of environmental variability on behavioural flexibility in a Mediterranean lizard. Behaviour. 158:1193–1223. doi:10.1163/1568539x-bja10088.

Deacon AE, Farrell AD, Fraser DF. 2015. Observations of a semi-aquatic spider attack: an overlooked fish predator in a well studied ecosystem? Living World J Trinidad Tobago Field Nat Club. 57–59.

Deacon AE, Jones FAM, Magurran AE. 2018. Gradients in predation risk in a tropical river system. Curr Zool. 64(2):213–221. doi:10.1093/cz/zoy004.

DePasquale C, Neuberger T, Hirrlinger AM, Braithwaite VA. 2016. The influence of complex and threatening environments in early life on brain size and behaviour. Proc R Soc B Biol Sci. 283(1823):20152564. doi:10.1098/rspb.2015.2564.

Dickey BF, McCarthy TM. 2007. Predator–prey interactions between crayfish (*Orconectes juvenilis*) and snails (*Physa gyrina*) are affected by spatial scale and chemical cues. Invertebr Biol. 126(1):57–66. doi:10.1111/j.1744-7410.2007.00076.x.

Dodson SI, Crowl TA, Peckarsky BL, Kats LB, Covich AP, Culp JM. 1994. Non-visual communication in freshwater benthos: an overview. J North Am Benthol Soc. 13(2):268–282. doi:10.2307/1467245.

Dolinsek IJ, Biron PM, Grant JWA. 2007. Assessing the effect of visual isolation on the population density of Atlantic salmon (*Salmo salar*) using GIS. River Res Appl. 23(7):763–774. doi:10.1002/rra.1024.

Dow SC. 2012. Uncertainty about uncertainty. In: Dow SC, editor. Foundations for New Economic Thinking: A Collection of Essays. London: Palgrave Macmillan UK. p. 72–82.

Dubois F, Drullion D, Witte K. 2012. Social information use may lead to maladaptive decisions: a game theoretic model. Behav Ecol. 23(1):225–231. doi:10.1093/beheco/arr179.

Dugatkin LA, Godin J-GJ. 1992. Predator inspection, shoaling and foraging under predation hazard in the Trinidadian guppy, *Poecilia reticulata*. Environ Biol Fishes. 34(3):265–276. doi:10.1007/BF00004773.

Dunlap AS, Stephens DW. 2016. Reliability, uncertainty, and costs in the evolution of animal learning. Curr Opin Behav Sci. 12:73–79. doi:10.1016/j.cobeha.2016.09.010.

Eiben B, Persons M. 2007. The effect of prior exposure to predator cues on chemically-mediated defensive behavior and survival in the wolf spider *Rabidosa rabida* (Araneae: Lycosidae). Behaviour. 144:889–906. doi:10.1163/156853907781492681.

Elvidge CK, Brown GE. 2015. Size-based differences determine the contextual value of risky information in heterospecific information use. Anim Behav. 102:7–14. doi:10.1016/j.anbehav.2015.01.009.

Elvidge CK, Chuard PJC, Brown GE. 2016. Local predation risk shapes spatial and foraging neophobia patterns in Trinidadian guppies. Curr Zool. 62(5):457–462. doi:10.1093/cz/zow013.

Elvidge CK, Macnaughton CJ, Brown GE. 2013. Sensory complementation and antipredator behavioural compensation in acid-impacted juvenile Atlantic salmon. Oecologia. 172(1):69–78. doi:10.1007/s00442-012-2478-6.

Elvidge CK, Ramnarine I, Brown GE. 2014. Compensatory foraging in Trinidadian guppies: Effects of acute and chronic predation threats. Curr Zool. 60(3):323–332. doi:10.1093/czoolo/60.3.323.

Endler JA. 1991. Variation in the appearance of guppy color patterns to guppies and their predators under different visual conditions. Vision Res. 31(3):587–608. doi:10.1016/0042-6989(91)90109-I.

Epp KJ, Gabor CR. 2008. Innate and learned predator recognition mediated by chemical signals in *Eurycea nana*. Ethology. 114(6):607–615. doi:10.1111/j.1439-0310.2008.01494.x.

Ferrari MCO. 2014. Short-term environmental variation in predation risk leads to differential performance in predation-related cognitive function. Anim Behav. 95:9–14. doi:10.1016/j.anbehav.2014.06.001.

Ferrari MCO, Brown GE, Chivers DP. 2018. Understanding the effect of uncertainty on the development of neophobic antipredator phenotypes. Anim Behav. 136:101–106. doi:10.1016/j.anbehav.2017.11.024.

Ferrari MCO, Chivers DP. 2013. Temporal dynamics of information use in learning and retention of predator-related information in tadpoles. Anim Cogn. 16(4):667–676. doi:10.1007/s10071-013-0602-6.

Ferrari MCO, Crane AL, Brown GE, Chivers DP. 2015. Getting ready for invasions: can background level of risk predict the ability of naïve prey to survive novel predators? Sci Rep. 5(1):8309. doi:10.1038/srep08309.

Ferrari MCO, Gonzalo A, Messier F, Chivers DP. 2007. Generalization of learned predator recognition: an experimental test and framework for future studies. Proc R Soc B Biol Sci. 274(1620):1853–1859. doi:10.1098/rspb.2007.0297.

Ferrari MCO, McCormick MI, Meekan MG, Chivers DP. 2015. Background level of risk and the survival of predator-naive prey: can neophobia compensate for predator naivety in juvenile coral reef fishes? Proc R Soc B Biol Sci. 282(1799):20142197. doi:10.1098/rspb.2014.2197.

Ferrari MCO, Vavrek MA, Elvidge CK, Fridman B, Chivers DP, Brown GE. 2008. Sensory complementation and the acquisition of predator recognition by salmonid fishes. Behav Ecol Sociobiol. doi:10.1007/s00265-008-0641-1.

Ferrari MCO, Wisenden BD, Chivers DP. 2010. Chemical ecology of predator–prey interactions in aquatic ecosystems: a review and prospectus. Can J Zool. 88(7):698–724. doi:10.1139/Z10-029.

Ferreira AS, Faria RG. 2021. Predation risk is a function of seasonality rather than habitat complexity in a tropical semiarid forest. Sci Rep. 11(1):16670. doi:10.1038/s41598-021-96216-8.

Feys J. 2016. Nonparametric tests for the interaction in two-way factorial designs using R. R J. 8(1):367–378. doi:10.32614/RJ-2016-027.

Feyten LEA, Brown GE. 2018. Ecological uncertainty influences vigilance as a marker of fear. Anim Sentience. 2(15). doi:10.51291/2377-7478.1311.

Feyten LEA, Crane AL, Ramnarine IW, Brown GE. 2021. Predation risk shapes the use of conflicting personal risk and social safety information in guppies. Behav Ecol. 32(6):1296–1305. doi:10.1093/beheco/arab096.

Feyten LEA, Demers EEM, Ramnarine IW, Brown GE. 2019. Predation risk assessment based on uncertain information: interacting effects of known and unknown cues. Curr Zool. 65(1):75–76. doi:10.1093/cz/zoy083.

Feyten LEA, Demers EEM, Ramnarine IW, Brown GE. 2022. Assessing effects of predator density and diversity on neophobia in Trinidadian guppies. Behav Processes. 201:104717. doi:10.1016/j.beproc.2022.104717.

Feyten LEA, Demers EEM, Ramnarine IW, Chivers DP, Ferrari MCO, Brown GE. 2019. Who's where? Ecological uncertainty shapes neophobic predator avoidance in Trinidadian guppies. Behav Ecol Sociobiol. 73(5):70. doi:10.1007/s00265-019-2687-7.

Fox J, Weisberg S. 2019. An R Companion to Applied Regression. Third. Thousand Oaks, CA: Sage. [accessed 2022 Jul 14]. https://socialsciences.mcmaster.ca/jfox/Books/Companion/index.html.

Giraldeau L-A, Valone TJ, Templeton JJ. 2002. Potential disadvantages of using socially acquired information. Philos Trans R Soc Lond B Biol Sci. 357(1427):1559–1566. doi:10.1098/rstb.2002.1065.

Golub JL, Vermette V, Brown GE. 2005. Response to conspecific and heterospecific alarm cues by pumpkinseeds in simple and complex habitats: field verification of an ontogenetic shift. J Fish Biol. 66(4):1073–1081. doi:10.1111/j.0022-1112.2005.00658.x.

Gonzalo A, López P, Martín J. 2007. Iberian green frog tadpoles may learn to recognize novel predators from chemical alarm cues of conspecifics. Anim Behav. 74(3):447–453. doi:10.1016/j.anbehav.2006.11.032.

Gonzalo A, López P, Martín J. 2010. Risk level of chemical cues determines retention of recognition of new predators in Iberian green frog tadpoles. Behav Ecol Sociobiol. 64(7):1117–1123. doi:10.1007/s00265-010-0927-y.

Granroth-Wilding HMV, Magurran AE. 2013. Asymmetry in pay-off predicts how familiar individuals respond to one another. Biol Lett. 9(3):20130025. doi:10.1098/rsbl.2013.0025.

Gratwicke B, Speight MR. 2005. The relationship between fish species richness, abundance and habitat complexity in a range of shallow tropical marine habitats. J Fish Biol. 66:650–667. doi:10.1111/j.0022-1112.2005.00629.x.

Greenberg LA, Paszkowski CA, Tonn WM. 1995. Effects of prey species composition and habitat structure on foraging by two functionally distinct piscivores. Oikos. 74(3):522–532. doi:10.2307/3545998.

Greenberg R, Mettke-Hofmann C. 2001. Ecological aspects of neophobia and neophilia in birds. In: Nolan V, Thompson CF, editors. Current Ornithology, Volume 16. Boston, MA: Springer US. p. 119–178.

Greggor AL, Jolles JW, Thornton A, Clayton NS. 2016. Seasonal changes in neophobia and its consistency in rooks: the effect of novelty type and dominance position. Anim Behav. 121:11–20. doi:10.1016/j.anbehav.2016.08.010.

Greggor AL, Masuda B, Flanagan AM, Swaisgood RR. 2020. Age-related patterns of neophobia in an endangered island crow: implications for conservation and natural history. Anim Behav. 160:61–68. doi:10.1016/j.anbehav.2019.12.002.

Griffin AS. 2004. Social learning about predators: a review and prospectus. Anim Learn Behav. 32(1):131–140. doi:10.3758/BF03196014.

Griffin AS, Netto K, Peneaux C. 2017. Neophilia, innovation and learning in an urbanized world: a critical evaluation of mixed findings. Curr Opin Behav Sci. 16:15–22. doi:10.1016/j.cobeha.2017.01.004.

Grüter C, Czaczkes TJ, Ratnieks FLW. 2011. Decision making in ant foragers (*Lasius niger*) facing conflicting private and social information. Behav Ecol Sociobiol. 65(2):141–148. doi:10.1007/s00265-010-1020-2.

Hall D, Suboski MD. 1995. Visual and olfactory stimuli in learned release of alarm reactions by zebra danio fish (*Brachydanio rerio*). Neurobiol Learn Mem. 63(3):229–240. doi:10.1006/nlme.1995.1027.

Harvey BC, Stewart AJ. 1991. Fish size and habitat depth relationships in headwater streams. Oecologia. 87(3):336–342. doi:10.1007/BF00634588.

Hasenjager MJ, Dugatkin LA. 2017. Fear of predation shapes social network structure and the acquisition of foraging information in guppy shoals. Proc R Soc B Biol Sci. 284(1867). doi:10.1098/rspb.2017.2020.

Hawkins LA, Magurran AE, Armstrong JD. 2004. Innate predator recognition in newly-hatched Atlantic salmon. Behaviour. 141(10):1249–1262. doi:10.1163/1568539042729694.

Heinen VK, Stephens DW. 2016. Blue jays, *Cyanocitta cristata*, devalue social information in uncertain environments. Anim Behav. 112:53–62. doi:10.1016/j.anbehav.2015.11.015.

Hervé M. 2022. **RVAideMemoire**: Testing and Plotting Procedures for Biostatistics. [accessed 2022 Jul 16]. https://CRAN.R-project.org/package=RVAideMemoire.

Hurlbert SH. 1971. The nonconcept of species diversity: A critique and alternative parameters. Ecology. 52(4):577–586. doi:10.2307/1934145.

Jacobs RA. 2002. What determines visual cue reliability? Trends Cogn Sci. 6(8):345–350. doi:10.1016/S1364-6613(02)01948-4.

Jenkins MR, Cummings JM, Cabe AR, Hulthén K, Peterson MN, Langerhans RB. 2021. Natural and anthropogenic sources of habitat variation influence exploration behaviour, stress response, and brain morphology in a coastal fish. J Anim Ecol. 90(10):2446–2461. doi:10.1111/1365-2656.13557.

Johnson DDP, Blumstein DT, Fowler JH, Haselton MG. 2013. The evolution of error: error management, cognitive constraints, and adaptive decision-making biases. Trends Ecol Evol. 28(8):474–481. doi:10.1016/j.tree.2013.05.014.

Jones KA, Godin J-GJ. 2010. Are fast explorers slow reactors? Linking personality type and anti-predator behaviour. Proc R Soc B Biol Sci. 277(1681):625–632. doi:10.1098/rspb.2009.1607.

Joyce BJ, Demers EEM, Chivers DP, Ferrari MCO, Brown GE. 2016. Risk-induced neophobia is constrained by ontogeny in juvenile convict cichlids. Anim Behav. 114:37–43. doi:10.1016/j.anbehav.2016.01.007.

Kaplan O, Lubow RE. 2001. Context and reminder effects in a visual search analog of latent inhibition. Learn Motiv. 32:137–153. doi:10.1006/lmot.2000.1077.

Katwaroo-Andersen J, Elvidge CK, Ramnarine I, Brown GE. 2016. Interactive effects of reproductive assets and ambient predation risk on the threat-sensitive decisions of Trinidadian guppies. Curr Zool. 62(3):221–226. doi:10.1093/cz/zow062.

Kawahara AY, Barber JR. 2015. Tempo and mode of antibat ultrasound production and sonar jamming in the diverse hawkmoth radiation. Proc Natl Acad Sci. 112(20):6407–6412. doi:10.1073/pnas.1416679112.

Kelley JL, Magurran AE. 2003a. Learned predator recognition and antipredator responses in fishes. Fish Fish. 4(3):216–226. doi:10.1046/j.1467-2979.2003.00126.x.

Kelley JL, Magurran AE. 2003b. Effects of relaxed predation pressure on visual predator recognition in the guppy. Behav Ecol Sociobiol. 54(3):225–232. doi:10.1007/s00265-003-0621-4.

Kendal RL, Coolen I, Laland KN. 2004. The role of conformity in foraging when personal and social information conflict. Behav Ecol. 15(2):269–277. doi:10.1093/beheco/arh008.

Kiesecker JM, Chivers DP, Blaustein AR. 1996. The use of chemical cues in predator recognition by western toad tadpoles. Anim Behav. 52(6):1237–1245. doi:10.1006/anbe.1996.0271.

Knight FH. 1921. Risk, Uncertainty and Profit. Rochester, NY: Social Science Research Network Report No. ID 1496192.

Komyakova V, Munday PL, Jones GP. 2013. Relative importance of coral cover, habitat complexity and diversity in determining the structure of reef fish communities. PLOS ONE. 8(12):e83178. doi:10.1371/journal.pone.0083178.

Koops MA. 1998. Misinformation and assessment uncertainty in the ecology of information use [PhD Thesis]. [Winnipeg, Manitoba]: University of Manitoba. [accessed 2020 Jun 4]. http://hdl.handle.net/1993/1722.

Koops MA. 2004. Reliability and the value of information. Anim Behav. 67:103–111. doi:10.1016/j.anbehav.2003.02.008.

Kovalenko KE, Thomaz SM, Warfe DM. 2012. Habitat complexity: approaches and future directions. Hydrobiologia. 685(1):1–17. doi:10.1007/s10750-011-0974-z.

Kurvers RHJM, van Oers K, Nolet BA, Jonker RM, van Wieren SE, Prins HHT, Ydenberg RC. 2010. Personality predicts the use of social information. Ecol Lett. 13(7):829–837. doi:10.1111/j.1461-0248.2010.01473.x.

Kuznetsova A, Brockhoff PB, Christensen RHB. 2017. **lmerTest** package: Tests in linear mixed effects models. J Stat Softw. 82(13). doi:10.18637/jss.v082.i13.

Laland KN. 2004. Social learning strategies. Anim Learn Behav. 32(1):4–14. doi:10.3758/BF03196002.

Lê S, Josse J, Husson F. 2008. **FactoMineR**: An R package for multivariate analysis. J Stat Softw. 25:1–18. doi:10.18637/jss.v025.i01.

Leduc AOHC, Kim J-W, Macnaughton CJ, Brown GE. 2010. Sensory complement model helps to predict diel alarm response patterns in juvenile Atlantic salmon (*Salmo salar*) under natural conditions. Can J Zool. 88(4):398–403. doi:10.1139/Z10-016.

Leduc AOHC, Nunes JACC, de Araújo CB, Quadros ALS, Barros F, Oliveira HHQ, Simões CRMA, Winandy GSM, Slabbekoorn H. 2021. Land-based noise pollution impairs reef fish behavior: A case study with a Brazilian carnival. Biol Conserv. 253:108910. doi:10.1016/j.biocon.2020.108910.

Leduc AOHC, Roh E, Breau C, Brown GE. 2007. Effects of ambient acidity on chemosensory learning: an example of an environmental constraint on acquired predator recognition in wild juvenile Atlantic salmon (Salmo salar). Ecol Freshw Fish. 16(3):385–394. doi:10.1111/j.1600-0633.2007.00233.x.

Lee W-S, Mangel M, Peres-Neto P. 2016. Environmental integration: Patterns of correlation between environmental factors, early life decisions, and their long-term consequences. Evol Ecol Res. 17:1–19.

Lehtonen J, Jaatinen K. 2016. Safety in numbers: the dilution effect and other drivers of group life in the face of danger. Behav Ecol Sociobiol. 70(4):449–458. doi:10.1007/s00265-016-2075-5.

Lenth RV. 2019. **emmeans**: Estimated Marginal Means, aka Least-Squares Means. [accessed 2022 Jan 23]. https://CRAN.R-project.org/package=emmeans.

Lima SL, Bednekoff PA. 1999. Temporal variation in danger drives antipredator behavior: the predation risk allocation hypothesis. Am Nat. 153(6):649–659. doi:10.1086/303202.

Lima SL, Dill LM. 1990. Behavioral decisions made under the risk of predation: a review and prospectus. Can J Zool. 68(4):619–640. doi:10.1139/z90-092.

Lima SL, Steury TD. 2005. Perception of predation risk: the foundation of non-lethal predator effects. In: Barbosa P, Castellanos I, editors. Ecology of predator-prey interactions. Oxford University Press, USA. p. 166–188.

Lucon-Xiccato T, Ferrari MCO, Chivers DP, Bisazza A. 2018. Odour recognition learning of multiple predators by amphibian larvae. Anim Behav. doi:10.1016/j.anbehav.2018.04.022.

Luttbeg B, Ferrari MCO, Blumstein DT, Chivers DP. 2020. Safety cues can give prey more valuable information than danger cues. Am Nat. 195(4):636–648. doi:10.1086/707544.

Magory Cohen T, Kumar RS, Nair M, Hauber ME, Dor R. 2020. Innovation and decreased neophobia drive invasion success in a widespread avian invader. Anim Behav. 163:61–72. doi:10.1016/j.anbehav.2020.02.012.

Magurran AE. 2005. Evolutionary Ecology: The Trinidadian Guppy. Oxford: Oxford University Press.

Magurran AE, Seghers BH. 1994. A cost of sexual harassment in the guppy, *Poecilia reticulata*. Proc Biol Sci. 258(1351):89–92. doi:10.1098/rspb.1994.0147.

Makino H, Masuda R, Tanaka M. 2015. Environmental stimuli improve learning capability in striped knifejaw juveniles: the stage-specific effect of environmental enrichment and the comparison between wild and hatchery-reared fish. Fish Sci. 81(6):1035–1042. doi:10.1007/s12562-015-0917-0.

Martin LB, Fitzgerald L. 2005. A taste for novelty in invading house sparrows, *Passer domesticus*. Behav Ecol. 16(4):702–707. doi:10.1093/beheco/ari044.

Mathis A, Chivers DP, Smith RJF. 1996. Cultural transmission of predator recognition in fishes: intraspecific and interspecific learning. Anim Behav. 51(1):185–201. doi:10.1006/anbe.1996.0016.

Mathis A, Smith RJF. 1993. Fathead minnows, *Pimephales promelas*, learn to recognize northern pike, *Esox lucius*, as predators on the basis of chemical stimuli from minnows in the pike's diet. Anim Behav. 46(4):645–656. doi:10.1006/anbe.1993.1241.

Mathis A, Vincent F. 2000. Differential use of visual and chemical cues in predator recognition and threat-sensitive predator-avoidance responses by larval newts (*Notophthalmus viridescens*). Can J Zool. 78(9):1646–1652. doi:10.1139/z00-090.

Mathot KJ, Wright J, Kempenaers B, Dingemanse NJ. 2012. Adaptive strategies for managing uncertainty may explain personality-related differences in behavioural plasticity. Oikos. 121(7):1009–1020. doi:10.1111/j.1600-0706.2012.20339.x.

Mazza V, Czyperreck I, Eccard JA, Dammhahn M. 2021. Cross-context responses to novelty in rural and urban small mammals. Front Ecol Evol. 9:661971. doi:10.3389/fevo.2021.661971.

McLachlan JR, Ratnayake CP, Magrath RD. 2019. Personal information about danger trumps social information from avian alarm calls. Proc R Soc B Biol Sci. 286(1899):20182945. doi:10.1098/rspb.2018.2945.

McNamara JM, Dall SRX. 2010. Information is a fitness enhancing resource. Oikos. 119(2):231–236. doi:10.1111/j.1600-0706.2009.17509.x.

McNicol D. 2004. A Primer of Signal Detection Theory. New York: Psychology Press.

Mettke-Hofmann C. 2014. Cognitive ecology: ecological factors, life-styles, and cognition. WIREs Cogn Sci. 5(3):345–360. doi:10.1002/wcs.1289.

Mettke-Hofmann C, Winkler H, Hamel PB, Greenberg R. 2013. Migratory New World blackbirds (Icterids) are more neophobic than closely related resident icterids. PLOS ONE. 8(2):e57565. doi:10.1371/journal.pone.0057565.

Mettke-Hofmann C, Winkler H, Leisler B. 2002. The significance of ecological factors for exploration and neophobia in parrots. Ethology. 108(3):249–272. doi:10.1046/j.1439-0310.2002.00773.x.

Meuthen D, Baldauf SA, Bakker TCM, Thünken T. 2016. Predator-induced neophobia in juvenile cichlids. Oecologia. 181(4):947–958. doi:10.1007/s00442-015-3478-0.

Michael SCJ, Patman J, Lutnesky MMF. 2021. Water clarity affects collective behavior in two cyprinid fishes. Behav Ecol Sociobiol. 75. doi:10.1007/s00265-021-03060-x.

Milliken FJ. 1987. Three types of perceived uncertainty about the environment: state, effect, and response uncertainty. Acad Manage Rev. 12(1):133. doi:10.2307/257999.

Mineka S, Cook M. 1986. Immunization against the observational conditioning of snake fear in rhesus monkeys. J Abnorm Psychol. 95(4):307–318. doi:10.1037/0021-843X.95.4.307.

Mitchell MD, Chivers DP, Brown GE, Ferrari MCO. 2016. Living on the edge: How does environmental risk affect the behavioural and cognitive ecology of prey? Anim Behav. 115:185–192. doi:10.1016/j.anbehav.2016.03.018.

Mitchell MD, McCormick MI, Ferrari MCO, Chivers DP. 2011. Friend or foe? The role of latent inhibition in predator and non-predator labelling by coral reef fishes. Anim Cogn. 14(5):707–714. doi:10.1007/s10071-011-0405-6.

Morand-Ferron J, Hermer E, Jones TB, Thompson MJ. 2019. Environmental variability, the value of information, and learning in winter residents. Anim Behav. 147:137–145. doi:10.1016/j.anbehav.2018.09.008.

Morrell LJ, Croft DP, Dyer JRG, Chapman BB, Kelley JL, Laland KN, Krause J. 2008. Association patterns and foraging behaviour in natural and artificial guppy shoals. Anim Behav. 76(3):855–864. doi:10.1016/j.anbehav.2008.02.015.

Morris-Drake A, Kern JM, Radford AN. 2016. Cross-modal impacts of anthropogenic noise on information use. Curr Biol. 26(20):R911–R912. doi:10.1016/j.cub.2016.08.064.

Munoz NE, Blumstein DT. 2012. Multisensory perception in uncertain environments. Behav Ecol. 23(3):457–462. doi:10.1093/beheco/arr220.

Nunes J de ACC, Sampaio CLS, Barros F. 2015. The influence of structural complexity and reef habitat types on flight initiation distance and escape behaviors in labrid fishes. Mar Biol. 162(3):493–499. doi:10.1007/s00227-014-2578-4.

Nyffeler M, Pusey BJ. 2014. Fish predation by semi-aquatic spiders: a global pattern. PLoS ONE. 9(6):e99459. doi:10.1371/journal.pone.0099459.

Oksanen J, Simpson G, Blanchet FG, Kindt R, Legendre P, Minchin P, O'Hara RB, Solymos P, Stevens MHH, Szoecs E, Wagner H, Barbour M, Bedward M, Bolker B, Borcard D, Carvalho G, Chirico M, De Caceres M, Durand S, Evangelista H, FitzJohn R, Friendly M, Furneaux B, Hannigan G, Hill M, Lahti L, McGlinn D, Ouellette M, Ribeiro Cunha E, Smith T, Stier A, Ter Braak C, Weedon J. 2022. **vegan**: Community Ecology Package. R Package Version. 2.6-4. [accessed 2022 Nov 24]. https://cran.r-project.org/web/packages/vegan/index.html.

Piyapong C, Krause J, Chapman BB, Ramnarine IW, Louca V, Croft DP. 2010. Sex matters: A social context to boldness in guppies (*Poecilia reticulata*). Behav Ecol. 21. doi:10.1093/beheco/arp142.

Preisser EL, Bolnick DI, Benard ME. 2005. Scared to death? The effects of intimidation and consumption in predator-prey interactions. Ecology. 86(2):501–509. doi:10.1890/04-0719.

Rankin CH, Abrams T, Barry RJ, Bhatnagar S, Clayton DF, Colombo J, Coppola G, Geyer MA, Glanzman DL, Marsland S, McSweeney FK, Wilson DA, Wu C-F, Thompson RF. 2009. Habituation revisited: an updated and revised description of the behavioral characteristics of habituation. Neurobiol Learn Mem. 92(2):135–138. doi:10.1016/j.nlm.2008.09.012.

Reader SM. 2003. Innovation and social learning: individual variation and brain evolution. Anim Biol. 53(2):147–158. doi:10.1163/157075603769700340.

Réale D, Reader SM, Sol D, McDougall PT, Dingemanse NJ. 2007. Integrating animal temperament within ecology and evolution. Biol Rev Camb Philos Soc. 82(2):291–318. doi:10.1111/j.1469-185X.2007.00010.x.

Relyea RA. 2002. Local population differences in phenotypic plasticity: predator-induced changes in wood frog tadpoles. Ecol Monogr. 72(1):77–93. doi:10.2307/3100086.

Rhoades E, Blumstein DT. 2007. Predicted fitness consequences of threat-sensitive hiding behavior. Behav Ecol. 18(5):937–943. doi:10.1093/beheco/arm064.

Ribiero PJJr, Diggle P, Christensen O, Schlather M, Bivand R, Ripley B. 2022. **geoR**: Analysis of Geostatistical Data. [accessed 2022 Nov 24]. https://CRAN.R-project.org/package=geoR.

Rieucau G, Giraldeau L-A. 2011. Exploring the costs and benefits of social information use: an appraisal of current experimental evidence. Philos Trans R Soc B Biol Sci. 366(1567):949–957. doi:10.1098/rstb.2010.0325.

Rilov G, Figueira WF, Lyman SJ, Crowder LB. 2007. Complex habitats may not always benefit prey: linking visual field with reef fish behavior and distribution. Mar Ecol Prog Ser. 329:225–238. doi:10.3354/meps329225.

Rodd FH, Reznick DN. 1997. Variation in the demography of guppy populations: the importance of predation and life histories. Ecology. 78(2):405–418. doi:10.2307/2266017.

Roggatz CC, Saha M, Blanchard S, Schirrmacher P, Fink P, Verheggen F, Hardege JD. 2022. Becoming nose-blind—Climate change impacts on chemical communication. Glob Change Biol. 28(15):4495–4505. doi:10.1111/gcb.16209.

Ross PM, Thrush SF, Montgomery JC, Walker JW, Parsons DM. 2007. Habitat complexity and predation risk determine juvenile snapper (*Pagrus auratus*) and goatfish (*Upeneichthys lineatus*) behaviour and distribution. Mar Freshw Res. 58(12):1144–1151. doi:10.1071/MF07017.

Roy T, Bhat A. 2016. Learning and memory in juvenile zebrafish: What makes the difference – population or rearing environment? Ethology. 122(4):308–318. doi:10.1111/eth.12470.

Salvanes AGV, Moberg O, Ebbesson LOE, Nilsen TO, Jensen KH, Braithwaite VA. 2013. Environmental enrichment promotes neural plasticity and cognitive ability in fish. Proc R Soc B Biol Sci. 280(1767):20131331. doi:10.1098/rspb.2013.1331.

Schmidt KA, Dall SRX, van Gils JA. 2010. The ecology of information: an overview on the ecological significance of making informed decisions. Oikos. 119(2):304–316. doi:10.1111/j.1600-0706.2009.17573.x.

Schmidt KA, Massol F, Szymkowiak J. 2022. Resurrecting Shannon's surprise: landscape heterogeneity complements information use and population growth. Oikos. 2022(10):e09305. doi:10.1111/oik.09305.

Searcy WA, Nowicki S. 2005. The Evolution of Animal Communication: Reliability and Deception in Signaling Systems. Princeton; Oxford: Princeton University Press.

Seppänen J-T, Forsman JT, Mönkkönen M, Thomson RL. 2007. Social information use is a process across time, space, and ecology, reaching heterospecifics. Ecology. 88(7):1622–1633. doi:10.1890/06-1757.1.

Shannon CE. 1948. A mathematical theory of communication. Bell Syst Tech J. 27:379–423, 623–656. doi:10.1002/j.1538-7305.1948.tb01338.x.

Shannon CE, Weaver W. 1949. The Mathematical Theory of Communication. Champaign, IL, US: University of Illinois Press.

Sih A. 1992. Prey uncertainty and the balancing of antipredator and feeding needs. Am Nat. 139(5):1052–1069. doi:10.1086/285372.

Sih A. 2013. Understanding variation in behavioural responses to human-induced rapid environmental change: a conceptual overview. Anim Behav. 85(5):1077–1088. doi:10.1016/j.anbehav.2013.02.017.

Sih A, Ziemba R, Harding KC. 2000. New insights on how temporal variation in predation risk shapes prey behavior. Trends Ecol Evol. 15(1):3–4. doi:10.1016/s0169-5347(99)01766-8.

Smolla M, Alem S, Chittka L, Shultz S. 2016. Copy-when-uncertain: bumblebees rely on social information when rewards are highly variable. Biol Lett. 12(6):20160188. doi:10.1098/rsbl.2016.0188.

Sol D. 2009. Revisiting the cognitive buffer hypothesis for the evolution of large brains. Biol Lett. 5(1):130–133. doi:10.1098/rsbl.2008.0621.

de Souza JF, Silveira MM, Barcellos HHA, Barcellos LJG, Luchiari AC. 2022. Sound stimulus effects on dusky damselfish behavior and cognition. Mar Pollut Bull. 184:114111. doi:10.1016/j.marpolbul.2022.114111.

Stankowich T, Blumstein DT. 2005. Fear in animals: a meta-analysis and review of risk assessment. Proc R Soc B Biol Sci. 272(1581):2627–2634. doi:10.1098/rspb.2005.3251.

Stephens DW. 1989. Variance and the Value of Information. Am Nat. 134(1):128–140. doi:10.1086/284969.

Stephenson JF. 2016. Keeping eyes peeled: guppies exposed to chemical alarm cue are more responsive to ambiguous visual cues. Behav Ecol Sociobiol. 70:575–584. doi:10.1007/s00265-016-2076-4.

Stroeymeyt N, Giurfa M, Franks NR. 2017. Information certainty determines social and private information use in ants. Sci Rep. 7(1):43607. doi:10.1038/srep43607.

Stynoski JL, Noble VR. 2012. To beg or to freeze: multimodal sensory integration directs behavior in a tadpole. Behav Ecol Sociobiol. 66(2):191–199. doi:10.1007/s00265-011-1266-3.

Szopa-Comley AW, Duffield C, Ramnarine IW, Ioannou CC. 2020. Predatory behaviour as a personality trait in a wild fish population. Anim Behav. 170:51–64. doi:10.1016/j.anbehav.2020.10.002.

Tebbich S, Teschke I. 2014. Coping with uncertainty: Woodpecker finches (*Cactospiza pallida*) from an unpredictable habitat are more flexible than birds from a stable habitat. PLOS ONE. 9(3):e91718. doi:10.1371/journal.pone.0091718.

Tejerina-Garro FL, Maldonado M, Ibañez C, Pont D, Roset N, Oberdorff T. 2005. Effects of natural and anthropogenic environmental changes on riverine fish assemblages: a framework for ecological assessment of rivers. Braz Arch Biol Technol. 48(1):91–108. doi:10.1590/S1516-89132005000100013.

Tonkin JD, Bogan MT, Bonada N, Rios-Touma B, Lytle DA. 2017. Seasonality and predictability shape temporal species diversity. Ecology. 98(5):1201–1216. doi:10.1002/ecy.1761.

Trimmer PC, Houston AI, Marshall JAR, Mendl MT, Paul ES, McNamara JM. 2011. Decision-making under uncertainty: biases and Bayesians. Anim Cogn. 14(4):465–476. doi:10.1007/s10071-011-0387-4.

Trompf L, Brown C. 2014. Personality affects learning and trade-offs between private and social information in guppies, *Poecilia reticulata*. Anim Behav. 88:99–106. doi:10.1016/j.anbehav.2013.11.022.

Tryjanowski P, Møller AP, Morelli F, Biaduń W, Brauze T, Ciach M, Czechowski P, Czyż S, Dulisz B, Goławski A, Hetmański T, Indykiewicz P, Mitrus C, Myczko Ł, Nowakowski JJ, Polakowski M, Takacs V, Wysocki D, Zduniak P. 2016. Urbanization affects neophilia and risk-taking at bird-feeders. Sci Rep. 6(1):28575. doi:10.1038/srep28575.

Valone TJ. 2006. Are animals capable of Bayesian updating? An empirical review. Oikos. 112(2):252–259. doi:10.1111/j.0030-1299.2006.13465.x.

Vieth W, Curio E, Ernst U. 1980. The adaptive significance of avian mobbing. III. Cultural transmission of enemy recognition in blackbirds: Cross-species tutoring and properties of learning. Anim Behav. 28(4):1217–1229. doi:10.1016/S0003-3472(80)80110-2.

Vilhunen S, Hirvonen H. 2003. Innate antipredator responses of Arctic charr (*Salvelinus alpinus*) depend on predator species and their diet. Behav Ecol Sociobiol. 55(1):1–10. doi:10.1007/s00265-003-0670-8.

Wagner RH, Danchin É. 2010. A taxonomy of biological information. Oikos. 119(2):203–209. doi:10.1111/j.1600-0706.2009.17315.x.

Walvoort D. 2022. **benthos**: Marine Benthic Ecosystem Analysis. [accessed 2022 Nov 24]. https://CRAN.R-project.org/package=benthos.

Webster MM, Laland KN. 2008. Social learning strategies and predation risk: minnows copy only when using private information would be costly. Proc R Soc B Biol Sci. 275(1653):2869–2876. doi:10.1098/rspb.2008.0817.

Weissburg M, Smee DL, Ferner MC. 2014. The sensory ecology of nonconsumptive predator effects. Am Nat. 184(2):141–157. doi:10.1086/676644.

Weissburg MJ, Zimmer-Faust RK. 1993. Life and death in moving fluids: Hydrodynamic effects on chemosensory-mediated predation. Ecology. 74(5):1428–1443. doi:10.2307/1940072.

White GE, Brown C. 2015. Cue choice and spatial learning ability are affected by habitat complexity in intertidal gobies. Behav Ecol. 26(1):178–184. doi:10.1093/beheco/aru178.

Wisenden BD. 2003. Chemically mediated strategies to counter predation. In: Collin SP, Marshall NJ, editors. Sensory Processing in Aquatic Environments. New York, NY: Springer. p. 236–251.

Wisenden BD, Harter KR. 2001. Motion, not shape, facilitates association of predation risk with novel objects by fathead minnows (*Pimephales promelas*). Ethology. 107(4):357–364. doi:10.1046/j.1439-0310.2001.00667.x.

Wobbrock JO, Findlater L, Gergle D, Higgins JJ. 2011. The aligned rank transform for nonparametric factorial analyses using only anova procedures. In: Proceedings of the SIGCHI Conference on Human Factors in Computing Systems. Vancouver, BC, Canada: Association for Computing Machinery. (CHI 2011). p. 143–146. doi:10.1145/1978942.1978963.

Yu AJ, Dayan P. 2005. Uncertainty, neuromodulation, and attention. Neuron. 46(4):681–692. doi:10.1016/j.neuron.2005.04.026.

Yunker WK, Wein DE, Wisenden BD. 1999. Conditioned alarm behavior in fathead minnows (*Pimephales promelas*) resulting from association of chemical alarm pheromone with a nonbiological visual stimulus. J Chem Ecol. 25(12):2677–2686. doi:10.1023/A:1020843322518.

Zhang Zonghang, Fu Y, Shen F, Zhang Zhen, Guo H, Zhang X. 2021. Barren environment damages cognitive abilities in fish: Behavioral and transcriptome mechanisms. Sci Total Environ. 794:148805. doi:10.1016/j.scitotenv.2021.148805.

Zimmermann H-J. 2000. An application-oriented view of modeling uncertainty. Eur J Oper Res. 122(2):190–198. doi:10.1016/S0377-2217(99)00228-3.

# I. Appendices

## **Appendix A:** *Chapter 2 Supplementary Materials*

**Table S2.1:** Experimental treatments for Experiment 1A and 1B, including the conditioning treatment names, the cues corresponding to said treatment, the test stimulus, and the sample size corresponding to each conditioning treatment × test stimulus combination.

| | *Treatment* | *Cues (order randomized)* | *Test Stimulus* | *Sample Size* |
|---|---|---|---|---|
| **Exp. 1A** | 1-cue | Lemon, Water, Water | Distilled Water (Control) | n = 12 |
| | | | Lemon Odour | n = 12 |
| | 2-cue | Lemon, Almond, Water | Distilled Water (Control) | n = 12 |
| | | | Lemon Odour | n = 12 |
| | 3-cue | Lemon, Almond, Aniseed | Distilled Water (Control) | n = 12 |
| | | | Lemon Odour | n = 12 |
| **Exp. 1B** | 1-cue | Lemon, Water, Water | Distilled Water (Control) | n = 12 |
| | | | Cherry Odour | n = 12 |
| | 2-cue | Lemon, Almond, Water | Distilled Water (Control) | n = 11 |
| | | | Cherry Odour | n = 11 |
| | 3-cue | Lemon, Almond, Aniseed | Distilled Water (Control) | n = 10 |
| | | | Cherry Odour | n = 12 |

**Table S2.2:** Experimental treatments for Experiment 2, including the conditioning treatment names, the cues corresponding to said treatment, the test stimulus, and the sample size corresponding to each conditioning treatment × test stimulus combination.

| *Treatment* | *Cue* | *Testing Stimulus* | *Sample Size* |
|---|---|---|---|
| Spatially Predictable | Lemon Odour | Distilled Water (Control) | n = 10 |
| | | Lemon Odour | n = 10 |
| Spatially Unpredictable | Lemon Odour | Distilled Water (Control) | n = 10 |
| | | Lemon Odour | n = 10 |
| Control | None | Distilled Water (Control) | n = 10 |
| | | Lemon Odour | n = 10 |

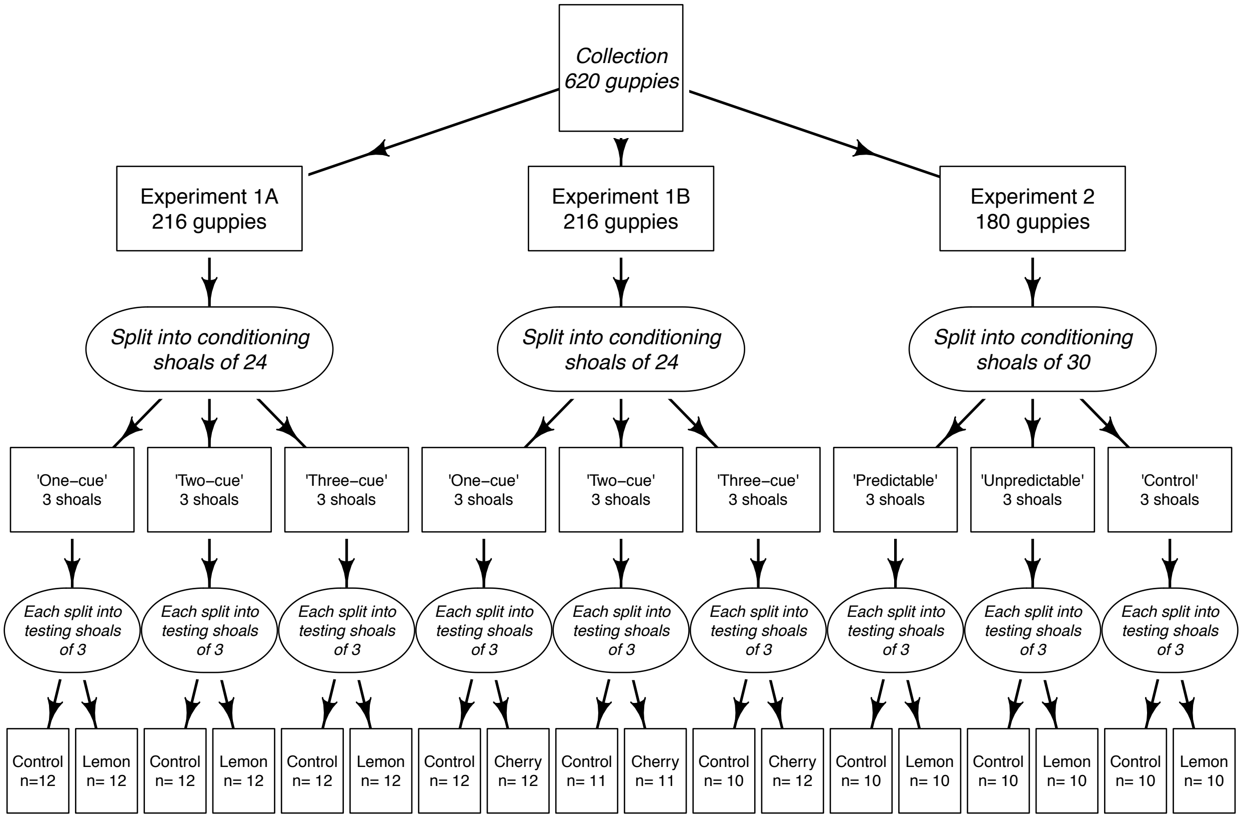


**Figure S2.1:** Experimental flow chart for Experiments 1A, 1B, and 2 demonstrating the total number of guppies collected and used, and sample sizes for each conditioning treatment × test stimulus combination.

**Appendix B:** *Chapter 5 Supplementary Materials*

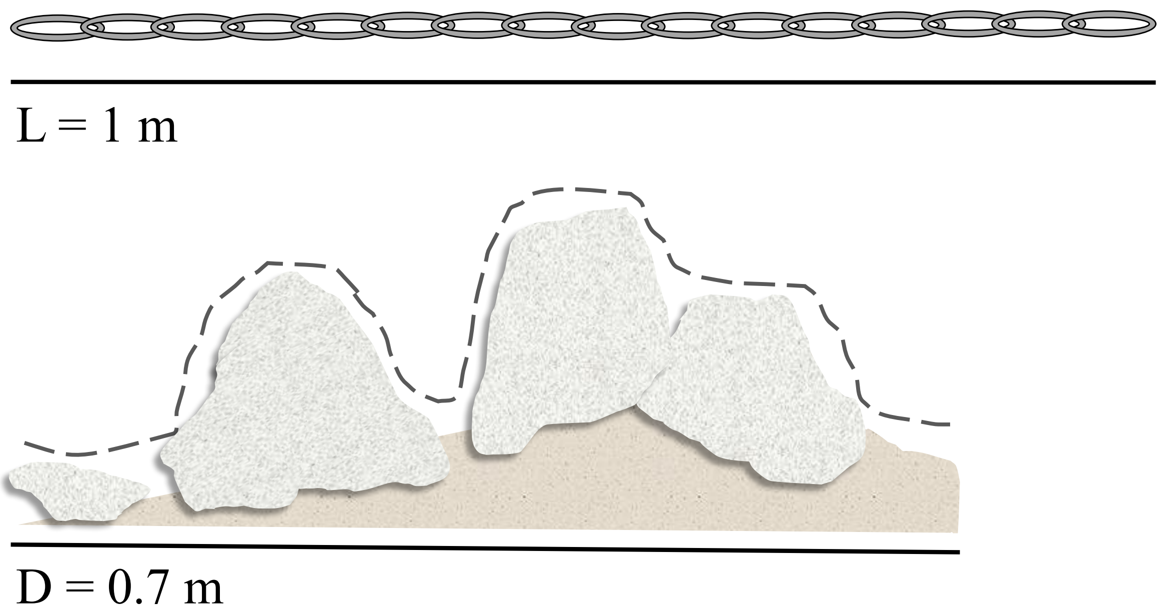


**Figure S5.1:** Schematic of rugosity measure, where a chain of original length (L) of 1 m was placed in a pool, and the linear length of the chain was measured (D). Rugosity was calculated as 1 - D/L. In this example, Rugosity would equal 0.3.

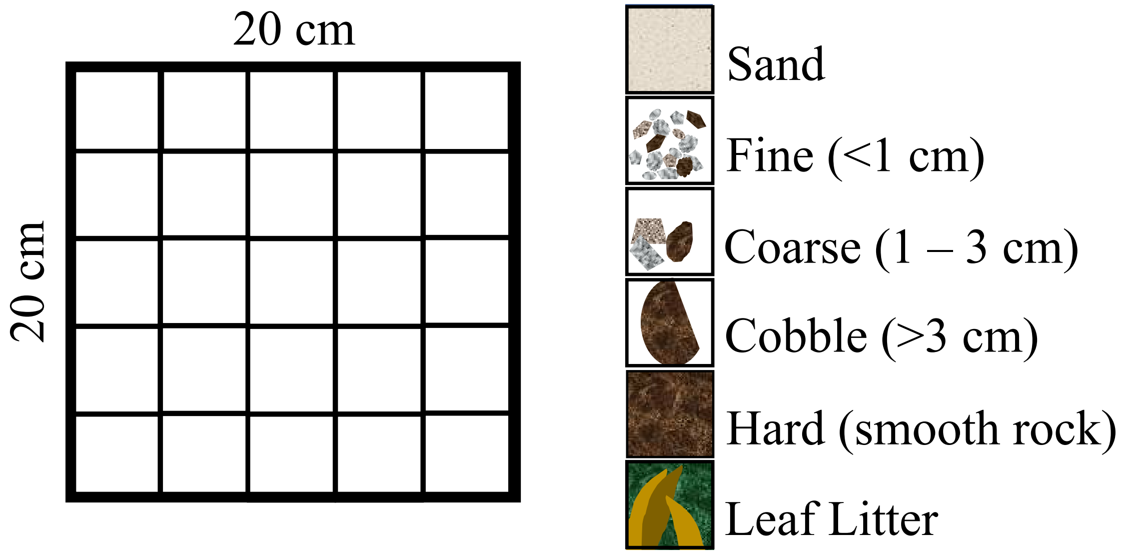


**Figure S5.2:** Schematic of the grid frame (20 cm × 20 cm) used for testing, along with the substrate classifications used.

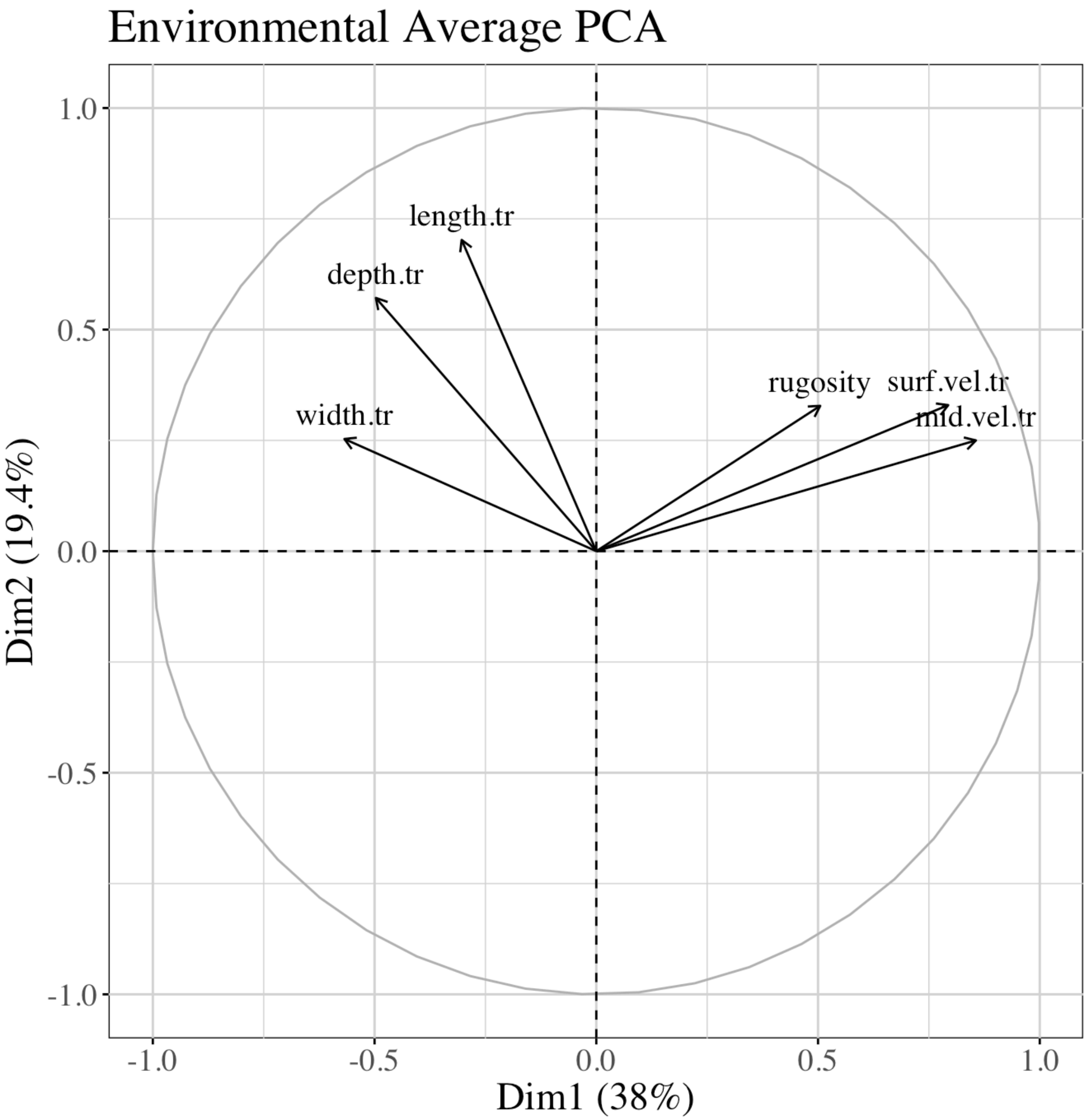


**Figure S5.3:** Biplot in Scaling Type 2 of the "average" environmental variables in PC1-PC2.Variables include the transformed average pool width (width.tr), transformed average pool depth (depth.tr), transformed pool length (length.tr), average substrate complexity (rugosity), transformed average surface water velocity (surf.vel.tr), and transformed average mid-depth water velocity (mid.vel.tr).

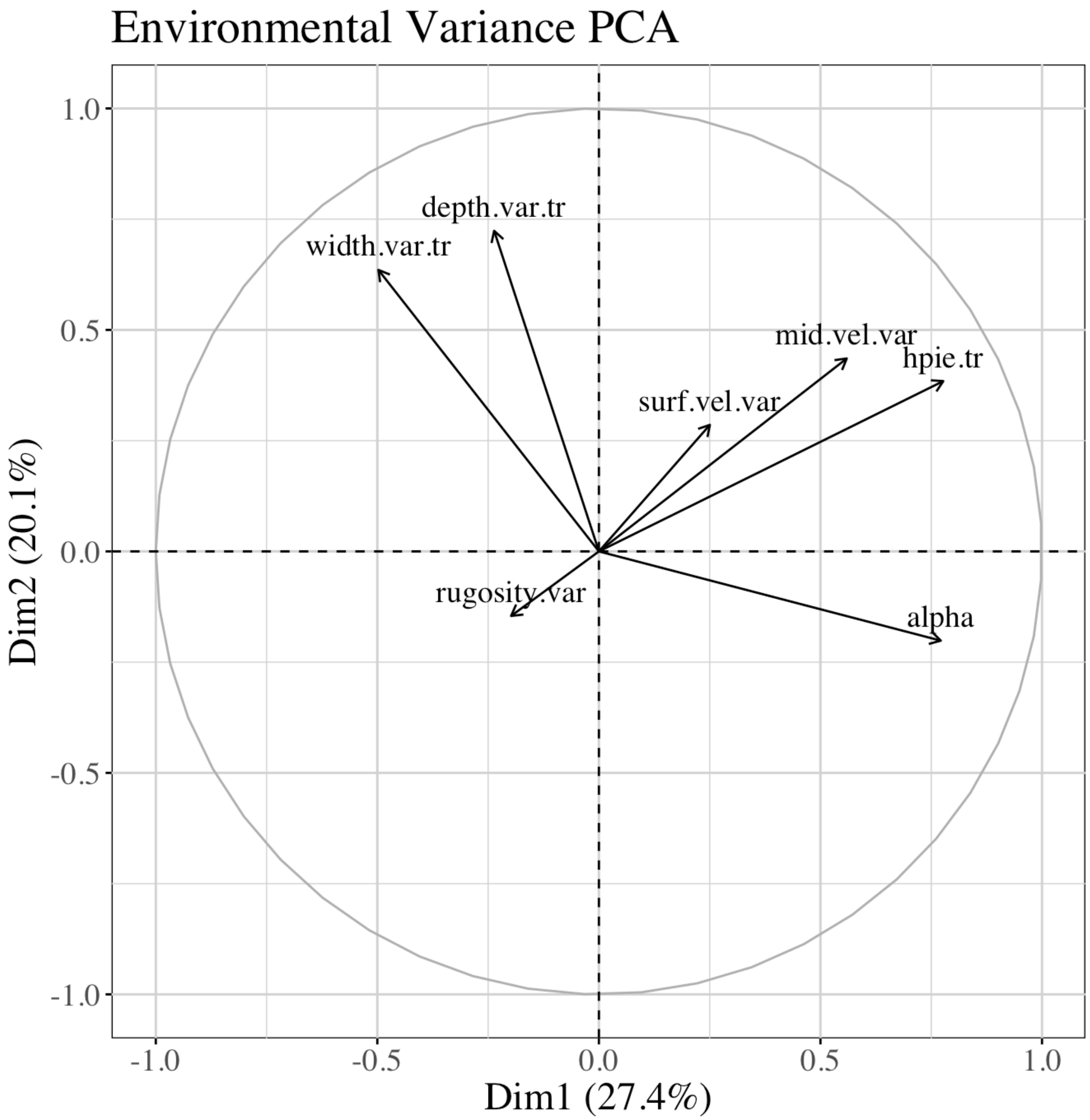


**Figure S5.4:** Biplot in Scaling Type 2 of the “variance” environmental variables in PC1-PC2.Variables include transformed pool width variance (width.var.tr), transformed pool depth variance (depth.var.tr), substrate complexity variance (rugosity.var), surface water velocity variance (surf.vel.var), and mid-depth water velocity variance (mid.vel.var), transformed substrate heterogeneity (hpie.tr), and substrate alpha diversity (alpha).