Impacts of Uncertainty of Predation Risk in Trinidadian Guppies

Félixe Dumaresq Synnott

A Thesis in

The Department of Biology

Presented in Partial Fulfillment of the Requirements

For the Degree of Master of Science (Biology) at

Concordia University

Montreal, Quebec, Canada

March 2025

CONCORDIA UNIVERSITY

SCHOOL OF GRADUATE STUDIES

This is to certify that the thesis prepared

By: Félixe Dumaresq Synnott

Entitled: Impacts of Uncertainty of Predation Risk in Trinidadian Guppies

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

Master of Science (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. Nicola Smith

____ External Examiner
Dr. Rassim Khelifa

____ Examiner
Dr. Robert Weladji

____ Examiner
Dr. Nicola Smith

____ Supervisor
Dr. Grant E. Brown

Approved by ____
Dr. Robert Weladji – Graduate Program Director

_________________________, 2025 ___________________________________
Dr. Pascale Sicotte, Dean of Faculty

GENERAL ABSTRACT

Impacts of Uncertainty of Predation Risk in Trinidadian Guppies

Félixe Dumaresq-Synnott, MSc

This study investigates how prey species, specifically Trinidadian guppies (*Poecilia reticulata*), cope with ecological uncertainty in predator-prey dynamics. Ecological uncertainty arises from conflicting and/or unreliable environmental cues, especially in ecosystems impacted by anthropogenic disturbances, which may directly influence prey decision-making and behaviour. This work explores how prey balance risk and safety in unpredictable environments, offering new insights into the adaptive strategies they use.

Chapter 1 looks at how conflicting safety and risk cues influence neophobia, the fear of novelty. The results show that guppies exposed to contradictory cues were more neophobic, exhibiting reduced movement, which supports the hypothesis that uncertainty leads to greater caution. Conversely, guppies conditioned to safety cues showed a preference for novelty, highlighting that they engage with new experiences when they feel that these are safe. These findings suggest that neophobia may be an adaptive response to environments where risk signals are unclear, particularly in areas disturbed by human activity.

Chapter 2 examines how anthropogenic disturbances contribute to ecological uncertainty which influence prey behaviour. Through field and lab experiments, it was found that guppies from high predation-risk and disturbed environments exhibited more caution and took longer to explore or return to disturbed areas. Guppies from low predation-risk environments, however, were more exploratory. This suggests that human disturbances increase uncertainty, driving prey to adopt more conservative, risk-avoidant strategies.

Ultimately, this study highlights how environmental unpredictability shapes risk-averse behaviours like neophobia. As human impacts on ecosystems continue to intensify, understanding ecological uncertainty is crucial for predicting long-term effects on biodiversity and ecosystem stability.

Acknowledgements

Most importantly, I would like to express my deepest appreciation and gratitude to my supervisor, Dr. Grant E. Brown. Your guidance, patience, and care—especially after my luggage was stolen in a foreign country—have been invaluable in helping me grow both as a person and as a scientist. Working under your wing since my undergraduate years has shown me just how dedicated you are to everyone around you; you make us feel like you have all the time for us even when we know deep down that you don't. I cannot thank you enough. I would also like to extend my heartfelt appreciations to my committee members, Dr. Robert Weladji and Dr. Nicola S. Smith, for your insightful advice and recommendations regarding my experimental protocol and research. Your contributions have greatly enhanced the quality of my work. This work was funded by Concordia University's Faculty of Arts and Sciences, the Department of Biology, and the Natural Sciences and Engineering Research Council of Canada (Discovery Grant) to Dr. Grant E. Brown. A special thank you to Dr. Indar W. Ramnarine and Kharran Deonarinesingh from the University of the West Indies, St. Augustine, Trinidad and Tobago, for your valuable assistance and lab space. To Dr. Adam L. Crane, thank you for sharing your knowledge and helping me grow as a biologist. I also wish to thank Abigail C. Nagl and Jade Morris for their contributions to my second chapter and for making my second field season unforgettable. To my friend, colleague, and short-term bedmate, Alix Brusseau—thank you for being an incredible role model and for guiding me throughout this academic journey. I aspire to be like you when I grow up. To my wonderful friends and fellow lab members in the Brown Lab (and honorary members too)—Alexander Levesque, Jenna Domenicano, Emily Campbell, April Mansfield, Grace Wallace, Sofia D'Angelo, Katie Moffat, Iris George, and Olivier Godin—thank you for your emotional support and friendship throughout these years. You have made this journey both memorable and rewarding. To Ivon Vassileva—thank you for taking such good care of our lab, the fish, and for keeping the ship running. To my best friends, Catherine Kayla Fortin and Stefanie Petrella—thank you for always being there for me with open arms and ears when the journey became challenging. Your support has meant the world to me. À toi, Maman, Martine Dumaresq, je tiens à te dire un immense merci pour ton amour inconditionnel, ta patience et ton soutien sans faille tout au long de mon parcours. Ta sagesse et ta gentillesse ont été mes phares dans cette aventure. For you, Dad, Daniel Synnott, I want to express my deepest thanks for your unwavering love, patience, and support throughout this journey. Your strength, sense of humor, and wisdom have always been a source of comfort and motivation for me. To my twin and first friend, Alexis Dumaresq Synnott—thank you for your support, even if you never quite understood why I spend so much time with fish without "actually" fishing, you're the best. To my favourite dog, Bagatelle—thank you for your love and cuddles and for reminding me that life is more than experiments and data. Finally, to my love, David Berger—thank you for your unconditional love, support, countless drives to Montreal, and for patiently listening to my endless talk about fish and science. I can't wait to see what our future holds.
And, of course, to the "millions fish"—thanks a million!

All work reported herein was conducted in accordance with Concordia University Animal Research Ethics Protocol AREC-30000255.

Contribution of Authors

Chapter 1
Study concept and design: F.D.S and G.E.B
Acquisition of data: F.D.S, A.J.B, A.L.C, and G.E.B
Analysis and interpretation of data: F.D.S and G.E.B
Drafting of manuscript: F.D.S
Critical revision: F.D.S and G.E.B

Chapter 2
Study concept and design: F.D.S and G.E.B
Acquisition of data: F.D.S, A.J.B, J.M, A.C.N, A.L.C, and G.E.B
Analysis and interpretation of data: F.D.S and G.E.B
Drafting of manuscript: F.D.S
Critical revision: F.D.S, A.J.B, A.L.C, and G.E.B

Table of Contents

List of Figures

List of Tables

General Introduction

Animals are constantly forced to make behavioural decisions that influence their life histories. These trade-offs, especially for prey species, involve allocating limited time and energy across competing demands, such as foraging, mating, and anti-predator behaviours (Lima & Dill, 1990; Brown & Godin, 2023). The ability to make these decisions depends on the quality of information gathered from the environment, which can be derived from two main sources: social information (gathered from interactions with conspecifics) and private information (gained through individual experiences) (Schmidt et al., 2010; Munoz & Blumstein, 2012; Crane et al., 2018). However, the reliability and consistency of this information can vary, leading to ecological uncertainty.

Ecological uncertainty is the inability to predict environmental events due to disturbances in information flow caused by both abiotic and biotic factors (Brown & Godin, 2023; Crane et al., 2024). It can arise from various sources, such as changes in the physical environment, the presence of unfamiliar stimuli, or conflicting information from different sources (Crane et al., 2024). Prey must adjust their anti-predator, foraging, competitive, and mating behaviours to cope with constantly changing and sometimes contradictory environmental cues (Lima & Dill, 1990; Sih, 1992). This adaptation is critical for maintaining fitness in an unpredictable world (Schmidt et al., 2010), as increased information-gathering behaviours driven by uncertainty can lead to heightened anti-predator responses. Predation risk and the ecological uncertainty of predation risk are different; predation risk references the level of risk (i.e., density, diversity, and abundance or predators and predation encounters) in an ecological setting whereas the ecological uncertainty of predation risk relates to the inability to accurately predict the level of predation risk (Crane et al., 2024). Under natural conditions, predation risk may be variable according to space, time, and natural environmental occurrences; however, anthropogenic disturbances may disrupt these and influence predator-prey interactions (Murphy et al., 2021), leading to ecological uncertainty (Crane et al., 2024).

One key behavioural response to ecological uncertainty is neophobia, or the fear of novelty (Brown et al., 2013). Neophobia plays a critical role in risk assessment, as animals faced with unfamiliar or uncertain conditions tend to exhibit more cautious behaviours. In prey species, this heightened caution can manifest as increased anti-predator responses when faced with ambiguous cues, such as novel objects or unfamiliar disturbances (Johnson et al., 2013). While this wariness reduces the likelihood of predation, it can also lead to missed opportunities for foraging and mating (Crane et al., 2024). The relationship between uncertainty and neophobia is central to understanding how prey cope with ecological unpredictability (Crane et al., 2024). When prey are exposed to conflicting information, such as competing cues about predators and/or safe foraging opportunities, their neophobic responses are likely to increase, reflecting heightened uncertainty and cautious decision-making. This increased wariness can be measured experimentally by observing how prey respond to novel stimuli. The aim of this research is to explore how ecological uncertainty influences predator-prey dynamics by examining the behavioural responses of prey to varying levels of risk and safety. Specifically, the study seeks to investigate the relationship between neophobia, risk assessment, and the uncertainty of environmental cues in Trinidadian guppies.

One ideal system for studying these dynamics is the freshwater ecosystems of Trinidad and Tobago, particularly the rivers in the Northern Range Mountains, where the Trinidadian guppy (*Poecilia reticulata*) is endemic. These small prey fish face a variety of predation pressures from larger species like the hart's rivulus (*Rivulus hartii*), pike cichlid (*Crenicichla alta*), and wolf fish (*Hoplias malabaricus*), which influence community structures through their predatory actions (Magurran, 2005).

These sites are very important to Trinidadian communities as they are easily accessible and allow Trinidadians to host several recreational activities (Deacon et al., 2015). Not unexpectedly, these activities cause much disturbance in the ecosystem and may introduce numerous chemical pollutants, create uncommon noises, and generate habitat modifications (Knight and Gutzwiller 1995, Deacon et al. 2015, Brusseau et al., 2023). Moreover, the diversity of habitats, from pristine, clear pools to disturbed, polluted waters, provides a unique opportunity to study how prey species respond to varying levels of ecological uncertainty (Deacon et al., 2015). Local disturbances in these ecosystems, such as falling fruit or human activities like swimming, can alter the flow of information in ways that introduce further uncertainty for the guppies. These disturbances can lead to unpredictable changes in the environment, such as unfamiliar odors or sudden changes in water chemistry, which confuse the prey's risk assessment and alter their behaviour. As a result, guppies are forced to constantly reassess the risks of predation, food availability, and other environmental factors, making them an ideal subject for studying how ecological uncertainty influences decision-making in prey animals.

The research is guided by three main hypotheses. First, in situations where prey receive contradictory information about safety and risk, increased neophobia will result, reflecting the heightened uncertainty faced by the prey (Johnson et al., 2013). This will be tested by subjecting guppies to a range of risk, safety, and uncertainty treatments and observing their anti-predator behaviours in response to novel cues. Second, prey exposed to high levels of background risk will exhibit greater uncertainty in response to novel stimuli compared to those with lower background risk. Lastly, prey from environments with higher anthropogenic disturbances will show more pronounced neophobic responses due to the continuous introduction of novel stimuli into their environments. These hypotheses will be tested through a combination of field and laboratory experiments, with the goal of providing both theoretical insights and practical applications for understanding predator-prey interactions and the role of ecological uncertainty in shaping prey behaviour.

The broader significance of this research lies in its potential to deepen our understanding of predator-prey dynamics in the context of ecological uncertainty and neophobia. By examining how prey respond to varying levels of risk and environmental disturbance, this study will contribute to the field of behavioural ecology and provide valuable insights into how animals cope with unpredictable environments. Furthermore, the findings may have broader implications for conservation biology, especially in the context of anthropogenic disturbances that alter natural habitats and create uncertainty for wildlife. This research aims to shed light on the complex ways in which animals balance safety and opportunity in uncertain environments, with a focus on the critical role of information in shaping decision-making processes. Chapter 1 highlights how behavioural decisions are strongly influenced by ecological uncertainty in risk and reward assessments. Meanwhile, chapter 2 emphasizes the belief that uncertainty is an understudied complex concept which humans may have a greater influence on than previously believed. By exploring these dynamics, we can better understand how prey species adapt to changing environments and the ecological consequences of those adaptations.

The following chapter will be submitted as:

Safety and Risk Associations: Uncertainty Shapes Behavioural Responses to Novelty in Trinidadian Guppies

Félixe Dumaresq-Synnott[1], Alix J.P. Brusseau[1], Adam L. Crane[2], & Grant E. Brown[1]
[1] Department of Biology, Concordia University, 7141 Sherbrooke St. West, Montreal, Québec, H4B 1R6
[2] Department of Mathematical and Natural Sciences, University of Arkansas at Monticello, 346 University Dr, Monticello, Arkansas, AR 71656

Chapter 1:

Safety and Risk Associations: Uncertainty Shapes Behavioural Responses to Novelty in Trinidadian Guppies

1.1. Introduction

Predation pressures drive numerous behavioural trade-offs within prey species (Lima & Dill, 1990; Brown & Godin, 2023). Indeed, spatially and temporally variable predation threats force prey to continually make trade-offs between the often-conflicting benefits of detecting and avoiding local threats and engaging in other fitness related activities such as foraging and mating (Lima & Dill 1990; Brown et al. 2006). The costs associated with making these decisions increase when prey cannot reliably recognize ecological threats (Dill, 1987). Thus, prey animals can only make context-appropriate behavioural decisions if they possess ecologically relevant and reliable information. Moreover, publicly available information about the condition of local microhabitats may either indicate risk (i.e., alarm cues) or safety (i.e., food odour) (Schmidt et al., 2010; Crane et al., 2018; Brown & Godin, 2023).

Several factors may influence the availability and/or reliability of information (Brown & Godin, 2023). Prey might become uncertain of local conditions when this information may be incomplete, conflicting, variable, or unreliable (Dall & Johnston, 2002; Crane et al., 2024), leading to greater uncertainty. This can influence animal behaviour where they increase in their information gathering activities to reduce uncertainty and become more risk-averse (Brown & Godin, 2023; Crane et al. 2024). Individuals face many different types of uncertainty within their environment; it is a multifaceted concept (Mathot et al., 2012). There exists uncertainty in mate choice; foraging opportunities; area use and exploration; competitive interactions; risk; and safety, to name a few. Uncertainty of predation risk occurs when prey are unsure about the existence and extent of a potential predation threat in their environment (Crane et al., 2024). Similarly, the uncertainty of safety may be created due to unreliable information regarding reward and safety, a concept that remains under-explored (Feyten et al., 2021; Crane et al. 2024). Most research relates to foraging or anti-predatory contexts, while minimal investigation has been done on safety-related aspects of ecology (Smolla et al., 2016; Feyten et al., 2023). Further research on how environmental information demonstrates safety could be valuable in understanding certain ecological behaviours (Luttbeg et al. 2020; Ferrari et al. 2017), especially when individuals face novel settings.

Neophobia, defined as the fear of novelty, is a phenotypic plastic response that is commonly observed in prey fishes (Brown et al., 2013; Crane et al., 2020). For prey fishes, various problems may arise when they become uncertain of the information they chemically and visually gather and therefore, they are uncertain of how to respond. As a result of increasing uncertainty, prey frequently find themselves in ambiguous scenarios resulting in an increase in the costs of deciding (Johnson et al., 2013), especially when risk is involved (Crane et al. 2024). Responding with an increase in anti-predator behaviour (i.e., risk-averse) towards a non-threating cue leads to the loss of foraging and mating opportunities (Johnson et al., 2013; Brown & Godin, 2023). Conversely, not responding to an actual predation risk can potentially lead to death (Johnson et al., 2013; Brown & Godin, 2023). Due to this, uncertainty may become an important driver of neophobia. Yet, little is known about this mechanism (Feyten et al., 2019). For instance, Trinidadian guppies are native to the freshwater rivers of the islands of Trinidad and Tobago. These rivers are of great importance to local communities and have multiple recreational and religious purposes (Deacon et al., 2015). However, these activities may influence the perception of risk and safety in these ecosystems via the introduction of novel information (Brusseau et al., 2024). This information could then be randomly paired with risk or safety cues (i.e., a novel smell is introduced during a predation or foraging event) and then induce uncertainty when later paired with other types of cues. Therefore, studying uncertainty becomes crucial to understand ecological interactions (Brown & Godin, 2023).

Risk is a common detailed concept when studying predator-prey interactions (Crane et al., 2024). The degree of risk may vary in the environment according to the distance and behaviour of a predator as well as the behaviour of other prey (Luttbeg et al., 2020). Evidently, it is more advantageous for a prey to view a predator since the indicators of risk are much greater and the uncertainty of such an encounter is much smaller, compared to not seeing a predator that is nearby (Luttbeg et al. 2020). Indeed, the closer the predator is to a prey, the clearer and more reliable the information is regarding the predator's distance and intention (Dugatkin & Godin, 1992; Fishman, 1999; Brown & Godin, 1999b). This allows for the prey to inspect the predator and reduce uncertainty regarding the situation (Crane et al., 2024). However, predator inspection also comes at a cost: the prey must survive the encounter to learn from it (Lima & Dill, 1990; Crane et al., 2020). To reduce this cost, prey can use chemosensory cues such as alarm cues which is released when the skin of a conspecific is damaged are a stronger indicator of risk (Kats & Dill 1998; Goldman et al., 2020). As alarm cues cannot be manipulated by the sender, they are a reliable source of predation risk in the surrounding environment of the receiver (Goldman et al., 2020).

Contrary to risk, the concept of safety and its ability to convey reliable predation information is much understudied. When predation risk is low, safety cues may provide more acute information about predation levels (Luttbeg et al., 2020). Observing a conspecific or a prey that share a predator forage or mate can be a sign of safety and might be an accurate depiction of the current predation level, especially if the observed individual occupies a dominant position in the social hierarchy (Luttbeg et al., 2020). Additionally, Luttbeg et al. (2020) found that prey have higher fitness if they are more attentive to safety cues rather than risk cues, which give more acute information on low levels of predation risk, as they give more acute information on high levels of predation risk. However, both types of cues, risk and safety, can be present at once. Therefore, uncertainty is much more likely to be experienced in any natural environment. Thus, uncertainty may influence anti-predator behaviour more than previously believed. For this study, we define safety cues as perceiving fitness-forward behaviours, like foraging and mating of conspecifics (either visually, auditorily, and/or chemically).

In the present experiment, we exposed 6 shoals of female Trinidadian guppies to 6 different treatments, one each, for 5 days. We created novelty within the prey's environment by exposing them to novel odours. These are chemosensory cues that the individuals have never encountered before, and therefore would elicit a neophobic response (Crane et al., 2020). The treatments were as follows: (1) alarm cues paired with alternating novel odours; (2) food odour paired with alternating novel odours (high safety); (3) alarm cues paired with water (known risk); (4) alternating alarm cues and food odour paired with alternating novel odours (high uncertainty); (5) alternating alarm cues and water paired with alternating novel odours (low uncertainty of predation risk); and (6) alternating food odour and water paired with alternating novel odours (low uncertainty of safety). On the sixth day, we tested the neophobic behaviour of these individuals by observing their anti-predator responses, as a proxy for experienced uncertainty. Moreover, 5 days later we retested them to conditions identical to those of their testing day to explore the retention of their neophobic behaviour and, thus, the persistence of uncertainty.

In scenarios of contradictory information regarding safety and risk, we hypothesized that neophobia arising from uncertain information should be influenced by conflicting risk and safety information. Here, the cost of non-response when the threat is real outweighs the cost of responding when the threat is not real. Hence, we predicted that pairing alarm cues with a novel odour should signify a "risky" environment, eliciting an anti-predator response in our prey fish and potentially conditioning them towards neophobia. Conversely, we also predicted that pairing food odour with a novel odour should represent a "safe" environment, resulting in no shift in normal behaviour and possibly even encouraging neophilia (attraction to novelty). Lastly, in the retention trials we predicted to observe the highest magnitude of neophobia (i.e., anti-predator response) in conditions with the greatest amount of contradicting of information. Ecological uncertainty arises from varied safety and risky cue associations within an environment (Feyten & Brown, 2018; Brown & Godin, 2023; Crane et al., 2024). By examining neophobia's strength, this study aims to understand how behaviours are shaped through uncertainty.

1.2. Methods

1.2.1. Model Organism

Trinidadian guppies are a small prey fish species naturally found in the rivers of Trinidad and Tobago. Prior to this study, female guppies were held in 275 L aquaria (~23 C°, 12-hour light-dark cycle, and fed ad libitum twice a day with Tetra^{TM} flakes). Females were chosen as a model for this study due to the strength of their response to conspecific chemical cues and strong anti-predator behaviours (Brusseau et al. 2024).

1.2.2. Stimulus Cues

We generated three cue types for this experiment: conspecific alarm cues, novel odours, and food odour. The alarm cues (noted as AC later) were made by euthanizing male and female conspecifics via a blow to the head followed by cervical dislocation. Afterwards, the heads and tails of these euthanized fish were removed which left the center mass of tissues to be used. These were placed in 100 mL of dechlorinated water, homogenized, filtered, and lastly diluted to a final concentration of 0.1 cm^2 mL^{-1}. This procedure follows the Concordia University Animal Research Ethics (Protocol #30000255). As previously demonstrated by other studies, this concentration is shown to elicit an anti-predator behaviour in Trinidadian guppies (Brown & Godin, 1999a). To preserve these cues for the duration of the experiment, they were divided into 30 mL aliquots, frozen at -20˚C, and each aliquot was thawed before use.

Moreover, six distinct novel odours were used: vanilla (NO_1), coconut (NO_2), almond (NO_3), mint (NO_4), orange (NO_5), and lemon (NO_6). Each odour was prepared by diluting 6 drops of essence (No Name^{TM} and Club House^{TM}) into 500 mL of dechlorinated water. These odours were never encountered by the test individuals prior to the experiment. Therefore, they have the ability to act as a novel stimulus and elicit neophobia in Trinidadian guppies (Crane et al., 2020). Similarly to the steps described previously, they were divided into 50 mL aliquots, frozen at -20˚C, and thawed prior to use. Novel odours 1 through 5 were used during the conditioning process whilst novel odour 6 was only introduce on the testing and retention days. Finally, the food odour (FO) was made by steeping 1g of fish food flakes, the same that the fish were fed with, into 0.5 L of dechlorinated water. The liquid obtained was then divided into 50 mL aliquots, frozen at -20˚C, and thawed before use.

1.2.3. Conditioning Process

We conditioned six shoals of guppies, each to a different treatment of risk or safety. For this, 6 individuals were placed into a 7.5-liter buckets filled with roughly 6 L of dechlorinated tap water (~ 25°C, pH 7.6) (Fig. 1). Each conditioning bucket was equipped with an air stone to which was attached a length of tubing to allow for the injection of conditioning cues without disturbing the fish. Alarm cues (AC) were injected to elicit a risky scenario, whereas food odours (FO) were injected to elicit safety. For each cue, we injected 2mL of it though the injection tube thanks to a syringe. Each block of conditioning gave an N of 3 per treatment combination (see below). A total of 9 testing blocks were conducted to ensure an adequate final sample size (N=27).

During each block, guppies were exposed to stimulus cues twice daily, at 10:00 (AM) and at 14:00 (PM), for five consecutive days. For each treatment, varying levels of risk or safety were paired with continuously alternating novel odours. The treatment exposures, explained in depth in Table S1.1, were as follows: (1) AC and NO, (2) FO and NO, (3) AC and water, (4) alternating FO and AC with NO, (5) alternating water and AC with NO, and lastly (6) alternating water and FO with NO (Table S1.1). For treatments 4, 5, and 6 the order of the exposure was alternated from one block to the next to control for the influence the initial exposure may have on subsequent behaviours. Regardless of risk treatment, each group received each NO twice over the course of the conditioning phase, with the stipulation that they did not receive the same NO in sequential exposures. Moreover, doing this also allowed us to pair a same novel odour with safety and risk (treatment 4), risk and water (treatment 5), and safety and water (treatment 6) (Table S1.1), during the same conditioning period and potentially further inducing uncertainty. Each individual treatment was created to condition the prey to different environments. Treatment (1) aimed to condition the fish to a high-risk situation, (2) to a safety situation, (3) to control for the AC exposures, (4) high uncertainty scenario, (5) to control for the number of AC exposures, and (6) to control for the number of FO exposures. Following each conditioning day, a complete water change was performed to prevent the accumulation of metabolites as well as limit the exposure to the cues.

1.2.4. Experimental Setup and Assays

The testing arenas were a series of 7.5-liter buckets identical to the conditioning setup, with intersecting lines creating four equal quadrants at the bottom (Figure S1.1). Using 2.50 cm PVC pipes, we assembled a rig around two individual testing buckets and fixed a mirror at an angle on top, allowing the observer to view the fish without being seen (Figure S1.1).

Two fish from the same treatment were placed in a testing arena (Figure 1.1). Only one focal fish was observed, identified thanks to its size or natural specific-coloured spots on their head. The movement behaviours, in the form of line crosses counted via a clicker, were observed for 4 minutes, followed by the injection of 2 mL of novel odour 6 (i.e., lemon). Following the injection movement was recorded for another 4 minutes. The intensity of the neophobic response was quantified as the magnitude of the anti-predator behaviour using movement. The movement index was quantified by counting the number of lines crossed by a focal individual during the 4-minute observation periods. Reduced movement is associated with anti-predator behaviour (Crane et al., 2022). Contrastingly, increased or unchanged movement should indicate normal calm behaviour.

1.2.5. Retention

After each testing day, the fish were returned to their original conditioning bucket, with the other fish hey were conditioned with, until the second testing day five days later, which assessed for retention of the neophobic behaviour. During this interim period, the fish were not exposed to any additional cues, were fed their regular diet, and water changes were performed every other day. During the retention testing day we followed the same procedure as during the previous assays (see previous section). Following the retention day, the fish were officially reintroduced to their initial 275 L holding tanks with other conspecifics.

1.2.6. Data Analysis

Initially, we ran a Generalized Linear Model to test the effect of background conditioning on the baseline (pre-stimulus) number of line crosses. We included conditioning group as a random factor to account for the non-independence of guppies conditioned in the same round. We then applied a Generalized Linear Mixed Model (GLMM) test for the effects of treatment. We included pre- vs. post-stimulus observation period as a repeated factor. We ran separate analyses for guppies tested on Day 1 vs. Day 6 post-conditioning. All analyses were conducted using SPSS (v. 29.0).

1.3. Results

Initially, we found no overall significant effect of treatment ($F_{5,47.05} = 1.13$, $P = 0.31$) or conditioning group ($F_{48,105} = 1.12$, $P = 0.312$), on baseline activity (number of lines crossed) demonstrating that our background conditioning treatment nor conditioning block did not influence pre-stimulus activity. Despite not finding any significant differences among pre-stimulus observations, there was sufficient variance, justifying the use of a repeated measures approach for subsequent analyses. Our repeated measures GLMM demonstrated a significant interaction between observation period (pre- vs. post-stimulus) and background conditioning treatment ($F_{5,153} = 6.18$, $P < 0.001$, Figure 1.2), but no significant main effect of observation period ($F_{1,153} = 0.14$, $P = 0.71$). The fish conditioned with continuous food odour paired with alternating novel odours had the greatest increase in movement post-stimulus injection. Alternatively, the random risk and safety treatment (where fish were conditioned to alternating alarm cues and food odours paired with alternating novel odours) had the greatest decrease in movement post-stimulus injection. Thus, each treatment had different movement outcomes pre- and post-stimulus in accordance with their risk, safety, or uncertainty exposure.

For guppies tested on Day 6 (retention), we found a significant main effect of observation period ($F_{1,138} = 11.05$, $P < 0.001$) but no observation period x treatment interaction ($F_{5,138} = 0.52$, $P = 0.76$; Figure 1.3). Thus, each treatment had no significant influence on movement demonstrating that the conditioning effect was considerably lost.

1.4. Discussion

This study aimed to examine how conflicting safety and risk information, conveyed through various odour cues, influences neophobia in prey fish. Our hypothesis was that contradictory cues would lead to an increase in neophobia due to the uncertainty they create regarding novel information. When testing the response of guppies tested on Day 1, we found a significant interaction between observation period (pre- vs. post stimulus injection) and treatment, with different treatments resulting in distinct baseline and post-stimulus movement patterns. Interestingly, baseline movement (pre-stimulus) reflected the conditioning process, while these differences were not significant, they do suggest that the fish were processing risk and safety information in a manner that influenced their baseline activity. For instance, fish exposed to consistently high-risk cues exhibited reduced movement before stimulus injection, particularly in the alarm cues-only treatment. Conversely, fish conditioned to safety cues showed more movement prior to the stimulus. Notably, fish exposed to the greatest uncertainty, where both risk and safety cues were conflicting, displayed the lowest baseline movement. This observation suggests that uncertainty may induce more cautious, risk-averse behaviour than even consistent high-risk exposure (Brown & Godin, 20203; Brusseau et al., 2024; Crane et al., 2024). Overall, these observations suggest that the fish adjusted their behaviour based on the information they learned during the conditioning phase. These results align with our hypothesis that greater uncertainty leads to stronger neophobic responses. Additionally, when exposed to consistent safety, fish showed an increase in post-stimulus movement, consistent with our prediction that novelty would be associated with safety, leading to neophilic behaviour.

The post-stimulus movement results on Day 1 provided further insight into how uncertainty influences neophobia. Fish in the treatments conditioned to risk-averse cues (the conditioning of the fish with continuous alarm cues paired with alternating novel odours, continuous alarm cues paired with water, and water alternating with alarm cues paired with alternating novel odours) crossed fewer lines after the stimulus, suggesting that novelty was perceived as a potential threat. In contrast, fish in the safety-conditioned treatments (the conditioning of the fish with continuous food odours paired with alternating novel odours and water alternating with food odours paired with alternating novel odours) exhibited more movement, indicating a preference for novelty, as predicted. Notably, the treatment exposed to the greatest uncertainty (conflicting cues of risk and safety) showed the most pronounced reduction in post-stimulus movement, further supporting our hypothesis that uncertainty induces stronger neophobic behaviour.

In line with previous studies on neophobia and risk assessment in prey species, our findings suggest that exposure to ambiguous or conflicting threat cues triggers increased caution and exploration avoidance (Feyten et al., 2019; Crane et al., 2022; Feyten et al., 2023). Research has demonstrated that animals in uncertain environments often exhibit heightened neophobia, as they prioritize avoiding potential risks over exploring novel stimuli (Feyten et al., 2019; Crane et al., 2022). This can be illustrated via the error management theory as described by Johnson et al. (2013), where prey must continuously make decisions about potential predation threats; where reacting to false-predation threats leads to the loss of opportunities and not reacting to true-predation threats can lead to injury or death. This aligns with our observation that the most pronounced neophobic behaviour occurred in the presence of conflicting risk and safety cues. Our study builds on this, showing that fish exposed to the highest uncertainty (conflicting cues) exhibited the most cautious behaviour, with reduced movement and exploration, which may reflect an adaptive strategy to reduce the likelihood of exposure to danger when faced with ambiguous environmental cues (i.e., neophobia) (Crane et al., 2022; Crane et al., 2024).

In contrast, research on neophilia, which is typically observed when animals associate novelty with safety or food, also offers an important comparison. For instance, Tryjanowski et al. (2016) found that great tits, *Parus major*, exhibited greater neophilia in urbanized areas compared to rural areas which allowed them to exploit uncertain food sources. Moreover, Franks et al. (2023) found that zebrafish, *Danio rerio*, exhibited indiscriminate neophilia to novel objects; the fish seemed to voluntarily seek cognitive stimulation when there was minimal environmental stimulation. This study does not delve into how anthropogenic disturbances may potentially impact fish cognitive stimulation; however, anthropogenic disturbances may increase environmental stimulation (as witnessed in Trinidadian Rivers) which could potentially reduce neophilia in affected fishes; in turn, this may exacerbate human impacts on behaviour. Here, our results illustrated in the safety-conditioned treatments, showed that the fish increased movement after exposure to novel odours associated with food, indicating neophilic tendencies as well. This may suggest that neophilia incentivizes the fish to move and explore, potentially leading them find a food source. While prior studies often focus on one cue type (e.g., food or predator), our study highlights how the presence of mixed or ambiguous signals might necessitate a more conservative, risk-averse strategy that prioritizes survival over exploration, even at the cost of missed opportunities.

The Day 6 retention trial did not yield significant results, although the data displayed a slight trend mirroring the initial trial. The lack of significant findings in the retention phase suggests two possibilities: (1) the conditioning process may not have been long enough to induce lasting neophobic responses, or (2) prey fish may not need to retain neophobic behaviour caused by uncertainty for long periods (e.g., 5 days). Thus, our second prediction, regarding the persistence of neophobia over time, was not fully supported. Future research could explore the duration over which uncertainty-driven neophobic responses persist and whether there is a threshold of uncertainty that impacts response longevity. Interestingly, the two treatments conditioned to food odours paired with alternating novel odours exhibited a trend to retention of neophobic responses, which may indicate that the fish place greater weight on safety cues when exposed to uncertainty. However, this effect was not substantial enough to draw definitive conclusions.

Overall, our results support the hypothesis that ecological uncertainty, particularly in risk and safety assessments, strongly influences neophobic behaviour in guppies. The significant neophobic responses observed in the presence of contradictory cues suggest that prey species may adapt to environments characterized by fluctuating or ambiguous threat levels. Neophobia, as a risk-averse strategy, may serve as a way to balance the costs of responding to false alarms (i.e., non-threats) against the potential costs of ignoring real threats. In unpredictable environments, where risk is not clearly defined, neophobic behaviour may enhance survival by reducing exposure to dangerous situations while minimizing missed opportunities.

Ecologically, these findings are particularly relevant in the context of increasing anthropogenic disturbance. Human-induced environmental changes—such as habitat fragmentation, the introduction of invasive species, and the impacts of climate change—are creating ecosystems with greater unpredictability. Such environments often present conflicting cues of risk and safety, amplifying ecological uncertainty. As a result, species inhabiting these modified ecosystems may face stronger selective pressures to adopt neophobic behaviours. This underscores the importance of understanding how uncertainty influences animal behaviour and survival strategies. By studying behaviour in uncertain environments, we can gain valuable insights into how organisms cope with fluctuating ecological conditions and anticipate the potential consequences for biodiversity.

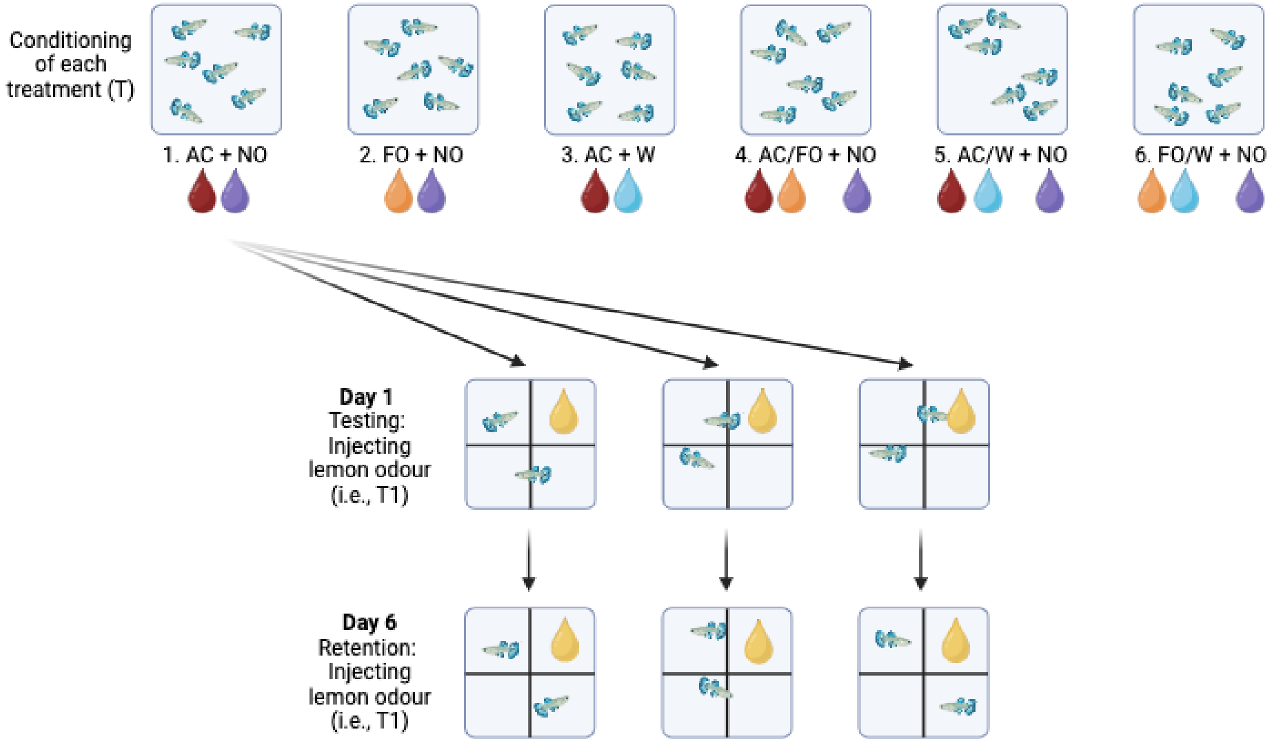

Figure 1.1. Conditioning set-up and experimental process (created using BioRender). The drop representations are as follows: red for AC, purple for NOs (regardless of odour), orange for FO, blue for water, and yellow for lemon odour (NO_6). The treatments were as follows: (1) alarm cues paired with alternating novel odours; (2) food odour paired with alternating novel odours (high safety); (3) alarm cues paired with water (known risk); (4) alternating alarm cues and food odour paired with alternating novel odours (high uncertainty); (5) alternating alarm cues and water paired with alternating novel odours (low uncertainty of predation risk); and (6) alternating food odour and water paired with alternating novel odours (low uncertainty of safety).

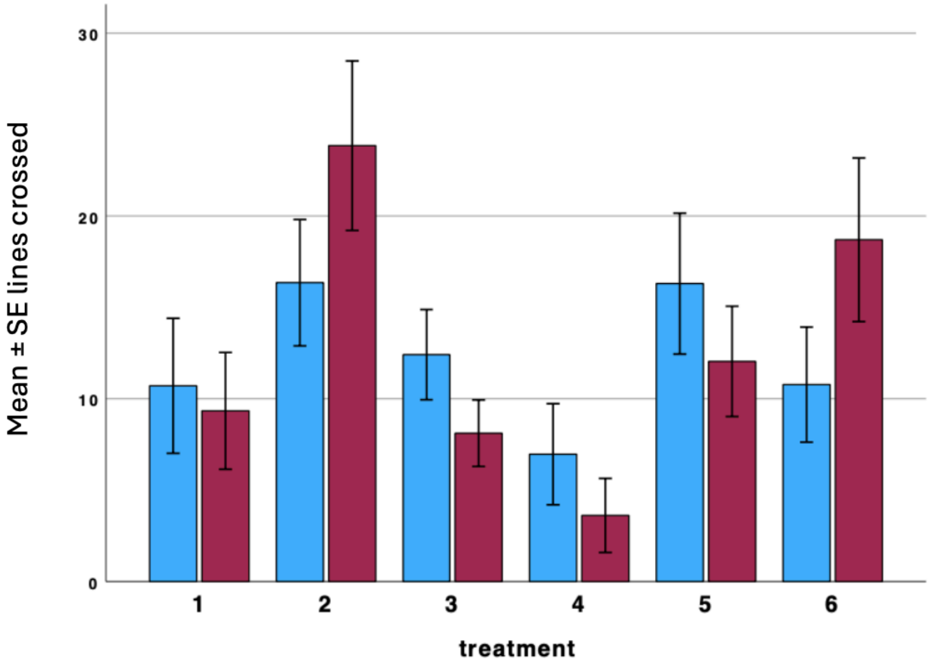

Figure 1.2. Mean (± SE) of line crosses pre- (blue) and post-stimulus injection (red) for Day 1 of the experiment. Sample size was 26-27 per treatment combination. The treatments were as follows: (1) alarm cues paired with alternating novel odours; (2) food odour paired with alternating novel odours (high safety); (3) alarm cues paired with water (known risk); (4) alternating alarm cues and food odour paired with alternating novel odours (high uncertainty); (5) alternating alarm cues and water paired with alternating novel odours (low uncertainty of predation risk); and (6) alternating food odour and water paired with alternating novel odours (low uncertainty of safety).

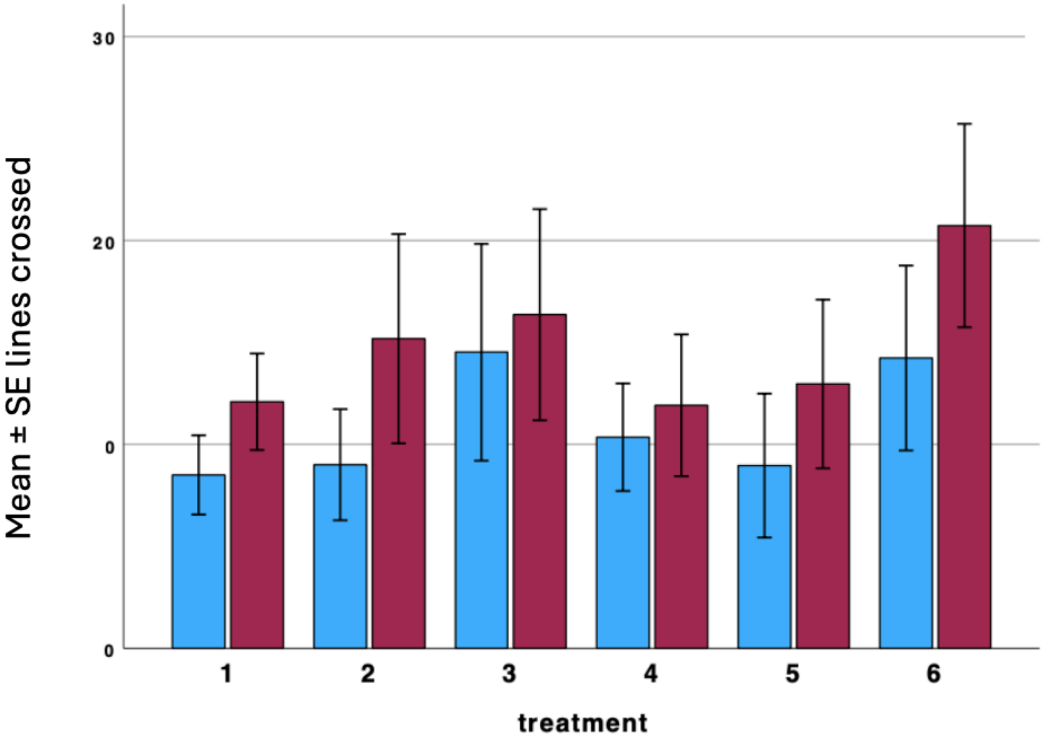

Figure 1.3. Mean (± SE) of line crosses pre- (blue) and post-stimulus injection (red) for day 6 (retention) of the experiment. Sample size was 22-26 per treatment combination. The treatments were as follows: (1) alarm cues paired with alternating novel odours; (2) food odour paired with alternating novel odours (high safety); (3) alarm cues paired with water (known risk); (4) alternating alarm cues and food odour paired with alternating novel odours (high uncertainty); (5) alternating alarm cues and water paired with alternating novel odours (low uncertainty of predation risk); and (6) alternating food odour and water paired with alternating novel odours (low uncertainty of safety).

Preface:

In the previous chapter, I examine how uncertainty is influenced by the availability of both risk and reward cues and how these effect neophobia. In Chapter 2, I further determine how uncertainty is subject to anthropogenic disturbances and levels of risk. In this chapter, I conduct a pair of experiments, in-situ and ex-situ, to examine the combined effect of human-induced disturbances and predation risk and how these influence the latency to explore (experiment 2) and return (experiment 1) to disturbed areas.

The following chapter will be submitted as:

Uncertainty Matters Under High Predation Risk: The Impact of Background Risk and Anthropogenic Disturbances on Trinidadian Guppies.

Félixe Dumaresq-Synnott[1], Alix J.P. Brusseau[1], Jade Morris[2], Abigail C. Nagl[2], Adam L. Crane[3] & Grant E. Brown[1]

[1] Department of Biology, Concordia University, 7141 Sherbrooke St. West, Montreal, Québec H4B 1R6
[2] Department of Biology, Missouri State University, Springfield, MO 65897, USA
[3] Department of Mathematical and Natural Sciences, University of Arkansas at Monticello, 346 University Dr, Monticello, Arkansas, AR 71656

Chapter 2:

Uncertainty Matters Under High Predation Risk: The Impact of Background Risk and Anthropogenic Disturbances on Trinidadian Guppies.

2.1. Introduction

Prey animals often face the challenge of balancing the need to avoid predators with the demands of foraging and mating (Lima & Dill, 1990; Brown et al., 2006; Brown & Godin, 2023). To manage these competing pressures, prey must make trade-offs (Dill, 1987) and rely on available information about local predation risks and foraging opportunities (Brown, 2003; Brown, Ferrari, & Chivers, 2011). However, this information is often unreliable or conflicting, which leads to ecological uncertainty (Brown & Godin, 2023; Munoz & Blumstein, 2012). Uncertainty of predation risk, when the threat of predation is unpredictable, can make decision-making difficult and costly. As a result, prey require information to reduce uncertainty, but gathering that information is also costly, particularly under risky conditions (Trimmer et al., 2011; Crane et al., 2022).

Uncertainty of predation risks arises when information is incomplete, conflicting, or unreliable (Dall & Johnstone, 2002; Crane et al., 2024), and it is shaped by both the availability and accuracy of information (Brown & Godin, 2023). However, environmental factors such as rapid changes or anthropogenic disturbances can affect information availability, perception, and processing of these cues (Weissburg et al., 2014). In situations where uncertainty about predation risk is high, prey face the challenge of making decisions based on ambiguous environmental cues. This often results in lost opportunities either by overreacting to non-threatening cues or failing to respond to actual threats (Johnson et al., 2013; Crane et al., 2024). For example, overly cautious behaviour can lead to missed foraging and mating opportunities (Johnson et al., 2013), while failure to react to a genuine predation risk can result in death (Johnson et al., 2013; Elvidge & Brown, 2014). Given the asymmetrical nature of these costs, prey may err on the side of caution (Johnson et al., 2013), resulting in increased neophobia (Crane et al., 2020).

When faced with ambiguous situations, prey often opt for the safest response, fearing all new stimuli to avoid potential risks (Feyten & Brown, 2018; Feyten et al., 2023). This uncertainty of predation risk drives neophobia, causing prey to prioritize fear over exploration and precise risk assessment (Feyten et al., 2023). While more information can reduce uncertainty, gathering that information can be costly in terms of time and energy, especially in risky environments. The effects of predation risk are also influenced by background risk, with Trinidadian rivers varying in predator populations. Some rivers are considered "high-risk" due to a higher density of predators, while others are "low-risk" with fewer predators. Prey from high-risk environments experience greater background predation risk, while those from low-risk rivers face less. Natural barriers that prevent predator dispersion often determine the presence of predators in a river (Deacon et al., 2015; Brusseau et al., 2024). These differences in predation risk shape prey behavior, as seen in Trinidadian guppies (*Poecilia reticulata*), which exhibit neophobia only in high-risk rivers (Brown et al., 2013; Brown et al., 2015).

Human activities lead to environmental disturbances, disrupting the ecological information available to surrounding organisms (Sih, 2013). In Trinidad, “liming sites,” where people gather for activities like cooking, swimming, and vehicle washing, cause significant disruptions, introducing pollutants, noise, and other disturbances that negatively affect local aquatic ecosystems (Deacon et al., 2015). These disturbances increase ecological uncertainty by altering environmental cues, which can make it harder for prey to assess predation risk and foraging opportunities (Knight & Gutzwiller, 1995; Deacon et al., 2015; Brusseau et al., 2024; Brusseau et al., under review). Disturbances also impact prey behaviour by increasing the uncertainty of predation risk. When environmental cues become unreliable, prey may misinterpret signals related to danger (Chivers et al., 2013; Candolin & Rahman, 2023; Feyten et al., 2023; Brusseau et al., 2024). Comparing disturbed liming sites with less impacted upstream areas allows us to test the impacts of disturbances contribute to the increased uncertainty in risk assessment (Deacon et al., 2015; Brusseau et al., 2024).

Here, we test the effects of uncertainty of predation risk affects prey behaviour in the context of both background risk and anthropogenic disturbances. Our first experiment assessed whether anthropogenic disturbances in high-risk environments increase behavioural uncertainty in response to novel physical disturbances. We compared guppies from high- versus low-predation-risk rivers and tested their responses to disturbances at or above anthropogenically impacted sites. Our second experiment explored how background risk and anthropogenic disturbances affect exploration and space use in a novel environment. This experiment was conducted in the laboratory, where we observed how guppies from different environments explored a new space. We hypothesized that prey from high-risk environments would exhibit more uncertainty in novel situations compared to prey from low-risk environments, due to their prior experiences with fluctuating risks (Brown et al., 2013; Brown et al., 2015). We also hypothesized that guppies from areas with frequent anthropogenic disturbances would show greater uncertainty due to the constant novelty in their environment. For the first experiment, we predicted that: (1) high-risk guppies would have a longer latency to return to the disturbed area, would inspect the disturbance less frequently, and would do so in shoals due to perceived predation threats; (2) fish from undisturbed sites would exhibit similar patterns to high-risk fish. For the second experiment, we predicted that: (3) high-risk guppies would have a longer latency to explore the novel arena and would explore it less due to greater uncertainty driven by neophobia; and (4) fish from undisturbed sites would show similar patterns due to the lack of novel disturbances in their environment. This study seeks to enhance our understanding of how prey animals adjust their behaviour in response to environmental uncertainty, particularly in contexts influenced by anthropogenic disturbances and varying predation risks.

2.2. Methods

2.2.1. Model Organism and Observation Sites

Field and laboratory observations were conducted in May 2024, with fish collected from two sites within each of two rivers in the Northern Range Mountains. We conducted in situ observations at disturbed sites (liming sites) and undisturbed sites located ~100 m upstream in the Lopinot and Upper Aripo Rivers. Natural barriers like small waterfalls and riffles helped distinguish liming sites from upstream areas that are less affected by disturbances (Deacon et al., 2015). The upstream sites are important for comparison because they offer a relatively undisturbed environment, serving as a baseline for studying how disturbances impact prey behaviour (Deacon et al., 2015; Brusseau et al., 2024).

The Lopinot River can be characterized as a high-risk river as it has several natural predators to the adult guppy (Magurran, 2005; Deacon et al., 2018), whilst the Upper Aripo River is considered a low-risk river for its lack of predators (Deacon et al., 2018). Guppies in high predation rivers are prey for larger species which influence community structures via predation (Magurran, 2005). These predators include, but are not limited to, the hart's rivulus (*Rivulus hartii*), pike cichlid (*Crenicichla alta*), wolf fish (*Hoplias malabaricus*), blue acara (*Aequidens pulcher*), and two-spotted sardine (*Astyanax bimaculatus*) (Magurran, 2005). Previous studies have shown that adult female guppies from the Lopinot River display a neophobic response to novel chemical cues, whereas those from the Upper Aripo River do not (Feyten et al., 2021, 2023). The downstream Aripo River was excluded as a 'high predation' site due to intensified pollution from human activities. Both the Lopinot and Aripo Rivers are located within the Caroni drainage basin and share similar habitat characteristics, including climate, geomorphology, vegetation, canopy coverage, and prey communities (Deacon et al., 2018). While microhabitat differences do exist within the streams, guppies were collected from sites with comparable features, except for the variation in predation and disturbance levels (Elvidge et al., 2016).

2.2.2. Experiment 1: In-situ Observations

We conducted behavioural observations at anthropogenically disturbed (i.e., ‘Liming sites’) and at undisturbed sites (i.e., above ‘liming sites) within the Lopinot (high predation) and Upper Aripo (low predation) Rivers. Observations were conducted at points at least 75 cm from the shoreline in pools with > 5 guppies present (visually assessed). At each point, we conducted a 2-minute pre-stimulus observation, during which, we recorded the number of guppies present (at 15 second intervals) within a 50 cm radius of the centre of observation area. We then presented a simulated overhead disturbance by dropping the rock stimulus and simultaneously injecting 60 mL of river water. Following the rock introduction, we began a 4-minute post-stimulus observation, during which we recorded: the latency of time for the first fish to return within the 50 cm radius of the rock, the number of fish within the 50 cm radius of the rock every 15 seconds, and the number of inspections. Inspections were defined as when an individual or a shoal are perceived within at least 10 cm of the rock, head directed toward the rock, either freezing or calmy swimming directly towards it. Within a site, observations conducted at least 10 m apart, moving upstream, to reduce the likelihood of repeat sampling. We conducted n = 14-15 observations at disturbed and undisturbed sites in each population, for a final sample size of N = 58.

2.2.3. Experiment 1: Statistical Analysis

As dependant variables, we calculated the proportional change in number of guppies present ((post – pre)/pre) and per capita inspections (number of inspections divided by the mean number of guppies present during the post-stimulus observation period) for each replicate. We then used a GLMM to test the effects of population (Lopinot vs. Upper Aripo River) and disturbance (disturbed vs. undisturbed sites), and their interactions on each behavioural metric. All analyses were conducted using SPSS v. 29.0.

2.2.4. Experiment 2: Ex-situ Observations

Like experiment 1, guppies were collected from the disturbed-low-risk and undisturbed-low-risk sites of the Upper Aripo river and from the disturbed-high-risk and undisturbed-high-risk sites of the Lopinot river. Adult female guppies were caught using a 3mm seine net within each of these locations and brought to the laboratory at the University of the West Indies, St Augustine. Females were selected as a model group due to the strength of their anti-predator response (Brusseau et al., 2024).

The observations were performed in 40 cm x 40 cm corrugated plastic arenas, sealed with aquatic safe silicone. On the floor of each arena, we created a 3x3 grid with 9 identical squares (Figure S2.1). At the top inner corner of the 4 external corner squares, we glued plastic plants to create shelter for the fish (Figure S2.1).

We placed a single female guppy in an opaque plastic cylinder within the center square of the arena and let the individual acclimate for 5 minutes. After this acclimation period, the cylinder was removed to expose the female to the entirety of the arena, we simultaneously started a 4-minute observation. During this time, we recorded the latency of the individual to exit the centre square, the number of seconds spent calmy swimming in the arena, and the number of lines crossed by the individual when moving within the arena. A line was considered crossed when he entire body of an individual had crossed over into another square. The number of lines crossed were later used to quantify an index of evenness of space use. When determining the index of evenness, we initially had to calculate the difference between the proportion of times that a guppy had entered each zone during the observations and the expected random proportion (0.11 if the 9 zones were used equally). Following this, the absolute values of these were summed to yield an overall index of space use evenness for the trial. The values ranged from 0, where are the squares of the arena were used equally, to 1.77, where the individual guppy spent the full observation period in one square (Crane et al., 2022). To present visually appealing data, we reversed these values (multiplying by −1) so that greater values represented more evenness. During this experiment, we conducted 25 observations for undisturbed-low-risk, 25 for disturbed-low-risk, 25 for undisturbed-high-risk, and 26 for disturbed-high-risk . Which gives a sample size of roughly N=25 for each treatment.

2.2.5. Experiment 2: Statistical Analysis

As dependent variables, we measured line crosses, time spent freezing, latency to leave the center, and evenness. We then used a Generalized Linear Mixed Model (GLMM) to test the effects of river (Lopinot vs. Upper Aripo River) and site (disturbed vs. undisturbed), and their interaction on each behavioural metric. River was treated as a fixed factor, while site was included as a random factor to account for site-specific variability. All analyses were conducted using SPSS v. 29.0.

2.3. Results

Experiment 1

For the proportional change in number of guppies present following the introduction of the acute rock disturbance, we found a significant main effect of population (P = 0.022; Table 2.1) but no effect of site nor River x Site interaction (Table 2.1). Within the Lopinot River, there was a greater decrease in the number of guppies present following the rock disturbance compared to the Upper Aripo River. Post-hoc GLMMs showed that while the change in number of guppies present did not differ between the disturbed vs. undisturbed sites in the Upper Aripo River ($F_{1,28}$ = 0.01, P = 0.99), we found a significant decrease following rock presentation at the undisturbed sites (vs. the disturbed sites) in the Lopinot River ($F_{1,28}$ = 5.14, P = 0.032). In both populations, guppies typically fled the observation area in response to the introduction of the acute disturbance. Our results suggest that at the low predation risk sites, guppies rapidly returned to the observation area.

For both the latency to first inspection and per capita inspection rates, we found significant River x Site interaction terms (P = 0.008 and P = 0.031 respectively; Figure 2.1; Table 2.1). In the Lopinot River (high predation), guppies tested at anthropogenically disturbed sites returned faster and inspected at higher rates compared to the upstream undisturbed sites (Figure 2.1). Conversely, the latency to inspection and inspection rates were similar at the disturbed vs. undisturbed sites in the Upper Aripo River (Figure 2.1).

Experiment 2

Overall, for the GLMM outputs of experiment 2 we found compelling results. For the mean time spent freezing, we found a significant interaction of River x Site (P=0.043; Table 2.2). Additionally, for the mean lines crossed, we found a significant effect of river (P=0.004; Table 2.2) and River x Site (P=0.014; Table 2.2). Within the undisturbed population of the Upper Aripo River, there were significantly more line crosses performed (Figure 2.2), suggesting more exploratory behaviour. Lastly, for the evenness of the lines crossed we found a significant effect of River x Site (P=0.005; Table 2.2).

We found that guppies tested in the Lopinot River (high predation) exhibited a trend to have higher line crosses, more even space use and less time freezing at anthropogenically disturbed vs. undisturbed sites (Figure 2.2). Conversely, guppies tested in the Upper Aripo (low predation) tended to have lower line crosses, less even space use and more time freezing at disturbed vs. undisturbed sites (Figure 2.2).

2.4. Discussion

Our combined results from the field and laboratory assays provide supporting evidence for our hypothesis that anthropogenic disturbances induce uncertainty of predation risk among prey, especially in environments where background risk (e.g., predation risk) is high. In these scenarios, guppies responded with more cautious, information-seeking behaviours when confronted with novel disturbances or environments. Both Experiment 1 and Experiment 2 are consistent with the hypothesis that disturbances, particularly in high-risk environments, lead to greater behavioural uncertainty as prey try to assess new, unpredictable risks.

In Experiment 1, we found no significant effects of river or site on per capita visits to the disturbed area, suggesting that guppies from both high and low predation environments inspected the disturbance area similarly. However, there were significant effects on the latency to return to the disturbed area. Guppies from undisturbed sites, particularly those from high predation rivers, exhibited longer latencies before returning to inspect the disturbance. This indicates that, while both high- and low-risk guppies may approach disturbances in a similar manner, their decision-making regarding how quickly to return is influenced by the perceived level of risk. In contrast, guppies from disturbed sites, regardless of predation background, returned more quickly, suggesting that disturbance-prone individuals are more uncertain and therefore less cautious in exploring their environment.

Similarly, the proportional change in guppy abundance post-disturbance was significantly greater in the high-risk, high-predation river (Upper Aripo) compared to the low-risk river. This suggests that guppies in riskier environments are more sensitive to disturbances, likely due to their heightened uncertainty regarding potential risks. Interestingly, no such significant change in abundance was observed for guppies from disturbed, low-risk sites. This discrepancy highlights the influence of background predation risk in shaping how prey species respond to disturbances, reinforcing the notion that high-risk environments amplify the perceived uncertainty of novel disturbances.

In Experiment 2, we observed a significant interaction between river and site regarding the latency to explore a novel arena. Guppies from low predation rivers, especially those from undisturbed sites, exhibited greater exploratory behaviour, as evidenced by a higher number of line crossings and less cautious exploration. In contrast, guppies from high predation rivers, particularly those from disturbed sites, exhibited longer latencies to initiate exploration and more uniform exploration patterns. This suggests that guppies from high-risk and disturbed environments adopt more cautious exploration strategies, likely as an adaptive response to greater environmental uncertainty.

Integrating the results from both experiments, it is clear that guppies from disturbed and high-risk environments exhibit greater behavioural uncertainty. While they may initially explore new disturbances more quickly (suggesting higher uncertainty; Crane et al. 2024), they also show greater caution in how and where they explore, likely as a strategy to assess potential risks. This aligns with the findings of Brusseau et al. (2024), who observed greater predator inspection rates in low-background-risk rivers and disturbed sites, supporting the idea that anthropogenic disturbances increase uncertainty by introducing novel, unpredictable elements into the environment. Additionally, our findings align with those of Chen & Koprowski (2015), who demonstrated that continuous anthropogenic activity, such as traffic and noise, can reduce animal abundance at disturbed sites. While our study focused on episodic aquatic disturbances, the results raise the interesting possibility that continuous disturbances might have even more profound effects on prey species.

Our results also suggest that the degree of neophobia observed in guppies from high predation rivers reflects an adaptive strategy to overestimate the likelihood of predation in novel situations. This fear of novelty in high-risk individuals has been demonstrated in other species, such as convict cichlids (*Amatitlania nigrofasciata*) (Brown et al., 2013; Joyce et al., 2016), Trinidadian guppies (*Poecilia reticulata*) (Elvidge et al., 2016), and wood frog tadpoles (*Lithobates sylvaticus*) (Mitchell et al., 2016). In our study, the increased latency to explore (Experiment 2) and return (Experiment 1) to disturbed areas may reflect a similar adaptive response, where guppies in high-risk environments perceive greater danger and respond by overestimating risk.

Recent studies suggest that anthropogenic disturbances may increase the uncertainty of predation risk among prey by reducing the availability of reliable information or altering the reliability of current environmental cues (Brusseau et al., 2024; Crane et al., 2024). In our study, the increased uncertainty observed in guppies from disturbed environments may directly result from such disturbances. Novel elements introduced by human activity, like chemical pollutants or physical habitat modifications, could cause prey species to experience greater uncertainty in assessing risk. This aligns with Brusseau et al. (2024), which highlights how anthropogenic disturbances create environments where information is less consistent or harder to interpret, forcing animals to adopt more cautious, exploratory behaviours. Furthermore, ecological uncertainty has been noted in other species exposed to anthropogenic disturbances. For example, golden jackals (*Canis aureus*) in Israel, living near human-impacted areas like villages, have smaller home ranges and core areas compared to those in less-disturbed habitats (Rotem et al., 2011). This suggests that individuals in human-impacted regions may alter their spatial behaviours due to increased uncertainty about resource availability or predation risk. Similarly, guppies in disturbed environments may modify their exploration and foraging patterns in response to heightened uncertainty.

Overall, our findings strongly support the hypothesis that anthropogenic disturbances and high background predation risk increase behavioural uncertainty in prey species. Guppies from disturbed or high-risk environments exhibit a combination of quicker, yet more cautious exploration, suggesting that they rely on risk-avoidance strategies when faced with novel or unpredictable environments. This is consistent with the broader concept of ecological uncertainty, where disturbances create environments that are more difficult to interpret, thus requiring prey species to alter their behaviours to cope with heightened unpredictability.

The concept of ecological uncertainty remains understudied but crucial, as it reflects how both biotic and abiotic factors—especially those influenced by human activity—interact to influence prey behaviour. Our study contributes to this understanding by highlighting how frequent, episodic disturbances introduce novel risks that prey species must navigate. As anthropogenic disturbances continue to shape ecosystems, it will be important to further explore how such uncertainties affect not only individual behaviours but also broader ecological interactions and community dynamics.

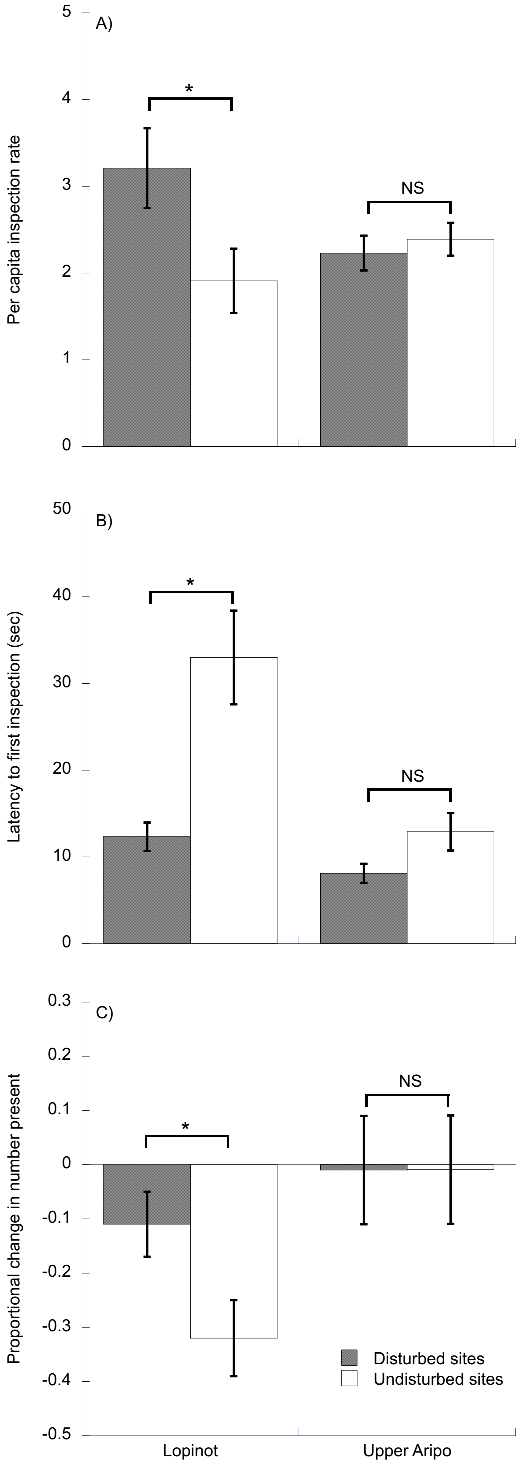

Figure 2.1. Mean (± SE) (a) per capita visits, (b) latency to return to disturbed area, and (c) proportional change in abundance pre- and post-disturbance. Guppies pre-exposed to high or low background risk were observed individually or as a member of a shoal. Blue bars = disturbed sites and red bars = undisturbed sites. Sample size was 15 per treatment combination for Experiment 1.

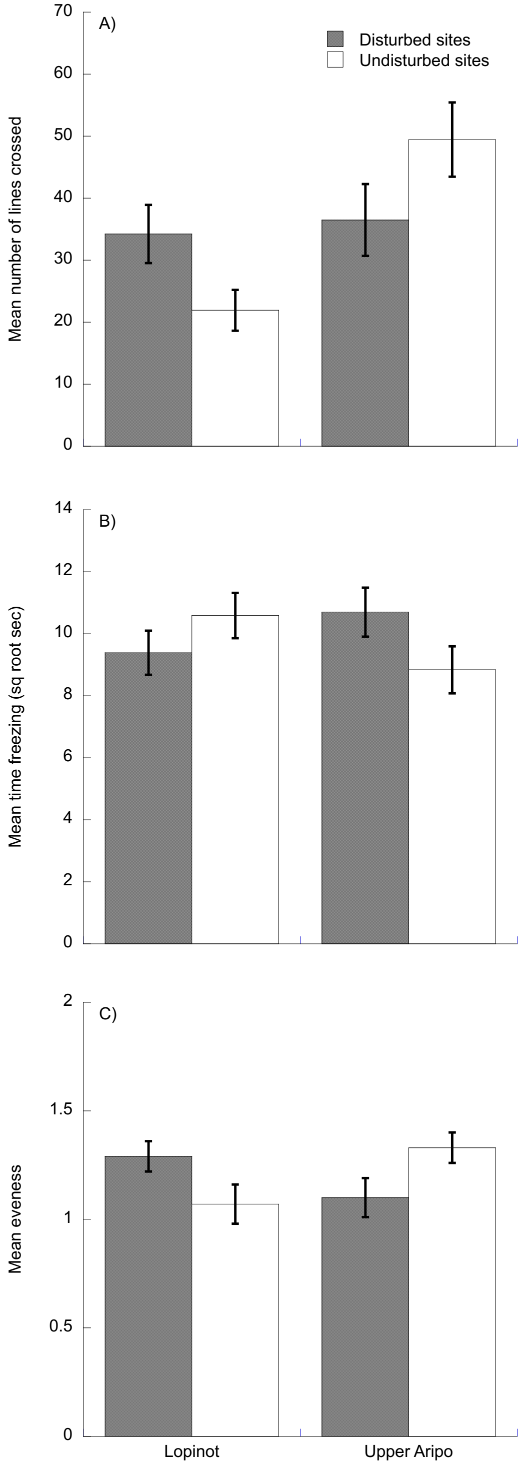

Figure 2.2. Mean (± SE) (a) latency to exit center square, (b) lines crossed, and (c) and evenness of area use for guppies pre-exposed to high or low background risk and tested individually as or a member of a shoal. Blue bars = disturbed sites and red bars = undisturbed sites. Sample size was 15 per treatment combination for Experiment 2.

Table 2.1. General Linear Mixed Model (GLMM) outputs for (a) per capita visits, (b) latency to return, and (c) proportional change in fish abundance relative to the occurrence of a physical disturbance in a pool for Experiment 1. Significant terms in bold.

	F value	Degrees of freedom	*P*
Per capita visits			
River	0.57	1, 54	= 0.45
Site	3.02	1, 54	= 0.09
River x Site	4.90	1, 54	**= 0.031**
Latency to return			
River	17.75	1, 54	**< 0.001**
Site	19.55	1, 54	**< 0.001**
River x Site	7.59	1, 54	**= 0.008**
Proportional Change			
River	5.53	1, 54	**= 0.022**
Site	1.46	1, 54	= 0.23
River x Site	1.44	1, 54	= 0.24

Table 2.2. General Linear Mixed Model (GLMM) outputs for (a) mean time (square-root transformed) spent freezing, (b) mean lines crossed, and (c) evenness in exploration of the arena in Experiment 2. Bolded *p* values are significant ($p < 0.05$).

	F value	Degrees of freedom	*P*
Mean time freezing			
River	0.09	1, 97	= 0.77
Site	0.19	1, 97	= 0.66
River x Site	4.20	1, 97	**= 0.043**
Mean lines crossed			
River	8.65	1, 97	**= 0.004**
Site	0.004	1, 97	= 0.95
River x Site	6.23	1, 97	**= 0.014**
Evenness			
River	0.16	1, 97	= 0.69
Site	0.008	1, 97	= 0.93
River x Site	7.08	1, 97	**= 0.005**

General Conclusion

Ecological uncertainty refers to the unpredictability and variability that organisms face in their environments, particularly in the association of safety and risky cues (Brown & Godin, 2023). This uncertainty arises when prey species are confronted with incomplete, conflicting, or unreliable information about their surroundings (Dall & Johnston, 2002, Crane et al., 2024). The inability to accurately assess threats or rewards increases the cognitive and survival costs for animals, forcing them to navigate trade-offs that can significantly influence their fitness (Lima & Dill, 1990). As the environmental cues animals rely on fluctuate, the uncertainty of risk assessment becomes more pronounced. This type of uncertainty has previously been underreported in ecological studies, but it is gaining increased attention in contemporary research. This shift in focus is crucial, as understanding how uncertainty shapes animal behaviour can provide insights into how organisms adapt to and cope with rapidly changing environments. Such knowledge is essential in assessing the long-term impacts of human activity on the planet's diverse and complex ecosystems.

In Chapter 1 of my study, I aimed to understand how uncertainty influences animal behaviour, particularly risk-averse behaviours like neophobia. My results suggest that uncertainty leads to more cautious behaviour, even more than a simulated high-risk environment. In my experiment, guppies, *Poecilia reticulata,* exhibited greater neophobic tendencies when exposed to uncertain risk and reward cues. This behaviour aligns with the chapter's hypothesis that uncertainty linked to fluctuating or contradictory environmental cues induces heightened caution and risk aversion. The significant neophobic behaviour I observed in response to uncertain information supports the idea that organisms adapt to unpredictable environments by adopting risk-averse strategies. This finding underscores the importance of understanding how ecological uncertainty drives life-history trade-offs in prey species, affecting their survival strategies and overall fitness.

In Chapter 2, I examined the role of anthropogenic disturbances in introducing ecological uncertainty. My combined field and laboratory experiments revealed that human-induced changes to habitats, such as physical disturbances or the introduction of novel elements, create environments that prey species perceive as more uncertain. For instance, guppies in disturbed habitats showed a delayed response in both exploring new areas (experiment 2) and returning to familiar, disturbed spaces (experiment 1), suggesting an adaptive response to the increased perceived danger in these environments. This tendency is particularly pronounced in habitats that are subject to frequent or unpredictable disturbances, where the cost of misjudging a threat could be fatal. To fully understand the impacts of ecological uncertainty, I believe it is essential to integrate both ex-situ (laboratory-based) and in-situ (field-based) research. While laboratory experiments allow for controlled manipulation of variables, field experiments provide insights into how animals respond to real-world conditions. In this chapter, the combination of these approaches offered a more comprehensive view of how uncertainty influences animal behaviour in both theoretical and actual environments.

As human activity continues to modify ecosystems at an alarming rate, the level of uncertainty in natural environments is likely to proportionally increase. Thus, compounding the challenges organisms face. Understanding how species cope with different types of uncertainty, whether driven by anthropogenic factors or natural variability, is vital for anticipating the potential impacts on biodiversity and ecosystem stability. The results of these experiments highlight the significant impact of environmental disturbances on animal behaviour. Species inhabiting modified ecosystems may face increased selective pressure to adopt more cautious behaviours, such as neophobia, due to the constantly changing ecological cues in these environments. This raises the urgent need to better understand how uncertainty affects survival strategies, as these behaviours are crucial for adapting to dynamic and potentially hostile environments. Given the complexity and diversity of uncertainties in Earth's ecosystems, understanding and addressing ecological uncertainty will become even more critical to preserving biodiversity and maintaining the health of the planet's habitats.

Bibliography

Brown, G. E. (2003). Learning about danger: Chemical alarm cues and local risk assessment in prey fishes. *Fish and Fisheries*, *4*(3), 227–234. https://doi.org/10.1046/j.1467-2979.2003.00132.x

Brown, G. E., Elvidge, C. K., Ramnarine, I., Ferrari, M. C. O., & Chivers, D. P. (2015). Background risk and recent experience influences retention of neophobic responses to predators. *Behavioral Ecology and Sociobiology*, *69*(5), 737–745. https://doi.org/10.1007/s00265-015-1888-y

Brown, G. E., Ferrari, M. C. O., & Chivers, D. P. (2011). Learning about Danger: Chemical Alarm Cues and Threat-Sensitive Assessment of Predation Risk by Fishes. In C. Brown, K. Laland, & J. Krause (Eds.), *Fish Cognition and Behavior* (2nd ed., pp. 59–80). Wiley. https://doi.org/10.1002/9781444342536.ch4

Brown, G. E., Ferrari, M. C. O., Elvidge, C. K., Ramnarine, I., & Chivers, D. P. (2013). Phenotypically plastic neophobia: A response to variable predation risk. *Proceedings of the Royal Society B: Biological Sciences*, *280*(1756). https://doi.org/10.1098/rspb.2012.2712

Brown, G. E., & Godin, J. J (1999a). Chemical alarm signals in wild Trinidadian guppies (*Poecilia reticulata*). *Canadian Journal of Zoology, 77*(4): 562-570.

Brown, G. E., & Godin, J.-G. J. (1999b). Who dares, learns: Chemical inspection behaviour and acquired predator recognition in a characin fish. *Animal Behaviour*, *57*(2), 475–481. https://doi.org/10.1006/anbe.1998.1017

Brown, G. E., & Godin, J. J (2023). Ecological uncertainty and anti-predator behaviour: an integrative perspective. *Frontiers in Ethology, 2*. https://doi.org/10.3389/fetho.2023.1238167.

Brown, G. E., Ferrari, M. C. O., Elvidge, C. K., Ramnarine, I. R., & Chivers D. P. (2023). Phenotypically plastic neophobia: a response to variable predation risk. *Proceedings of the Royal Society B: Biological Sciences, 280*(1756). https://doi.org/10.1098/rspb.2012.2712.

Brown, G. E., Rive, A. C., Ferrari, M. C. O., & Chivers, D. P. (2006). The dynamic nature of antipredator behaviour: Prey fish integrate threat-sensitive antipredator responses within background levels of predation risk. *Behavioural Ecology and Sociobiology*, *61*(1), 9–16. https://doi.org/10.1007/s00265-006-0232-y

Brusseau, A.J.P., Feyten, L. E. A., Crane, A. L., & Brown G. E. (2024). Exploring the effects of anthropogenic disturbance on predator inspection activity in Trinidadian guppies. *Current Zoology,* 70(1), 109-111. https://doi.org/10.1093/cz/zoad002.

Brusseau, A. J. P., Feyten, L. E. A., Groves, V., Felismino, M. E. L., Cao Van Truong, D., Crane, A. L., Ramnarine, I. W., & Brown, G. E. (2023). Sex and background risk influence responses to acute predation risk in Trinidadian guppies. *Behavioral Ecology*, *34*(5), 898–906. https://doi.org/10.1093/beheco/arad055

Candolin, U., & Rahman, T. (2023). Behavioural responses of fishes to anthropogenic disturbances: Adaptive value and ecological consequences. *Journal of Fish Biology*, *103*(4), 773–783. https://doi.org/10.1111/jfb.15322

Chivers, D.P., Dixson, D.L., White, J.R., McCormick, M.I., Ferrari, M.C.O. (2013). Degradation of chemical alarm cues and assessment of risk throughout the day. *Ecol Evol*, *3*(11), 3925–3934. doi:https://doi.org/10.1002/ece3.760.

Chen, H. L., & Koprowski, J. L. (2015). Animal occurrence and space use change in the landscape of anthropogenic noise. Biological Conservation, 192, 315–322. https://doi.org/10.1016/j.biocon.2015.10.003

Crane, A. L., Brown, G. E., Chivers, D. P., & Ferrari, M. C. O. (2020). An ecological framework of neophobia: From cells to organisms to populations. *Biological Reviews*, *95*(1), 218–231. https://doi.org/10.1111/brv.12560

Crane, A. L., Demers, E. E., Feyten, L. E. A., Ramnarine, I. W., & Brown G. E. (2022). Exploratory decisions of Trinidadian guppies when uncertain about predation risk. *Animal Cognition, 25*(3), 581–87. https://doi.org/10.1007/s10071-021-01575-4.

Crane, A. L., Feyten, L. E. A., Preagola, A. A., Ferrari, M. C. O., & Brown G. E. (2024). Uncertainty about predation risk: a conceptual review. *Biological Reviews, 99*(1), 238-252. https://doi.org/10.1111/brv.13019.

Crane, A. L., Bairos-Novak, K. R., Sacco, L. H., & Ferrari, M. C. O. (2018). The socially mediated recovery of a fearful fish paired with periodically replaced calm models. *Proceedings of the Royal Society B: Biological Sciences, 285*(1888), 20180739. https://doi.org/10.1098/rspb.2018.0739.

Dall, S. R. X. (2010.). Managing Risk: The Perils of Uncertainty. *Ecology of Behavior*. Oxford University Press.

Dall, S. R. X., & Johnstone, R. A. (2002). Managing uncertainty: information and insurance under the risk of starvation. Edited by R. A. Johnstone and S. R. X. Dall. *Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences, 357*(1427), 1519–26. https://doi.org/10.1098/rstb.2002.1061.

Deacon, A. E., Jones, F. A. M., & Magurran, A. E. (2018). Gradients in predation risk in a tropical river system. *Current Zoology*, *64*(2), 213–221. https://doi.org/10.1093/cz/zoy004

Deacon, A. E., Shimadzu, H., Dornelas, M., Ramnarine, I. W., & Magurran, A. E. (2015). From species to communities: The signature of recreational use on a tropical river ecosystem. *Ecology and Evolution*, *5*(23), 5561–5572. https://doi.org/10.1002/ece3.1800

Dill, L. M. (1987). Animal decision making and its ecological consequences: the future of aquatic ecology and behaviour. *Canadian Journal of Zoology, 65*, 803–811. https://doi.org/10.1139/z87-128

Dugatkin, L. A., & Godin, J.-G. J. (1992). Predator inspection, shoaling and foraging under predation hazard in the Trinidadian guppy, *Poecilia reticulata*. *Environmental Biology of Fishes*, *34*(3), 265–276. https://doi.org/10.1007/BF00004773

Elvidge, C. K., Chuard, P. J. C., & Brown, G. E. (2016). Local predation risk shapes spatial and foraging neophobia patterns in Trinidadian guppies. *Current Zoology*, *62*(5), 457–462. https://doi.org/10.1093/cz/zow013

Elvidge, C. K., Ramnarine, I., & Brown, G. E. (2014). Compensatory foraging in Trinidadian guppies: Effects of acute and chronic predation threats. *Current Zoology*, *60*(3), 323–332. https://doi.org/10.1093/czoolo/60.3.323

Ferrari, M. C. O., Brown, G. E., & Chivers, D. P. (2018). Understanding the effect of uncertainty on the development of neophobic anti-predator phenotypes. *Animal Behaviour, 136*, 101–6. https://doi.org/10.1016/j.anbehav.2017.11.024.

Feyten, L. E. A., & Brown, G. E. (2018). Ecological uncertainty influences vigilance as a marker of fear. *Animal Sentience*, *15*(7). https://doi.org/10.51291/2377-7478.1311

Feyten, L. E. A., Crane, A. L., Ramnarine, I. W., and Brown, G. E. (2021). Predation risk shapes the use of conflicting personal risk and social safety information in guppies. Edited by Ulrika Candolin. *Behavioural Ecology, 32*(6), 1296–1305. https://doi.org/10.1093/beheco/arab096.

Feyten, L. E. A., Demers, E. E., Ramnarine, I. W., Chivers, D. P., Ferrari, M. C. O., & Brown, G. E. (2019). Who's where? Ecological uncertainty shapes neophobic predator avoidance in Trinidadian guppies. *Behavioural Ecology and Sociobiology, 73*(5), 70. https://doi.org/10.1007/s00265-019-2687-7.

Feyten, L. E. A, Ramnarine, I. W., & Brown, Grant E. (2023). Microhabitat conditions drive uncertainty of risk and shape neophobic responses in Trinidadian guppies, *Poecilia reticulata*. Ecology and Evolution, 13(9), doi: 10.1002/ece3.10554.

Fishman, M. A. (1999). Predator Inspection: Closer Approach as a Way to Improve Assessment of Potential Threats. *Journal of Theoretical Biology*, *196*(2), 225–235. https://doi.org/10.1006/jtbi.1998.0834

Franks, B., Gaffney, L. P., Graham, C., & Weary, D. M. (2023). Curiosity in zebrafish (*Danio rerio*)? Behavioral responses to 30 novel objects. *Frontiers in Veterinary Sciences,* 9:1062420. doi: 10.3389/fvets.2022.1062420

Goldman, J. A., Feyten, L. E. A., Ramnarine, I. W., & Brown, G. E. (2020). Sender and receiver experience alters the response of fish to disturbance cues. *Current Zoology*, *66*(3), 255–261. https://doi.org/10.1093/cz/zoz050

Johnson, D. D. P., Blumstein, D. T., Fowler, J. H., & Haselton, M. G. (2013). The evolution of error: error management, cognitive constraints, and adaptive decision-making biases. *Trends in Ecology & Evolution*, *28*(8), 474–81. https://doi.org/10.1016/j.tree.2013.05.014.

Joyce, B. J., Demers, E. E. M., Chivers, D. P., Ferrari, M. C. O., & Brown, G. E. (2016). Risk-induced neophobia is constrained by ontogeny in juvenile convict cichlids. *Animal Behaviour*, *114*, 37–43. https://doi.org/10.1016/j.anbehav.2016.01.007

Kats, L. B., & Dill, L. M. (1998). The scent of death: Chemosensory assessment of predation risk by prey animals. *Écoscience*, *5*(3), 361–394. https://doi.org/10.1080/11956860.1998.11682468

Knight, R.L., Gutzwiller, K.J., editors. 1995. Wildlife and recreationists: coexistence through management and research. Washington, D.C: Island Press.

Lima, S. L., & Dill, L. M. (1990). Behavioural decisions made under the risk of predation: a review and prospectus. *Canadian Journal of Zoology, 68*(4), 619–40. https://doi.org/10.1139/z90-092.

Magurran, A. E. (2005). *Evolutionary Ecology*. Oxford University Press. https://doi.org/10.1093/acprof:oso/9780198527855.001.0001

Magurran, A. E., & Phillip, D. A. T. (2001). Implications of Species Loss in Freshwater Fish Assemblages. *Ecography,* 24(6), 645-650. https://doi.org/10.1034/j.1600-0587.2001.240603.x

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

Mitchell, M. D., Chivers, D. P., Brown, G. E., & Ferrari, M. C. O. (2016). Living on the edge: How does environmental risk affect the behavioural and cognitive ecology of prey? *Animal Behaviour*, *115*, 185–192. https://doi.org/10.1016/j.anbehav.2016.03.018

Munoz, N. E., & Blumstein, D. T. (2012). Multisensory perception in uncertain environments. *Behavioral Ecology*, *23*(3), 457–462. https://doi.org/10.1093/beheco/arr220

Murphy, A., Diefenbach, D. R., Ternent, M., Lovallo, M., & Miller, D. (2021). Threading the needle: How humans influence predator–prey spatiotemporal interactions in a multiple-predator system. *Journal of Animal Ecology*, *90*(10), 2377–2390. https://doi.org/10.1111/1365-2656.13548

Rodd, F. H., Hughes, K. A., Grether, G. F., & Baril, C. T. (2002). A possible non-sexual origin of mate preference: Are male guppies mimicking fruit? *Proceedings of the Royal Society of London. Series B: Biological Sciences*, *269*(1490), 475–481. https://doi.org/10.1098/rspb.2001.1891

Rotem, G., Berger, H., King, R., (Kutiel), P. B., & Saltz, D. (2011). The effect of anthropogenic resources on the space-use patterns of golden jackals. *The Journal of Wildlife Management*, *75*(1), 132–136. https://doi.org/10.1002/jwmg.9

Schmidt, K. A., Dall, S. R. X., & Van Gils, J. A. (2010). The ecology of information: an overview on the ecological significance of making informed decisions. *Oikos, 119*(2), 304–16. https://doi.org/10.1111/j.1600-0706.2009.17573.x.

Sih, A. (1992). Prey Uncertainty and the Balancing of Antipredator and Feeding Needs. *The American Naturalist*, *139*(5), 1052–1069. https://doi.org/10.1086/285372

Sih, A. (2013). Understanding variation in behavioural responses to human-induced rapid environmental change: A conceptual overview. *Animal Behaviour*, *85*(5), 1077–1088. https://doi.org/10.1016/j.anbehav.2013.02.017

Smith, B. R. & Blumstein, B. T. (2010). Behavioural types as predictors of survival in Trinidadian guppies (*Poecilia reticulata*), *Behavioural Ecology*, *21*(5), 919- 926. https://doi.org/10.1093/beheco/arq084

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

Trimmer, P. C., Houston, A. I., Marshall, J. A. R., Mendl, M. T., Paul, E. S., & McNamara, J. M. (2011). Decision-making under uncertainty: Biases and Bayesians. *Animal Cognition*, *14*(4), 465–476. https://doi.org/10.1007/s10071-011-0387-4

Tryjanowski, P., Møller, A. P., 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, J. J., Polakowski, M., Takacs, V., Wysocki, D., & Zduniak, P. (2016). Urbanization affects neophilia and risk-taking at bird-feeders. *Scientific Reports*, *6*(1), 28575. https://doi.org/10.1038/srep28575

Appendices

Appendix A: Chapter 1 Supplementary Materials

Table S1.1. The conditioning process for the six individual treatments over the course of 5 days, with two exposures a day. AC=Alarm Cues, NO=Novel Odours (the number corresponds to the odour), FO=Food Odours, and W=Water. The treatments were described as follows: (1) alarm cues paired with alternating novel odours; (2) food odour paired with alternating novel odours (high safety); (3) alarm cues paired with water (known risk); (4) alternating alarm cues and food odour paired with alternating novel odours (high uncertainty); (5) alternating alarm cues and water paired with alternating novel odours (low uncertainty of predation risk); and (6) alternating food odour and water paired with alternating novel odours (low uncertainty of safety).

	Day 1 AM	Day 1 PM	Day 2 AM	Day 2 PM	Day 3 AM	Day 3 PM	Day 4 AM	Day 4 PM	Day 5 AM	Day 5 PM
1	**AC+NO$_1$**	**AC+NO$_2$**	**AC+NO$_3$**	**AC+NO$_4$**	**AC+NO$_5$**	**AC+NO$_1$**	**AC+NO$_2$**	**AC+NO$_3$**	**AC+NO$_4$**	**AC+NO$_5$**
2	**FO+NO$_1$**	**FO+NO$_2$**	**FO+NO$_3$**	**FO+NO$_4$**	**FO+NO$_5$**	**FO+NO$_1$**	**FO+NO$_2$**	**FO+NO$_3$**	**FO+NO$_4$**	**FO+NO$_5$**
3	**AC+W**	**AC+W**	**AC+W**	**AC+W**	**AC+W**	**AC+W**	**AC+W**	**AC+W**	**AC+W**	**AC+W**
4	**AC+NO$_1$**	**FO+NO$_2$**	**AC+NO$_3$**	**FO+NO$_4$**	**AC+NO$_5$**	**FO+NO$_1$**	**AC+NO$_2$**	**FO+NO$_3$**	**AC+NO$_4$**	**FO+NO$_5$**
5	**AC+NO$_1$**	**W+NO$_2$**	**AC+NO$_3$**	**W+NO$_4$**	**AC+NO$_5$**	**W+NO$_1$**	**AC+NO$_2$**	**W+NO$_3$**	**AC+NO$_4$**	**W+NO$_5$**
6	**FO+NO$_1$**	**W+NO$_2$**	**FO+NO$_3$**	**W+NO$_4$**	**FO+NO$_5$**	**W+NO$_1$**	**FO+NO$_2$**	**W+NO$_3$**	**FO+NO$_4$**	**W+NO$_5$**

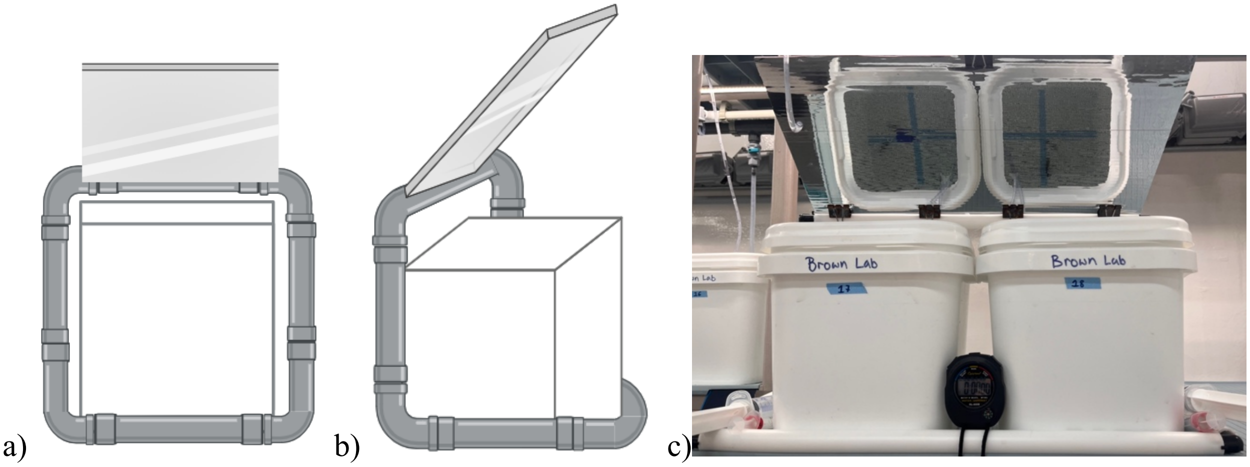

Figure S1.1. Novel observational set up where: a) front view of the sketch for a single bucket (created using BioRender), b) side view for a single bucket (created using BioRender), c) actual in-lab testing set up with two buckets, the fish's reflections are able to be viewed in the mirror above the buckets. This novel set-up prevents the observer from disturbing the test individuals.

Appendix B: Chapter 2 Supplementary Materials

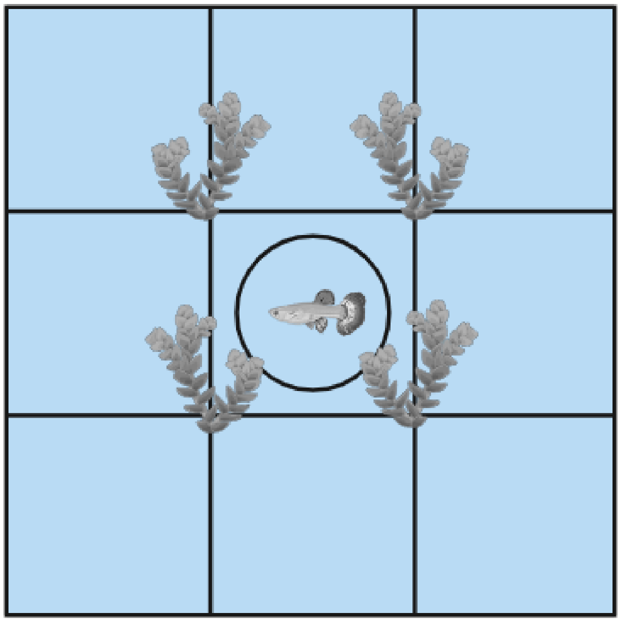

Figure S2.1: Schematic of the grid frame (40 cm x 40 cm) used for testing in Experiment 2.