

**THE EFFECT OF UNCERTAIN PREDATION RISK ON THE  
EXPRESSION OF NEOPHOBIA IN CONVICT CICHILDS  
*ARCHOCENTRUS NIGROFASCIATUS***

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## Abstract

Predation exerts a pervasive and unforgiving selection pressure such that it can influence prey life history, morphology, physiology, and behaviour. The combined effects of climate change, anthropogenic disturbances, and invasive species can lead to short- and long-term changes in how prey mitigate predation risk. As a result, many animals are likely experiencing increasing amounts of novelty and uncertainty about their environment. In a predation context, uncertainty can be linked to the relevance of the antipredator response to the information contained within a predator-related cue. Increased uncertainty may have consequential impacts on the decision-making and risk-assessment abilities of various prey species. Thus, understanding how prey manage uncertainty and the ecological factors driving uncertainty is fast becoming a pressing issue for ecologists and conservation biologists. Neophobia, or the fear of novel stimuli, has been proposed as a way for prey to respond to increased uncertainty without the costs of learning unknown predator-specific information. While neophobia was initially presumed to emerge from high levels of predation risk, recent evidence has shown that uncertainty, instead of the level of risk, is likely the key driver of neophobia. However, the specific ecological factors that drive uncertainty and, by extension, neophobic responses remain unclear. Using juvenile convict cichlids (*Archocentrus nigrofasciatus*), I explored the extent to which neophobia emerges in response to the different factors that contribute to the uncertainty associated with predation risk. These factors were namely the unreliability of risk assessment cues and the temporal unpredictability of risk. Upon investigating the effect of cue reliability on neophobia, I demonstrated that neophobia is maintained after repeated encounters with unknown (i.e., unreliable) alarm cues from an unrelated species following exposures to known and highly reliable conspecific alarm cues. While I failed to find an effect of temporal predictability of risk on neophobia, I found that a lack of temporal pattern in predation risk induces neophobia in prey regardless of the level of risk intensity. My thesis identifies how certain factors that relate to how predation risk is perceived shape the uncertainty that prey experience and the resulting neophobic response. Faced with a rapidly changing world, understanding how prey manage novel cues provides valuable knowledge that can inform conservation and management efforts of ecologically and socioeconomically important key species.

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## List of abbreviations

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<b>Abbreviation</b>	<b>Definition</b>
<b>AC</b>	Alarm cues
<b>ANCOVA</b>	Analysis of covariance
<b>ANOVA</b>	Analysis of variance
<b>CAC</b>	Conspecific alarm cues
<b>CDC</b>	Conspecific disturbance cues
<b>GAC</b>	Guppy alarm cues
<b>NO</b>	Novel odour
<b>PAC</b>	Peacock cichlid alarm cues
<b>W</b>	Water

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# Chapter 1: Introduction

## 1.1. General information

Predation exerts a pervasive and unforgiving selection pressure on prey populations and can shape multiple facets of an individual's life, including life history, morphology, and behaviour (Beckerman et al., 2007; Chivers & Smith, 1998; Hoverman et al., 2005). Prey can alter the timing of life history shifts in response to predation. For example, amphibian and fish embryos tend to hatch earlier and emerge at smaller sizes in the presence of aquatic predators (Chivers et al., 2001; Kusch & Chivers, 2004; Warkentin et al., 2001). Lethal and nonlethal cues from predators have also been shown to induce plasticity in the age and size of amphibians and insects at metamorphosis (Benard, 2004; Chivers et al., 1999). By reducing the time it takes to reach maturity, these aquatic prey species can lessen the likelihood of predation at a life stage when they have minimal morphological defence mechanisms. Changes in life history switch points can also induce shifts in growth and reproductive patterns. For example, freshwater snails and *Daphnia* have been shown to grow faster and delay reproduction when exposed to predators that fed on conspecifics (Crowl & Covich, 1990; Riessen, 1999). Predation can also affect morphology. Prey that have a long history of coexistence with a specific predator and experience a stable level of predation can develop canalized morphological defences such as protective armour, defensive spines, crypsis or aposematic colouration (Edgell et al., 2009; Nunes et al., 2014). Conversely, inducible morphological defences are known to occur in prey that experience spatially and temporally variable risks, wherein the protective trait emerges in the presence of predators but is reduced or absent in low or no predation situations (Bourdeau, 2010; Harvell, 1990; Nunes et al., 2014). These short-term adaptive responses are more likely to emerge when reliable cues from predators are available, predation risk is unpredictable and the fitness gains are balanced by the costs (Harvell, 1990; Nunes et al., 2014). Moreover, the plasticity of short-term, adaptive responses to predation can also be observed in the behaviour of prey.

Predator-induced behavioural changes have been widely documented in the literature. Because prey can immediately change its behaviour when faced with acute predation threats, behaviour is argued to be the most plastic out of all the responses to predators (Brown & Godin, 2023). Prey often display antipredator behaviours through increased vigilance, grouping behaviour, deterrence behaviours, aggression and/or avoidance (e.g., camouflage, reduced activity, seeking refuge or fleeing) (Chivers & Smith, 1998; Lima & Dill, 1990). Engaging in

any of these behaviours can increase an individual's chances of survivorship and reproductive success but it can also incur losses in expended energy and opportunities for foraging, mating, parental care, and territorial defence. Conversely, the opposite would increase a prey's susceptibility to predation. Hence, prey must balance the trade-offs between these costs and benefits when deciding the timing, intensity, and mode of response to a predation threat (Brown & Godin, 2023; Lima & Dill, 1990). More importantly, this task hinges on the availability of reliable information in the environment such that prey can be sure about the state of their local environment and achieve optimal behavioural decisions.

## **1.2. Uncertainty in prey decision-making**

Throughout the individual's life history, prey are faced with the fundamental challenge of avoiding predation while optimizing fitness trade-offs. Formulating solutions requires that animals solve multidimensional problems with factors that interact over space and time. This task involves stimulus acquisition, perception, processing, and cost-sensitive behavioural responses (Stephens, 2008). Individuals are expected to execute behavioural strategies that strike a balance between benefits and energy costs (Lima & Dill, 1990), but the optimal choice is not always apparent to decision-makers. Hence, decision-makers often deal with uncertainty in their choices. Uncertainty in decision-making can be defined as an animal's perception of unpredictability and unknowns (Crane et al., 2020a). To better understand this concept, the uncertainty experienced by the decision-maker and the uncertainty caused by the environment must be delineated. Animals need temporal and spatial knowledge of their environment, as well as their own capabilities and limitations to decide and to act. How much they know about these factors contributes to their level of 'internal uncertainty', or their confidence in their own ability to assign probabilities to events (Crane et al., 2023b). This type of uncertainty can come from the decision maker's lack of understanding about their response options, their own perceptual processing, their response limitations, and the costs of performing a certain action (Milliken, 1987). On the other hand, animals can also experience uncertainty from the outside world due to the complexity and the changes in the environment. This can be referred to as 'external uncertainty' whereby decision-makers are unsure about the environment or a particular environmental parameter (Duncan, 1972; Milliken, 1987). Specifically in ecology, Dall (2010) coined this type of uncertainty as "ecological uncertainty", or the 'moment-to-moment degree to

which events are determined by factors that are out of an individual's control or immediate experience'. This paper also describes two types of uncertainty based on reducibility, with reducible uncertainty being attributed to the decision-maker's own action whereas irreducible uncertainty depends on the underlying internal and external processes that are inherently untraceable at spatial and temporal scales. Prey are simultaneously confronted with all these types of uncertainty. While they can sample unknown information and/or maintain energy reserves and safe niches to negotiate ambient uncertainty, problems emerge when uncertainty about the environment increases.

In a predation risk context, uncertainty can be linked to the validity of the antipredator response following the detection of a cue (Ferrari et al., 2018). As uncertainty increases, a prey's ability to assign accurate probabilities to events may be impaired. A high degree of uncertainty can result in a mismatch between the information detected from a cue and the response elicited. Unsurprisingly, any discrepancy in the cue-response patterns can exacerbate the costs of making behavioural decisions (Dall et al., 2005; Damien & Tougeron, 2019; Luttbeg & Trussell, 2013). Over-responding to perceived danger may result in lost opportunities to forage or mate whereas under-responding may lead to injury or death. Although prey have efficient learning paradigms to identify and respond to threats from active predators, experiential learning can have life-threatening consequences due to failure to survive first encounters (Brown, 2003). This is especially the case in novel environments where risks are unknown. As prey undergo ontogenetic and habitat shifts, they are continually transported into novel environments throughout their lifespan. Moreover, prey are increasingly exposed to anthropogenic changes that are often not uniform or consistent with natural environmental variance, introducing unpredictability in environmental parameters (Thompson et al., 2013). Understanding how species manage uncertainty can provide valuable insight into the study of cognitive ecology and is fast becoming a pressing issue for ecologists and conservation biologists.

### **1.3. Neophobia**

While there are no objective measures of how animals perceive uncertainty in their environment, researchers circumvent this issue by exposing animals to variations in stimuli and measuring their behavioural responses. Novel cues are typically involved in this process because of their inherent uncertainty. Many studies have examined the effect of uncertain predation risk

on over-responses toward novel cues. A fearful response to novel stimuli is known as neophobia. In a predation risk context, neophobia has been demonstrated as a plastic antipredator strategy that allows prey to cope in conditions when known information is not readily available (Crane et al., 2020a). When prey encounter a novel and threatening stimulus, showing a neophobic response has substantial advantages whereas failing to respond to the threat (i.e., a false negative) is highly costly and may result in severe injury or death. Conversely, when prey encounter a novel and non-threatening stimulus, a neophobic response (i.e., a false positive) may incur smaller costs through losing opportunities but individuals can exploit those opportunities if they fail to respond. As predicted by the Error Management Theory, this asymmetry in costs between a false negative and a false positive renders individuals biased towards being cautious (Blumstein & Bouskila, 1996; Johnson et al., 2013). Moreover, prey can improve survivorship in novel environments by negotiating the costs of learning in the form of failed first encounters (Brown et al., 2013; Elvidge et al., 2016). Hence, showing a fear response to a novel stimulus can be a good strategy for coping with the general stress related to uncertain risk until the threat is identified and/or the novel environment becomes more familiar and stable (Crane et al., 2020a).

Historically, neophobia was presumed to emerge from exposure to high-risk environments because prey are likely to encounter threatening over non-threatening cues (Feyten et al., 2019a). Encountering a novel cue in a high-risk environment may be treated as potentially risky whereas it may be ignored in low-risk environments. Alternatively, individuals are ostensibly more uncertain in risky environments due to the frequency of predation threats and unawareness of the timing and the identity of the next predator to attack. Hence, it can be argued that uncertainty, instead of elevated predation risk, may act as the key driver for the expression of neophobic phenotypes (Brown & Godin, 2023; Crane et al., 2020a; Ferrari et al., 2018). A neophobic prey assumes that novel stimuli are dangerous even if they may not be. If neophobia develops after repeated exposures to unpredictable or unknown information but not under predictable or stable conditions, then we may conclude that this behaviour was caused by uncertainty.

Several studies have examined the role that uncertain predation risk plays in shaping the expression of neophobia across different aquatic groups. For example, Ferrari et al. (2018) induced uncertainty from incomplete information about predation risk in the form of injured

conspecific cues that do not include information about a specific predator and from predator diversity through injured conspecific cues paired with a different predator odour for each day of the background exposure period. They found that uncertainty induced neophobia in wood frog (*Lithobates sylvaticus*) tadpoles whereas those that were certain about the identity of the predator due to repeated exposures to a single predator odour did not develop neophobic responses. However, Crane & Ferrari (2016) demonstrated that uncertainty from incomplete information and certainty from repeated exposures to a single predator odour paired with injured conspecific cues both induced neophobia in fathead minnows (*Pimephales promelas*). While these results may be species-specific effects, conflicting results in the literature warrant further investigation into the effects of uncertain predation risk on prey decision-making.

#### **1.4. Study system**

##### *Convict cichlids*

The convict cichlid (*Archocentrus nigrofasciatus*) is a member of the family Cichlidae. It is a small freshwater fish (generally <100 mm standard length), characterized by their black stripes and is native to Central America (Ishikawa & Tachihara, 2010). It can be found in lakes and streams across the Pacific slope of Guatemala to Costa Rica and the Atlantic coast of Honduras and Panama. This species is generally omnivorous and feeds on plants, algae, small fish, crustaceans, insects, and worms. However, their small size renders them vulnerable to an array of aquatic and terrestrial predators such as fishes, birds, and invertebrates. This species possesses chemical alarm cues that are released upon injury to the skin or muscle. Additionally, juveniles can effectively respond to conspecific alarm cues regardless of the presence of parental care and the ontogenetic stage of the alarm cue sender (Brown et al., 2004; Pollock et al., 2005). Animals at this life history stage are under intense predation pressure due to their small size and are at a sensitive period of cognitive development.

Convict cichlids have been widely used as test species for cognitive and behavioural studies. This species is easily bred in aquaria and requires low maintenance, making them highly suitable for laboratory-oriented studies. Moreover, juveniles have repeatable and heritable individual differences in personality traits and behavioural syndromes under the pressure of predation threats and novel environments (Mazué et al., 2015). Cichlids are typically observed in pairs as singletons are generally inactive (Brown et al., 2013). The antipredator responses of

cichlids may include dashing (rapid bursts of apparently disoriented swimming), freezing, increased shoaling coherence, reduced frequency of aggressive interactions, and increased shelter use. Hence, juvenile convict cichlids are an ideal test species for understanding prey decision-making and have become a well-studied system for exploring the chemical ecology of predator-prey interactions.

## **1.5. Research objectives**

My overall objective is to understand the effect of uncertain predation risk on prey decision-making and neophobia. In my thesis, I present a series of experiments divided into two data chapters, to test the hypothesis that uncertainty, instead of elevated risk, drives the expression of neophobia. I induced uncertainty from the temporal unpredictability of risk and the unreliability of chemical risk assessment cues. I used juvenile convict cichlids to answer the following questions:

*How do unreliable chemical risk cues affect the onset of neophobia?* Prey require reliable public information to assess the relevance of a threat and reduce their uncertainty about predation risk (Brown & Godin, 2023; Feyten et al., 2019b). Reliable risk assessment cues are immediately linked to acute predation risk and thus, should provide prey with the least uncertainty about risk (Feyten & Brown, 2018). In Chapter 2, I tested whether juvenile convict cichlids express a graded intensity of neophobic responses based on the reliability of chemical risk cues in their environment.

*How does the temporal predictability of risk affect the expression of neophobia?* The temporal predictability of risk shapes a prey's certainty about risk and the intensity of antipredator behaviours across temporal scales (Lima & Bednekoff, 1999; Sih et al., 2000). If a threat is predictable, then individuals may exhibit minimal antipredator behaviours prior to the presence of the predator and only become vigilant when the threat emerges. If a threat is unpredictable, then prey might engage in long bouts of vigilance. In Chapter 3, I parsed out the effects of uncertainty associated with temporal unpredictability of risk from the certainty related to learning about the temporal patterns of risk on the onset of neophobia. I tested the effect of unpredictable risk schedule and unpredictable test time to investigate how temporally unpredictable risk affected the intensity of induced neophobia.

## **1.6. Anticipated significance**

My research is focused on understanding how uncertainty about predation risk affects prey decision-making, specifically the decision to engage in neophobic predator avoidance. When prey are uncertain about the threats in their local environment, they develop adaptive antipredator behaviours such as neophobia. Assuming novel stimuli as risky in a high-risk environment is likely adaptive due to the high ratio of threatening over non-threatening stimuli. Additionally, I aimed to demonstrate how uncertainty, as opposed to elevated background risk levels, influences the expression of neophobia. Research on cognitive ecology and antipredator strategies can improve various conservation efforts such as reintroduction. For example, inducing neophobia in naïve, captive individuals prior to reintroduction is likely to increase survivorship when confronted by multiple novel predators in the wild (Crane et al., 2020a). More importantly, addressing questions about the effects of uncertain predation risk on neophobia will shed light on how prey may cope with increasing ecological uncertainty associated with climate change, invasive species, and anthropogenic habitat modification.

## **Chapter 2: The effect of chemical risk cue reliability on the expression of predator neophobia**

### **2.1. Introduction**

Prey that fail to respond appropriately to predation risk may lose their life. Consequently, predation acts as a selective pressure that favours prey that can display adaptive responses. The benefit of an adaptive response is affected by the reliability of environmental cues (Donaldson-Matasci et al., 2013; Yoshimura et al., 2013). Moreover, the reliability of a cue is determined by how tightly correlated a specific cue and a particular event are over past repeated encounters (Bairos-Novak et al., 2019a; Crane et al., 2023b). Because predation threats can vary over spatial and temporal scales, variation in cue reliability becomes an unavoidable aspect of natural environments. This leads to a fluctuation in the degree of uncertainty prey experience when making decisions. As a result, prey must periodically re-assess the reliability of environmental cues. For example, prey may perceive the sight of a predator as risky or non-threatening depending on the time of day and spatial location of the encounter (Munoz & Blumstein, 2012; Roth II & Lima, 2007). Also, predator kairomones convey highly reliable information about the predator's hunger state and diet but are unreliable when they are carried away by the current or persist after the predator has left the vicinity (Crane et al., 2023a; Van Buskirk et al., 2014). Moreover, some animals may undergo migration or short-term movements throughout their ontogeny and are continually exposed to novel environments wherein predation-related cues are likely unreliable (Ferrari et al., 2018). Hence, parsing out reliable cues from unreliable ones is paramount to optimize energetic costs associated with plastic antipredator behaviours.

Prey experience an array of stimuli that potentially indicate a predation threat. However, to assign a specific cue as reliable, they must possess a certain level of knowledge or familiarity about the information contained in that cue. Individuals can either innately recognize or learn to recognize a particular cue (Crane & Ferrari, 2015; Ferrari et al., 2010). For example, a wide variety of aquatic prey possesses an innate fear response to alarm cues, which are chemicals from conspecifics that are only released upon injury to the skin or muscle (Chivers & Smith, 1998; Ferrari et al., 2010). Prey can also develop a learned fear response to a novel predator cue through a one-time pairing with alarm cues, indicating that these cues are reliable and honest indicators of local risks (Batabyal et al., 2014; Chivers & Smith, 1998; Lucon-Xiccato et al., 2020). Thus, “known” risk cues are more reliable than “unknown” or novel cues because the



former is likely to represent a consistent acute risk (Feyten & Brown, 2018). In habitats or foraging patches wherein prey have grown familiar, have adequate foraging opportunities and are exposed to a stable predator guild, prey are expected to experience a low degree of uncertainty. However, as uncertainty about the environment increases, individuals are presumed to encounter more cues with decreasing reliability (Dall et al., 2005; Koops, 2004). In such cases, prey would potentially benefit from increasing vigilance towards novel cues. For example, Trinidadian guppies from a high-predation environment were shown to have longer delays in predator inspection when exposed to two novel cues (a visual cue and a chemical cue) as opposed to when one or two cues were known, demonstrating the additive effect of novel cues on perceived predation risk (Feyten et al., 2019b). These results evoked the idea that the cognitive rules used by prey to assess predation risk are determined by their informational environment (Luttbeg & Trussell, 2013). Consequently, any changes to the ecology of information are likely to affect their capacity for adaptive behaviour (Dall et al., 2005; Schmidt et al., 2010).

In aquatic environments, olfaction is a crucial aspect of risk assessment and predator avoidance through the detection of semiochemicals, or chemicals that facilitate intra- and interspecific communication (Ferrari et al., 2010). While alarm cues are generally a highly reliable indicator of an imminent threat, these cues can still generate uncertainty among prey because they lack information about the predator's identity (Ferrari et al., 2018). Its reliability may also fluctuate depending on the sender's phylogenetic and biological association with the receiver. Previous evidence showed taxonomic conservatism of alarm cues, wherein the more closely related species are, the stronger heterospecific alarm cue responses are (Dalesman et al., 2007; Hume & Wagner, 2018). During a predation event, conspecifics may learn to associate risk with heterospecific alarm cues when their alarm cues are paired with those of unrelated species. By learning to recognize heterospecific alarm cues, members of the same prey guild can "eavesdrop" to fine-tune their antipredator responses and acquire a better sense of their predator's diet and preferences (Elvidge & Brown, 2015; Groves et al., 2022). Additionally, prey may use disturbance cues as a source of information about predator activity and risk assessment (Crane et al., 2021; Goldman et al., 2020a). Disturbance cues are chemicals released by disturbed but uninjured emitters and induce behavioural changes akin to predator avoidance (Crane et al., 2021). However, unlike alarm cues, disturbance cues are not specific to predation events only. It

can be released through several non-threatening or threatening events such as competition, confinement or crowding, acidic pulse, and exposure to stressful conditions (e.g., heat, food deprivation, cortisol exposure) (Abreu et al., 2016; Bett et al., 2016; Giaquinto & Hoffmann, 2012; Hazlett, 1985). Moreover, disturbance cue signalling is highly context-dependent. Receivers have been shown to respond with different intensities depending on the ambient level of nitrogenous waste (Brown et al., 2012; Vavrek et al., 2008), as well as the background risk experience, diet, audience, familiarity with, and the group size of the donors (Bairos-Novak et al., 2017, 2019a, 2019b; Crane et al., 2020c; Goldman et al., 2019, 2020b, 2022). Rather than being tightly correlated with risk, these cues may serve as an early warning signal to prime prey for a more intense response to subsequent risk-related information. Individuals may need to initially evaluate the validity of the cues in relation to predation and thus, are potentially a less reliable indicator of risk compared to alarm cues (Crane et al., 2022; Ferrari et al., 2008b).

While several studies have investigated how cue reliability, and its concomitant certainty, induces an enhanced adaptive antipredator behaviour (Bourdeau, 2010; Brown et al., 2014; Ferrari et al., 2016), the way animals cope with decreasing risk cue reliability is often overlooked. Theoretical evidence suggests that the reliability of risk assessment cues will decrease as ecological uncertainty increases (Dall et al., 2005; Koops, 2004). Additionally, Error Management Theory posits that individuals are more likely to engage in risk-averse strategies as risk becomes more uncertain (Blumstein & Bouskila, 1996; Johnson et al., 2013). Hence, prey may develop neophobia as a cognitive bias to minimize the probability of incurring mistakes with the greatest costs under circumstances of decreasing cue reliability. In the following study, I investigated the onset of neophobia in predator-naïve convict cichlids (*Archocentrus nigrofasciatus*) exposed to uncertain predation risk generated from different chemical risk cues. The goal of this experiment is to determine whether cichlids develop neophobia with graded intensity based on the reliability of chemical risk cues. I used convict cichlids as donors of conspecific alarm cues and disturbance cues, peacock cichlids (*Cichla ocellaris*) as donors of confamilial alarm cues and guppies (*Poecilia reticulata*) as donors of alarm cues from an unrelated species (i.e., novel alarm cues). Out of all these chemical risk cues, I expected conspecific alarm cues to be the most highly reliable indicator of risk and novel alarm cues to be the least reliable to cichlids. I hypothesized that neophobia can only occur when chemical risk cues are somewhat reliable, such that fish repeatedly exposed to highly reliable conspecific alarm

cues will elicit the strongest neophobic response whereas exposure to unreliable novel alarm cues will not generate neophobia.

## **2.2. Methodology**

### ***Test species***

This research took place at Concordia University. Juvenile convict cichlids were obtained from a laboratory colony. This population descended from crosses of the laboratory stock population and wild fish from Costa Rica. Cichlids were housed in 37-L glass aquaria with dechlorinated tap water at 25°C, an air stone, artificial plants, and a gravel substrate. They were fed, in excess, twice a day with bloodworms around 09:00-10:00 h and brine shrimp (*Artemia spp.*) around 14:00-15:00 h. The photoperiod was maintained at 12:12 h light: dark cycle.

### ***Stimulus collection***

#### ***Convict cichlid alarm cues (CAC)***

Convict cichlid alarm cues were collected from 10 sub-adult donors (standard length: mean  $\pm$  S.D. = 10.38  $\pm$  1.99 cm). Cichlids were euthanized via cervical dislocation followed by spiking using a scalpel blade (in accordance with the Canadian Council on Animal Care and Concordia University Animal Research Ethics protocol # 30000255). Skin fillets were removed from the flanks and placed in 100 mL of chilled dechlorinated tap water. Then, this mixture was homogenized using an immersion blender (2-speed Immersion Blender, The Black and Decker Corp., Middleton, USA) and filtered through glass wool to discard any remaining tissues. I collected a total of 208.10 cm<sup>2</sup> in a final volume of 2075 mL, yielding a solution of 0.1 cm<sup>2</sup> of skin per 1 mL of dechlorinated water. This concentration has been shown to reliably elicit an antipredator response in juvenile convict cichlids (Brown et al., 2016; Wisenden & Sargent, 1997). I standardized the alarm cues from different species based on the concentration of the area of skin used, with the exception of guppy alarm cues (see below), to ensure that cichlids were exposed to similar levels of predation risk across all prey species used in this study. The solution was then stored as 20 mL aliquots and frozen at -20 °C until use.

### *Convict cichlid disturbance cues (CDC)*

Disturbance cues were collected using recently established methods (Bairos-Novak et al., 2019b; Goldman et al., 2019). I placed 15 donor fish (standard length: mean  $\pm$  S.D. =  $2.35 \pm 0.63$  cm) in a 20-L tank filled with 9.5 L of dechlorinated tap water. This concentration is similar to the one used to induce an antipredator response in the same species (Goldman et al., 2019). The tanks were wrapped in an opaque garbage bag to prevent visual disturbance prior to stimulus collection. I allowed them to acclimate for 24 hours without food to prevent chemical interference from dietary cues. I also affixed an air stone in the back of the tank, which was removed 1 hour before collection. After the acclimation period, I chased the fish with an esocid predator model (15 cm) connected to a glass rod for 60 seconds while avoiding any contact with the fish to prevent the release of alarm cues. I then waited for another minute before softly mixing the water inside the tank. I removed 500 mL from the tank to be used as disturbance cues. This solution was stored in 50 mL aliquots and immediately frozen at  $-20$  °C until use. Based on previous studies, frozen disturbance cues remain effective at simulating increased background risk when compared to freshly made cues (Goldman et al., 2020a; Rivera-Hernández et al., 2022).

### *Peacock cichlid alarm cues (PAC)*

Peacock cichlids, *C. ocellaris*, were obtained from a hatchery (La Maison du Poisson, Montreal, Canada). Alarm cues were collected from 5 donors (standard length: mean  $\pm$  S.D. =  $6.8 \pm 0.14$  cm). In total, I obtained  $43.13 \text{ cm}^2$  of skin in a final volume of 430 mL which provided a concentration of  $0.1 \text{ cm}^2$  of skin per mL. The rest of the stimulus preparation was identical to that of convict cichlid alarm cues.

### *Guppy alarm cues (GAC)*

Donor *P. reticulata* were collected from a laboratory stock population that descended from wild-caught individuals from a site on the Upper Aripo River, Trinidad. To obtain alarm cues, we euthanized 72 donors (standard length: mean  $\pm$  S.D. =  $1.83 \pm 0.18$  cm) via cervical dislocation and decapitation. The head, tail and visceral contents were then removed, and the remaining carcasses were homogenized in dechlorinated tap water. The solution was diluted to achieve a final concentration of  $0.1 \text{ cm}^2$  of donor biomass per mL. Due to the small size of

guppies, collecting alarm cues from skin fillets was impractical and would be to the detriment of the quality of the cues. Thus, I used donor biomass as the basis of the alarm cue concentration. Then, it was stored in 20 mL aliquots at -20 °C until use. This method of preparation was based on previous studies that have consistently induced neophobic behaviour in Trinidadian guppies (Crane et al., 2021; Goldman et al., 2020a).

#### *Novel odour*

For the novel chemical cue, I added 6 drops of orange extract (Club House, McCormick & Company, Inc., London, Canada) in 500 mL dechlorinated tap water. Neophobic prey fish can reliably detect and respond to synthetic novel odour at this concentration (Brown et al., 2020; Elvidge et al., 2016). Orange odour was freshly prepared before testing trials.

#### ***Experimental protocol***

This experiment consisted of a 6 x 2 design, whereby fish were exposed to CAC+CAC, CAC+CDC, CAC+PAC, CAC+GAC, CAC+W, or W+W, then tested with water or novel odour to determine the expression of neophobia. It had two phases: an exposure period followed by two testing trials. The exposure period consisted of exposures to different cues of varying reliability that occurred thrice a day for 3 days. Cichlids were exposed to 15 mL of CAC or a water (W) control for the initial five exposures. Then, they received 15 mL of CAC, CDC, PAC, GAC, or W for the last four exposures. Thus, cichlids were given either of the six background exposure treatments: a) conspecific alarm cues only (CAC+CAC), conspecific alarm cues followed by disturbance cues (CAC+CDC), conspecific alarm cues followed by confamilial peacock cichlid alarm cues (CAC+PAC), conspecific alarm cues followed by novel alarm cues from guppies (CAC+GAC), conspecific alarm cues followed by water (CAC+W) or water only (W+W) (Table 2.1).

To test the onset of neophobia, cichlids were exposed to 5 mL of orange odour or a water control one day post-exposure period.

**Table 2.1. Schematic diagram of exposure treatments used in Experiment 1. Cichlids experienced background risk from exposure to different risk cues three times a day for 3 days.**

Treatment	Exposure period		
	Day 1	Day 2	Day 3
a) CAC+CAC	CAC, CAC, CAC	CAC, CAC, CAC	CAC, CAC, CAC
b) CAC+CDC	CAC, CAC, CAC	CAC, CAC, CDC	CDC, CDC, CDC
c) CAC+PAC	CAC, CAC, CAC	CAC, CAC, PAC	PAC, PAC, PAC
d) CAC+GAC	CAC, CAC, CAC	CAC, CAC, GAC	GAC, GAC, GAC
e) CAC+W	CAC, CAC, CAC	CAC, CAC, W	W, W, W
f) W+W	W, W, W	W, W, W	W, W, W

*Exposure procedure*

Randomly selected groups of 16 cichlids were placed into a series of 12-L opaque, white pails containing 5 L of dechlorinated tap water and a gravel substrate. The pails also had a single air stone to which a 2-m long piece of airline tubing was attached for gently injecting the stimuli into the pails. I covered the pails with blue opaque plastic and secured them with binder clips to minimize external disturbance. The photoperiod was kept at 12:12 h light: dark cycle and the temperature was maintained between 24-25 °C. Cichlids were fed immediately after being transferred into the pails and were left to acclimate for 1 hour. All exposures occurred at 11:00, 13:00 and 15:00 h each day for 3 days. Prior to injecting the stimuli in the tank, I slowly withdrew and discarded 60 mL of water from the injection tubes to remove any stagnant water. I then withdrew but retained another 60 mL of water for flushing the stimuli into the pail. Throughout the exposure period, cichlids were fed twice a day with a pinch of crushed bloodworms, 1 hour before the first exposure and 1 hour after the last exposure of the day. I also facilitated a 50% water change using a shaker siphon 30 minutes after the last exposure, ensuring to softly wiggle the siphon tube to deter fish from the suction.

*Testing procedure*

Test tanks consisted of a series of 24-L glass aquaria filled with 20 L of dechlorinated tap water, a gravel substrate, and a single air stone. Tanks were wrapped with blue plastic on three

sides, whereas the side facing the observer was covered with a piece of window tinting to prevent visual disturbance. Additionally, I fastened a 2-m long piece of airline tubing to the air stone to introduce experimental cues and minimize disturbance to the fish.

Cichlid pairs were transferred into the tanks wherein they acclimated for at least 1 hour. They were tested in pairs as previous reports have shown that singletons are generally inactive (Brown et al., 2013). Each pair was size matched to minimize aggression towards each other. All trials were conducted between 09:00 and 17:00 h. Each trial consisted of a 5 minute pre-stimulus and a 5 minute post-stimulus injection observation period. Cichlids were fed commercial flakes shortly before starting the pre-stimulus period. I then withdrew and discarded 60 mL of water from the injection tubes, followed by another withdrawal of 60 mL of water for flushing the stimuli into the tank.

At 10-sec intervals during the observation periods, I recorded the occurrence of certain behaviours exhibited by the pair for most of the 10-sec interval: dashing (rapid erratic darting), freezing (centre of the body not moving), foraging (moving and striking at food regardless of the rate of movement or strikes), or calm swimming (not engaged in the other behaviours or stereotypic pacing; Crane et al., 2020d). All trials were performed blind to the exposure treatments, and the order of testing was partially randomized with odd-numbered tanks receiving water and even-numbered tanks receiving novel odour. I tested a total of 185 cichlid pairs (standard length: mean  $\pm$  S.D. = 1.17  $\pm$  0.23 cm) with n=13-17 per treatment combination (fish were never tested more than once).

### ***Statistical analysis***

Preliminary data exploration revealed a size effect on certain behaviours, such as calm swimming (one-way ANCOVA, size:  $F_{1,42.9}=12.9$ ,  $P=0.001$ ) and foraging (one-way ANCOVA, size:  $F_{1,30.4}=4.15$ ,  $P=0.05$ ), although the size effect was constant across treatment (treatment\*size:  $P>0.40$ , for both variable). To remove this inconsistent effect across variables and to simplify the analysis, I combined these two behavioural measures by adding their respective occurrences and produced a new variable, which I labelled ‘calm foraging’, for which no size effect existed (one-way ANCOVA, size:  $F_{1,31.7}=1.91$ ,  $P=0.18$ ). A multivariate approach was considered to analyze the remaining variables. However, upon inspection, I found that freezing behaviour appeared to code for the inverse of calm foraging (Pearson correlation, pre-observation:  $r=-0.97$ ,  $P<0.001$ ;

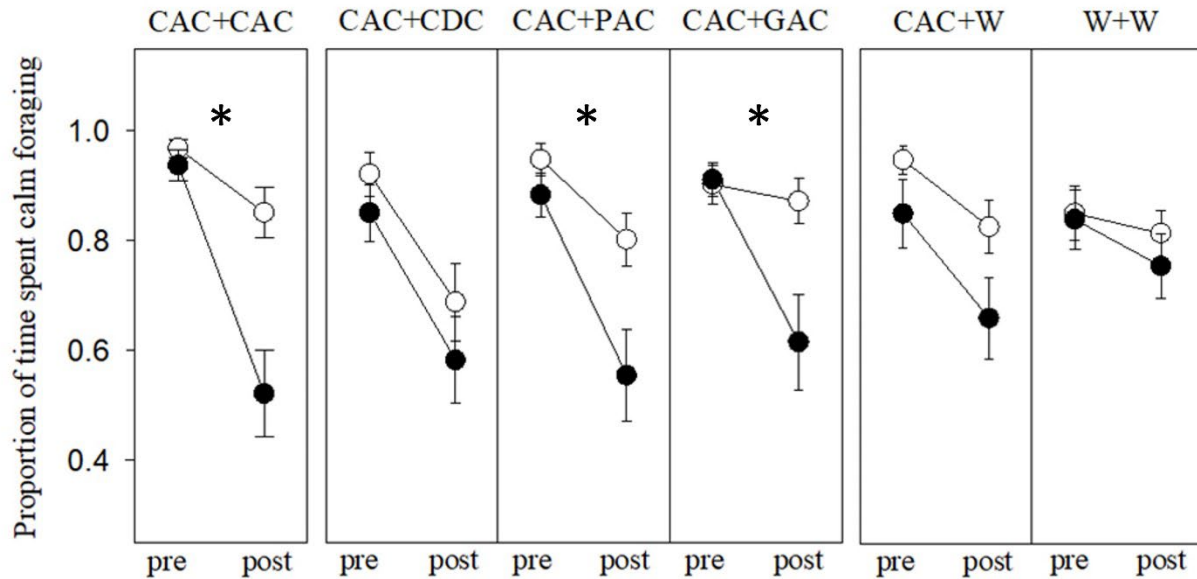
post-observation:  $r=-0.51$ ,  $P<0.001$ ), and the occurrence of dashing behaviour was too infrequent to be considered in a traditional Principal Component Analysis. Therefore, the analysis was carried out on ‘calm foraging’ only, which occurrence was predicted to decrease as the fish’s antipredator behaviour increased. Indeed, reduced time spent moving and foraging are both documented antipredator behaviour in juvenile cichlids (Brown et al., 2006; Ferrari et al., 2010).

Data for calm foraging was converted to proportions of occurrence by dividing the number of 10-sec intervals calmly foraging over the total number of intervals. For most groups, the data for the proportion of time spent calmly foraging for the post-observation period met the assumptions of normality (Kolmogorov-Smirnov test,  $P>0.05$ ) and homoscedasticity (Levene’s test,  $F_{47,137}=1.36$ ,  $P=0.09$ ), but not for the pre-observation period. Although it had a non-normal distribution, a repeated measures ANOVA is a test robust against deviation from normality. It is also important to note that this analysis had a lower power due to the unequal variance inherent in the pre-observation period data. Nonetheless, it is the best possible statistical test for this type of data. Thus, I used a 3-way nested repeated measures ANOVA to analyze the effects of the fixed factors (risk exposure treatment [CAC+CAC, CAC+CDC, CAC+PAC, CAC+GAC, CAC+W, W+W], the test cue [water or novel odour], time [pre-stimulus or post-stimulus period], their interactions and the exposure pail as the random factor. I used a Type I SS model due to the hierarchical structure of the experimental design wherein the exposure pail served as the nest and the unit of replication in the analysis. To interpret significant interactions, I split the data by exposure treatment and investigated the effect of cue and time on the response of the fish. All analyses were performed in SPSS 25 with  $\alpha = 0.05$ .

### 2.3. Results

There was a significant three-way interaction between all three fixed factors ( $F_{5,155} = 2.33$ ,  $P = 0.045$ , Figure 2.1), indicating that cichlids differed in their response to the test cues between the pre- and post-stimulus periods depending on the exposure treatment. Post hoc testing revealed that exposure to CAC+CAC ( $F_{1,25} = 11.3$ ,  $P = 0.003$ ), CAC+PAC ( $F_{1,28} = 6.29$ ,  $P = 0.018$ ) and CAC+GAC ( $F_{1,21} = 8.48$ ,  $P = 0.008$ ) significantly decreased their calm foraging behaviour upon exposure to a novel odour compared to the water control. However, fish from the CAC+CDC ( $F_{1,28} = 0.22$ ,  $P = 0.639$ ), CAC+W ( $F_{1,28} = 1.34$ ,  $P = 0.257$ ) and W+W ( $F_{1,25} = 0.60$ ,  $P = 0.446$ ) did not differ in their response to the test cues.





**Figure 2.1. Mean ( $\pm$ s.e.) proportion of time spent calm foraging for cichlids before (pre) and after (post) injection of water (white circles) or a novel odour (black circles) one day after exposure to conspecific alarm cues only (CAC+CAC), conspecific alarm cues followed by disturbance cues (CAC+CDC), conspecific alarm cues followed by confamilial peacock cichlid alarm cues (CAC+PAC), conspecific alarm cues followed by novel alarm cues from guppies (CAC+GAC), conspecific alarm cues followed by water (CAC+W) or water only (W+W). Asterisks denote significant differences at  $\alpha=0.05$ . Sample sizes were  $n = 13-17$  per treatment combination.**

## 2.4. Discussion

In this study, there was no significant difference in how juvenile cichlids responded to water or a novel odour after repeated exposures to the water control, indicating that those fish did not show neophobia (i.e., a fear of a novel stimulus). However, those that received conspecific alarm cues only (CAC+CAC) showed a marked neophobic response. This was expected as a strong neophobic response can be induced from a minimum of six consecutive exposures to alarm cues (Brown et al., 2015). Although five exposures were the minimum number of alarm cues exposure in my experiment, the concentration used was ecologically high enough to theoretically induce neophobia in the other treatment groups. However, exposures to different types of risk assessment cues after neophobia has been established drove the variation in cichlids' fear response to a novel odour. For fish that received water after the alarm cue exposures (i.e., CAC+W group), I did not observe a marked neophobia which suggested that subsequent exposures to water weakened any neophobic responses. This group served as a

negative control for the other risk assessment cues such that it demonstrated how fish responded when they opted to ignore the second cue.

Fish that received subsequent exposures to heterospecific alarm cues showed a marked antipredator response to a novel odour. This demonstrates that these risk cues provided cichlids with information about risk. Although there are no studies that tested the capacity of convict cichlids to recognize alarm cues from confamilial peacock cichlids, it is known that heterospecific alarm cue recognition emerged as a product of phylogenetic conservatism through evolution from a common ancestor and/or learning during periods of shared risk (i.e., same prey guild) (Elvidge & Brown, 2015; Wisenden & Sargent, 1997). The more closely related the two species are, the stronger heterospecific alarm cue responses are. Several prey populations have consistently been shown to recognize and display antipredator responses to confamilial alarm cues but not to alarm cues from unrelated heterospecifics (Dalesman et al., 2007; Hume & Wagner, 2018; Mitchell et al., 2012; Pollock & Chivers, 2004).

Given that fish from CAC+PAC and CAC+GAC groups showed similar neophobic responses, I can conclude that cichlids did not ignore the heterospecific alarm cues; otherwise, their response to a novel odour should have been similar to that of the CAC+W group. Rather, these cues contributed to the maintenance of neophobia. It may have provided convict cichlids risk-related information that they recognized due to the taxonomic conservatism of alarm cues. However, convict cichlids and guppies are known to be allopatric unrelated species (Goldman et al., 2019); thus, cichlids should not have been able to recognize guppy alarm cues. Studies have demonstrated that convict cichlids increased feeding and/or decreased shelter use as a response to alarm cues from unrelated species (Pollock et al., 2005; Wisenden & Sargent, 1997). Instead, guppy alarm cues may have served as a novel odour that promoted the waning of neophobia. Brown et al. (2015) found that the neophobic responses of high-risk guppies waned after five consecutive exposures to a particular novel odour but not to a new novel odour. In this experiment, fish in the CAC+GAC group received four consecutive exposures to guppy alarm cues. If four consecutive exposures to novel alarm cues can induce the waning of neophobic response from a lack of negative reinforcement, then this neophobic response is likely the result of neophobia being triggered by a new novel stimulus.

Fish that received disturbance cues as a second risk cue not only elicited a fear response to a novel odour but also to the water control. While this may suggest an experimental artefact,

this may also indicate that exposure to conspecific alarm cues followed by disturbance cues caused a generalized fear towards disturbance (i.e., injection). Disturbance cues are considered to be a known cue to most prey populations (Crane et al., 2021). It can serve as an early warning signal to potential threats (Goldman et al., 2020a). Thus, it may have primed individuals to increase vigilance to the disturbance caused by the injection of the cue, and not the cue per se. Alternatively, these cues can also generate uncertainty about the source of the threat due to the generalized nature of events from which it is elicited (Crane et al., 2021). Indeed, uncertainty and the reliability of public information are expected to have interacting effects on the stress responses of prey (Feyten & Brown, 2018). Individuals may increase vigilance but maintain a tempered stress response because the cost of mounting a high-stress response and depleting energy stores is likely greater than any potential benefit. To confirm the credibility of the threat, prey may develop heightened responses to non-threatening cues (e.g., water disturbance or novel odour) and/or exploratory behaviours towards uncertain threats. This is analogous to the uncertainty caused by degrading cues wherein the information that it contains may change over time and, by extension, how prey respond to such cues (Crane et al., 2023a; Van Buskirk et al., 2014). For example, western grey kangaroos are known to approach aged dingo (predator) scents enough such that these predator cues have been labelled as an ‘attractant’ (Parsons et al., 2018). Hence, the reliability of risk assessment cues in an uncertain environment may result in the decoupling of the physiological correlates of fear and vigilance behaviour (Feyten & Brown, 2018).

How prey mitigate predation risk in a novel or changing environment is a fundamental question in biology. My results indicate that developing a fear response towards a non-threatening novel stimulus may serve as an adaptive strategy against the varying reliability of risk assessment cues. Moreover, the intensity at which these responses are expressed may be commensurate to how reliable a particular risk assessment cue is to receivers. These findings expand on the theoretical framework of the uncertainty hypothesis, demonstrating that uncertainty about risk is the key driver of neophobia.

## **Chapter 3: The effect of temporal predictability of risk on the expression of predator neophobia**

### **3.1. Introduction**

The predation risk that prey experience across their evolutionary and life history is tightly linked to the presence of predators. Consequently, a prey's vulnerability to risk changes depending on the feeding patterns and abundance of their coexisting predators (Ferrari & Chivers, 2009). At an individual's level, predation risk can vary moment-to-moment and over daily and seasonal cycles. Prey have been shown to adjust their activity levels based on the fluctuating levels of predation across a diel cycle. For example, the movement patterns and activity of Yellowstone elk (*Cervus elaphus*) have been observed to rise and fall along with the crepuscular activity of wolves (*Canis lupus*), allowing prey to use wolf hotspots during predator downtimes (Kohl et al., 2018). Juvenile lemon damselfish (*Pomacentrus moluccensis*) tempered their foraging activity during periods of high risk but not when predation risk was low or absent (Bosinger et al., 2012). Such changes in antipredator response across temporal scales strongly suggest that prey can fine-tune their behaviour and exhibit flexible strategies to make the best out of short-term cost-benefit trade-offs (Ferrari et al., 2015). More importantly, these studies highlight that in some systems with predictable risk patterns, temporal variability of risk can drive behavioural responses. Prey can track temporal patterns of risk through experiential learning.

Temporal threat-sensitive learning – the ability to learn the pattern of risk exposure through time – allows prey to gain more certainty about the state of its local environment and modulate behavioural responses based on periods of safety and danger (Luttbeg et al., 2020). For instance, Lima & Bednekoff (1999) proposed the risk allocation hypothesis to explain behavioural decision-making under variable conditions. In this framework, prey are predicted to alter the intensity of antipredator responses based on the level and frequency of active predation to optimize behavioural trade-offs. However, this hypothesis holds only when prey are given enough time to learn the predictability pattern of their predators (reviewed in Ferrari et al., 2009). Hence, individuals that can track relevant threats over time should be able to make better-informed decisions compared to those that cannot (Crane & Ferrari, 2017). Ferrari et al. (2015) discovered the phenomenon of time-sensitive neophobia by exposing wood frog (*Lithobates sylvaticus*) embryos to risk in the morning or evening and testing the behaviour of tadpoles after

hatching. Tadpoles only elicited neophobia at times that matched their embryonic exposure time, demonstrating the importance of predictability of risk in fine-tuning the timing of neophobic responses. However, this mechanism requires that risk remains temporally constant. To some degree, the temporal aspect of risk allows prey to assess the relevance of a piece of information (Brown et al., 2020). Conversely, some predators may take advantage of this strategy and maintain spatial and temporal unpredictability to increase their chances of success (Roth II & Lima, 2007). The question remains: do prey allocate vigilance differently if predation risk is unpredictable as opposed to predictable? Brown et al. (2020) showed that northern red-bellied dace (*Chrosomus eos*) developed stronger neophobia when exposed repeatedly to a random risk schedule compared to those receiving risk on a fixed schedule. While these results may support the uncertainty hypothesis, it can be argued that neophobia was induced by how certain prey were about the timing (or lack of timing) of risk. However, testing the intensity of neophobic responses at times when prey do not expect risk has largely been overlooked.

In the following study, I investigated how temporal predictability of risk affected the intensity of neophobia in juvenile convict cichlids. The objective of these experiments was to 1) test the effects of temporal risk patterns on the onset of neophobia by exposing convict cichlids to a fixed or random risk schedule and 2) measure the intensity of neophobic responses when cichlids are expecting risk (i.e., predictable risk) as opposed to not expecting risk (i.e., unpredictable risk) by testing fish at times that matched or did not match the fixed risk schedule. I hypothesized that risk predictability, rather than temporal learning of risk, drives the intensity of neophobic responses. If uncertainty about risk drives neophobia, then I expected cichlids to elicit a stronger neophobic response when exposed to a random instead of a fixed risk schedule. Also, I expected them to elicit a stronger neophobic response when they are not expecting risk to occur than when risk is predictable and fish are expecting a threat to occur.

## **3.2. Methodology**

### ***Test species***

Experiment 2 was conducted at the same time as Experiment 1. Hence, the origin and housing conditions of cichlids were identical to that of Experiment 1.

### ***Stimulus collection***

### *Convict cichlid alarm cues*

Convict cichlid alarm cues were collected from 13 sub-adult donors (standard length: mean  $\pm$  S.D. = 11.22  $\pm$  1.61 cm). The rest of the stimulus preparation was identical to that of Experiment 1. In total, I obtained 259.07 cm<sup>2</sup> of skin in a final volume of 2590 mL of dechlorinated water.

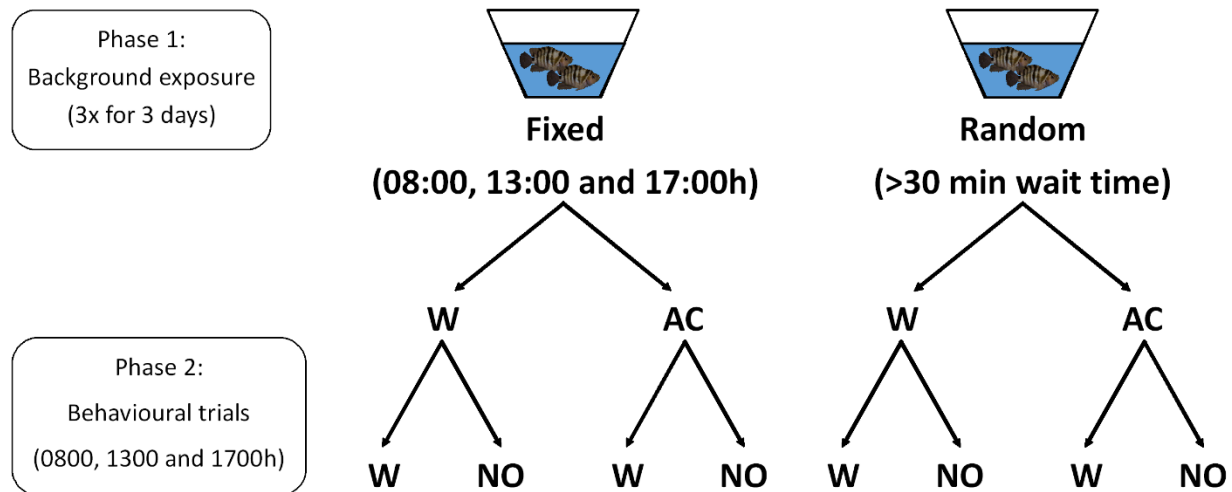
### *Novel odour*

Similar methods to Experiment 1 were used to generate the novel chemical cue.

## ***Experimental protocol***

### *Experiment 2.1: Does temporal risk schedule affect the onset of neophobia?*

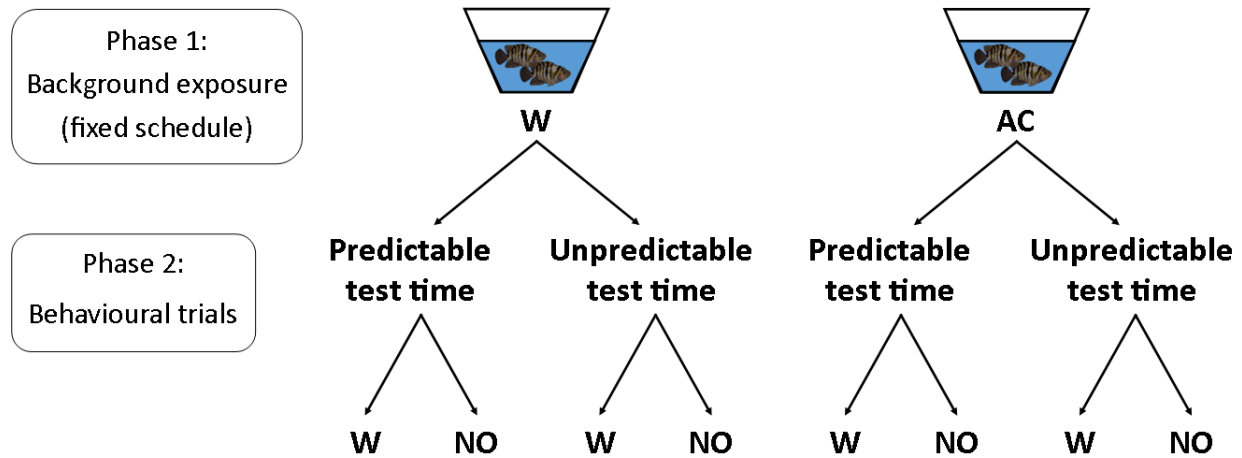
This experiment consisted of a 2 x 2 x 2 fully factorial design, whereby fish were exposed to one of two background exposure cues (water or alarm cues) in one of two schedules (fixed or random) for 3 days. After this exposure phase, the fish were tested for their responses to one of two cues (water or novel odour) at times that matched the fixed risk schedule (Figure 2.1).



**Figure 3.1. Experimental design for Experiment 2.1. Cichlids experienced background risk from exposure to water (W) or alarm cues (AC) under a fixed or random schedule (see methods for exposure details). One day after the end of the exposure period, the behaviour of cichlids was measured before and after injection of water (W) or a novel odour (NO) at times that matched the fixed risk schedule.**

*Experiment 2.2: Does the temporal predictability of risk affect the intensity of neophobic responses?*

This experiment was also a 2 x 2 x 2 fully factorial design. Cichlids that received one of the two background exposure cues (water or alarm cues) under the fixed schedule. One day after the exposure period, fish were tested for their response to one of two test cues (water or novel odour) at times that matched (i.e., predictable test time) or did not match (i.e., unpredictable test time) the fixed risk schedule (Figure 3.2).



**Figure 3.2. Experimental design for Experiment 2.2. Cichlids experienced background risk from exposure to water (W) or alarm cues (AC) under a fixed schedule. One day after the end of the exposure period, the behaviour of cichlids was measured before and after injection of water (W) or a novel odour (NO) at times that matched (predictable test time) or did not match (unpredictable test time) the fixed risk schedule.**

Both experiments were conducted simultaneously and included two phases: an exposure period followed by a test trial. The exposure period consisted of administering 15 mL of alarm cues or a dechlorinated water control in a fixed or random schedule, thrice a day for 3 days. Cichlids were exposed to the stimulus at 08:00, 13:00, and 17:00 h under the fixed schedule. For the random schedule, fish received the stimulus at random times of the day between 08:00 and 17:00 h with the proviso that each exposure is provided with a minimum of 30 minutes between consecutive exposures and the temporal patterns could not be identical over two consecutive days. Random times were determined by assigning a number to a specific hour and choosing a set of three times each day using a random number generator.

To test the effect of temporal predictability on the onset of neophobia, I exposed cichlids to 5 mL of orange odour or water 1 day after the exposure period at times that matched or did not match the fixed schedule.

#### *Exposure procedure*

Randomly selected groups of 16 cichlids were placed into a series of 24-L glass aquaria filled with 12 L of dechlorinated tap water, a gravel substrate, and a single airstone. I placed a piece of window tinting on the side of the tank facing the observer to minimize visual disturbance. Additionally, I covered the three other sides with blue opaque plastic to prevent visual communication between tanks. I also fastened a 2-m long piece of airline tubing to the airstone for injecting stimuli into the tanks. Cichlids were immediately fed with bloodworms after being transferred to the tanks and acclimated for 24 hours prior to the start of the exposure period. The cue injection process, feeding regimen, water change procedure, photoperiod, and water temperature were similar to that of Experiment 1. To optimize the number of fish used, I used lower replicates for water exposure (N = 6-8 per treatment combination) and higher replicates for alarm cue exposure (N= 15-17 per treatment combination) as I did not expect any response from the water control group.

#### *Testing procedure*

The setup of the test tanks, testing protocol, and behavioural assay for Experiment 2 was similar to that of Experiment 1. I tested a total of 198 cichlid pairs (standard length: mean  $\pm$  S.D. =  $0.97 \pm 0.21$  cm).

#### ***Statistical analysis***

Similar to Experiment 1, only calm swimming and foraging behaviours were included in the analysis and the same data manipulations were performed. For most groups, the data for proportion of time spent calmly foraging for the post-observation period met the assumptions of normality (Kolmogorov-Smirnov test,  $P > 0.1$ ) and homoscedasticity (Levene's test,  $F_{93,85} = 1.21$ ,  $P = 0.186$ ), but not for the pre-observation period data. Because the same assumptions were met as those of Experiment 1, I applied the same rationale to use a 4-way repeated measures ANOVA with Type 1 sum of squares. For Experiment 2.1, risk schedule (fixed or random), background



cue (water or alarm cues), test cue (water or novel odour), observation period (pre- or post-stimulus) and their interactions were the fixed factors. For Experiment 2.2, the background exposure cue, the test cue, the test time (predictable or unpredictable test time), the observation period and their interactions served as the fixed factors. The exposure tank was the random factor (i.e., nest) for both experiments. All analyses were performed in SPSS 25 with  $\alpha = 0.05$ .

### 3.3. Results

#### *Experiment 2.1: Does temporal risk schedule affect the onset of neophobia?*

There was no significant interaction among all fixed factors (Table 3.1). Rather, there was a background cue x observation period interaction ( $F_{1,59} = 8.79, P = 0.004$ ), indicating that cichlids decreased calm foraging more after exposure to alarm cues than to water. As expected, exposure to alarm cues created a high-risk environment in which cichlids mounted an antipredator response after receiving the test cues. However, background cue did not interact with the other fixed factors (Table 3.1). Background cue was expected to significantly interact with the other cues because exposure to water represented a procedural no-risk control whereas alarm cue exposure was high-risk. However, the lack of interaction revealed that fish perceived the water control as risky. Nonetheless, I found a significant 3-way interaction between risk schedule, test cue and observation period ( $F_{1,59} = 6.49, P = 0.013$ ) which indicated that risk schedule affected neophobia. To further investigate the response pattern of the fish across exposure groups, I looked at the effect of background cue, test cue and observation period on the fish receiving risk under a fixed schedule only and on those receiving risk on a random schedule. I failed to find a significant interaction between all three fixed factors (Table 3.2). However, for the ‘fixed’ group, background cue interacted with observation period ( $F_{1,34} = 7.67, P = 0.009$ ). This showed that cichlids did not elicit a fear response to a novel odour after repeated exposures to water, marking a non-neophobic response in the control group (Figure 3.3a). In comparison, fish showed a fear response to both water and a novel odour after alarm cue exposures which indicated that they developed a generalized fear towards disturbance (i.e., injection; Figure 3.3b). For the ‘random’ group, I did not find a background cue x observation period interaction; rather, test cue interacted with observation period ( $F_{1,25} = 8.58, P = 0.007$ ). This indicated that cichlids developed neophobia when exposed to a random schedule, regardless of background risk (Figure 3.3c-d).

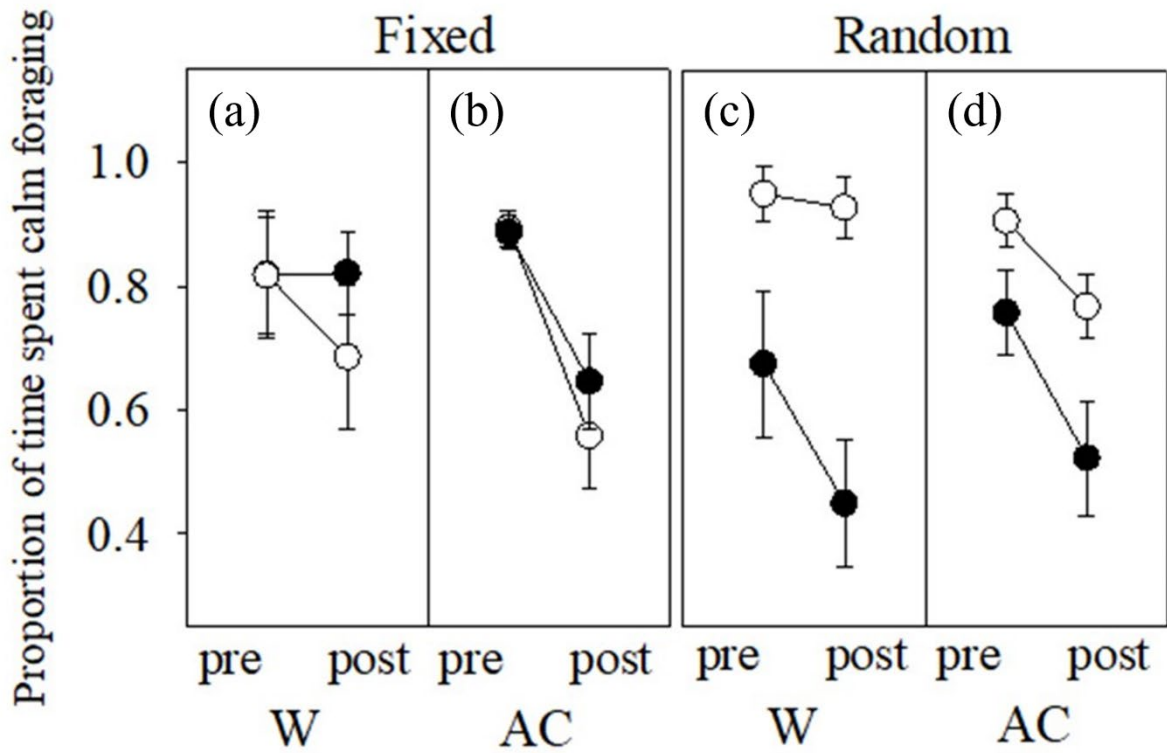


Figure 3.3. Mean  $\pm$  s.e. proportion of time spent calm foraging of cichlids before (pre) and after (post) injection of water (white circles) or a novel odour (black circles) at times that matched the fixed schedule one day after exposure to water (W) or alarm cues (AC) under a fixed or random schedule.

**Table 3.1. Repeated measures ANOVA output analyzing the effects of four fixed factors (risk schedule [fixed or random], background cue [water or alarm cues], test cue [water or novel odour] and observation period [pre- or post-stimulus]) and their interactions, with background tank as a random factor (nested design with Type I sum of squares). Bold type and asterisks represent significant terms of interest.**

Model term	<i>F</i>	<i>df</i>	<i>P</i>
Observation period	70.5	1, 59	<0.001
Risk schedule x Observation period	0.70	1, 59	0.405
Background cue x Observation period	8.79	1, 59	<b>0.004*</b>
Test cue x Observation period	0.002	1, 59	0.962
Risk schedule x Background cue x Observation period	2.79	1, 59	0.100
Risk schedule x Test cue x Observation period	6.49	1, 59	<b>0.013*</b>
Background cue x Test cue x Observation period	0.06	1, 59	0.804
Risk schedule x Background cue x Test cue x Observation period	0.54	1, 59	0.464
Tank x Observation period	1.24	20, 59	0.255

**Table 3.2. Post-hoc repeated measures ANOVA output analyzing the effect of three fixed factors (background cue [water or alarm cues], test cue [water or novel odour] and observation period [pre- or post-stimulus]) and their interactions, with background tank as a random factor (nested design with Type I sum of squares) for each risk schedule (fixed or random). Bold type and asterisks represent significant terms of interest.**

Risk Schedule	Model term	<i>F</i>	<i>df</i>	<i>P</i>
Fixed	Observation period	32.4	1, 34	<0.001
	Background cue x Observation period	7.67	1, 34	<b>0.009*</b>
	Test cue x Observation period	2.03	1, 34	0.164
	Background cue x Test cue x Observation period	0.07	1, 34	0.792
	Tank x Observation period	1.06	10, 34	0.420
Random	Observation period	59.5	1, 25	<0.001
	Background cue x Observation period	1.52	1, 25	0.229
	Test cue x Observation period	8.58	1, 25	<b>0.007*</b>
	Background cue x Test cue x Observation period	1.20	1, 25	0.283
	Tank x Observation period	2.32	10, 25	0.043

*Experiment 2.2: Does the temporal predictability of risk affect the intensity of neophobic responses?*

Similar to Experiment 2.1, no significant interaction among all fixed factors was found (Table 3.3). Test cue was expected to affect calm foraging behaviour since exposure to a novel odour should elicit a neophobic response in fish that experienced a high-risk environment (i.e., alarm cues) but not in fish that experienced a no-risk environment (i.e., water). However, test cue did not interact with any of the fixed factors (Table 3.3). This indicated that cichlids' change in calm foraging behaviour across observation periods reflected their fear response to mechanical disturbance (i.e., injection) rather than their neophobic responses. Rather, I found a background cue x risk predictability x observation period interaction ( $F_{1,78} = 11.5, P = 0.001$ ) which indicated that fear responses differed based on risk intensity and risk predictability. To further investigate this response pattern, I looked at the effect of risk predictability, test cue and observation period on the background cues separately (i.e., fish receiving water and those receiving AC). The 'water' group was expected to not render any significant interactions because it represented a

procedural no-risk environment, whereas the three fixed factors were expected to affect the calm foraging behaviour of the ‘alarm cues’ group as cichlids should be able to fine-tune their antipredator response based on the temporal patterns of risk. For the ‘alarm cues’ group, I found a risk predictability x observation period interaction ( $F_{1,25} = 1.52, P = 0.010$ ) but surprisingly, the same interaction was observed in the ‘water’ group ( $F_{1,34} = 7.67, P = 0.027$ ; Table 3.4). As mentioned in Experiment 2.1, this interaction in the ‘water’ group confirmed that exposure to water induced a risky environment. Cichlids that were tested at an unpredictable time showed a generalized fear of disturbance whereas those tested at a predictable time did not elicit a fear response (Figure 3.4a-b). On the other hand, the interaction in the ‘alarm cues’ group demonstrated that cichlids developed a stronger fear response when risk was predictable versus unpredictable (Figure 3.4c-d). This revealed that high-risk cichlids were able to keep track of the temporal patterns of risk and modulate their antipredator response accordingly.

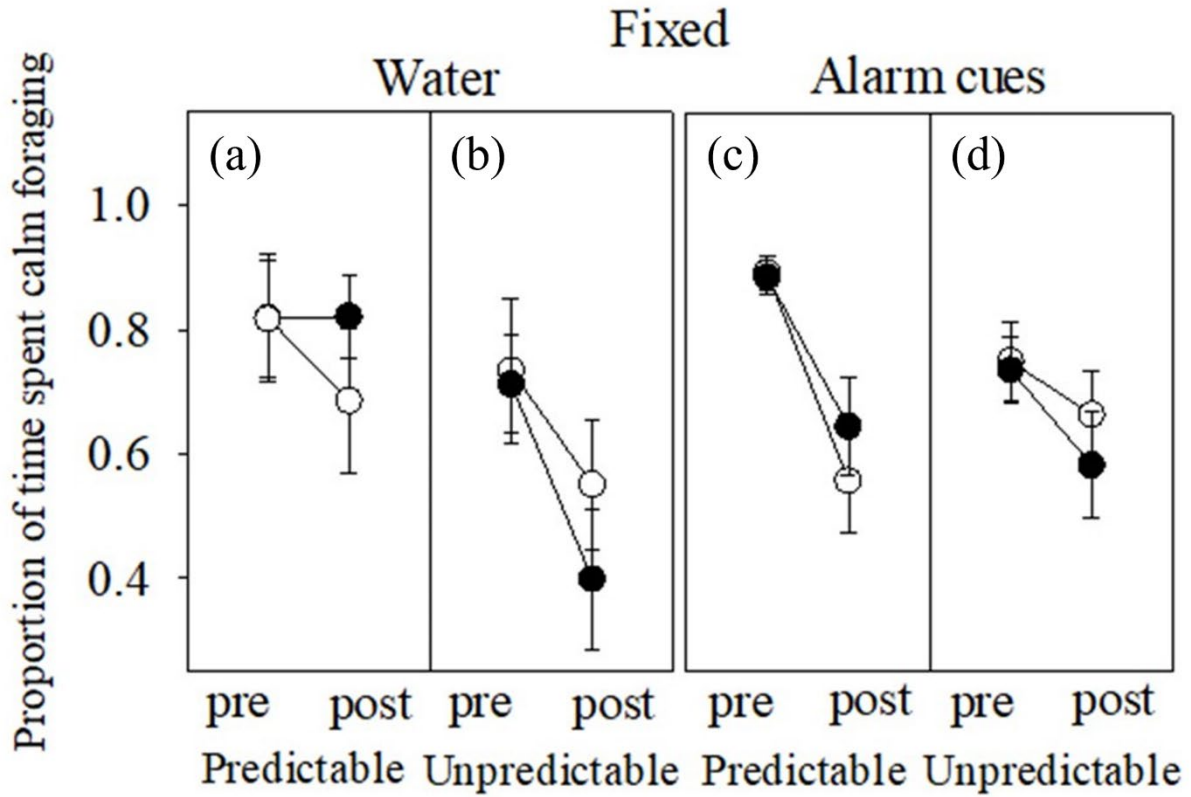


Figure 3.4. Mean  $\pm$  s.e. proportion of time spent calm foraging of cichlids before (pre) and after (post) injection of water (white circles) or a novel odour (black circles) when risk is expected (i.e., predictable) or not expected (i.e., unpredictable) one day after exposure to water or alarm cues under a fixed schedule.

**Table 3.3. Repeated measures ANOVA output analyzing the effects of four fixed factors (background cue [water or alarm cues], risk predictability [predictable or unpredictable time], test cue [water or novel odour] and observation period [pre- or post-stimulus]) and their interactions, with background tank as a random factor (nested design with Type I sum of squares). Bold type and asterisks represent significant terms of interest.**

Model term	<i>F</i>	<i>df</i>	<i>P</i>
Observation period	58.5	1, 78	<0.001
Background cue x Observation period	0.66	1, 78	0.419
Risk predictability x Observation period	0.91	1, 78	0.343
Test cue x Observation period	0.02	1, 78	0.887
Background cue x Risk predictability x Observation period	11.5	1, 78	<b>0.001*</b>
Background cue x Test cue x Observation period	0.038	1, 78	0.846
Risk predictability x Test cue x Observation period	3.86	1, 78	0.053
Background cue x Risk predictability x Test cue x Observation period	0.25	1, 78	0.619
Tank x Observation period	1.66	10, 78	0.105

**Table 3.4. Post-hoc repeated measures ANOVA output analyzing the effect of three fixed factors (risk predictability [predictable or unpredictable time], test cue [water or novel odour] and observation period [pre- or post-stimulus]) and their interactions, with background tank as a random factor (nested design with Type I sum of squares) for each background cue (water or alarm cues). Bold type and asterisks represent significant terms of interest.**

Background cue	Model term	<i>F</i>	<i>df</i>	<i>P</i>
Water	Observation period	32.4	1, 34	<0.001
	Risk predictability x Observation period	7.67	1, 34	<b>0.027*</b>
	Test cue x Observation period	2.03	1, 34	0.957
	Risk predictability x Test cue x Observation period	0.07	1, 34	0.115
	Tank x Observation period	1.06	10, 34	0.110
Alarm cues	Observation period	59.5	1, 25	<0.001
	Risk predictability x Observation period	1.52	1, 25	<b>0.010*</b>
	Test cue x Observation period	8.58	1, 25	0.797
	Risk predictability x Test cue x Observation period	1.20	1, 25	0.205
	Tank x Observation period	2.32	10, 25	0.205

### 3.4. Discussion

In Experiment 2.1, cichlids that received water under a fixed schedule did not show a fear response to a novel odour, demonstrating the behaviour of non-neophobic fish. This was expected as exposure to water under any circumstance should not simulate a presence of risk. However, fish that received water under a random schedule elicited a fear response to a novel odour. This suggests that cichlids did not necessarily perceive exposure to water as non-risky. Rather, the disturbance caused by the injection of water during the exposure period may have created a low-risk (but not ‘no-risk’) environment. Hence, the results are influenced by the effects of background risk level, as well as the uncertainty caused by the temporal variability of risk. In fish that experienced low background risk, risk schedule affected calm foraging behaviour based on the test cues. Those that experienced a fixed schedule did not develop neophobia whereas those under the random schedule developed a marked neophobic response. In contrast, risk schedule did not affect cichlids’ response to the test cues when they were exposed to a high background risk. Fish showed a decrease in their calm foraging behaviour whether they received



the water control or a novel odour, demonstrating that cichlids developed a generalized fear of disturbance (i.e., injection). Another study also failed to find an effect of risk temporal variability in cichlids exposed to high background risk, but not in low-risk fish (Ferrari et al., 2008a). Alternatively, the effect of background risk could have interfered with the timing of risk. Brown et al. (2020) explored various sources of uncertainty and found a stronger effect from risk intensity than the timing of risk. Because alarm cues only signal the presence of risk but not its source, this uncertainty compounded the effect of the stressors acting on high-risk cichlids, impeding their ability to gauge the timing of risk. Indeed, cichlids under the random schedule were neophobic compared to cichlids under the fixed schedule, regardless of background risk level. Similar findings were found in northern red-bellied dace wherein they developed stronger neophobic responses after exposure to a random as opposed to a fixed risk schedule (Brown et al., 2020). Overall, the results of this experiment indicate that risk intensity influences the effect of risk temporality on neophobia.

In Experiment 2.2, I attempted to tease apart the effect of time of testing from the effect of timing of risk on neophobia. Cichlids under the fixed schedule were tested for their response to the test cues at times when they were expecting risk to occur (i.e., predictable time) versus when they were not expecting any risk to occur (i.e., unpredictable time). While the control (water + predictable time) group did not show a marked neophobic response, fish that received water and tested at an unpredictable time elicited a fear response to both water and a novel odour, or a generalized fear of injection. This confirmed that exposure to water simulated a low-risk environment rather than the absence of risk. Similarly, alarm-cue-treated cichlids showed a generalized fear of injection regardless of testing time. However, fish tested at an unpredictable time had a weaker fear response than those tested at a predictable time (see slopes in Figure 3.4). This suggests that cichlids can keep track of predation risk within a short amount of time and mount a strong antipredator response accordingly. Indeed, lemon damselfish, a common prey in coral reef habitats, was observed to reduce foraging during the peak activity of rock cod, a common predator, across a 24-hour diel cycle (Bosiger & McCormick, 2014). Any type of disturbance during a known high-risk period may imply an imminent threat so it is best to engage in antipredator behaviour. Additionally, these results are in line with recent studies that described time-dependent antipredator behaviours within prey populations (Crane et al., 2019; Ferrari et al., 2015). Prey tend to show stronger antipredator responses in times when they are anticipating

risk as opposed to when they are not expecting any threats to occur. Although I failed to find an effect of temporal predictability on neophobia, these results highlight the importance of risk intensity and timing of risk in the ability of prey to anticipate a threat and fine-tune their antipredator response.

To investigate further methodological artefacts, I found an effect of time of testing in the random schedule group. This indicates that external disturbances heavily interfered with the effect of risk temporality. Because all exposure protocols were done in the same room, fish that received risk treatments in a random schedule were also affected by disturbances from the fixed schedule. Moreover, the absence of a predictability effect on neophobia may have been the result of a short exposure period. Ferrari et al. (2009) found that prey cannot make optimal decisions about temporal patterns of risk if the exposure period was 3 days or less. Conversely, I did not perform water changes in between exposures which may have caused cichlids to treat the three-exposure sequence as a continuous event, rather than discrete events of exposure to high predation risk. Future research should consider the relationship between predictability and intensity of risk, as well as ensure a suitable exposure period for learning the temporal patterns of risk.

Temporal threat-sensitive learning has been explored in amphibians (Crane et al., 2019; Ferrari et al., 2015) and fish (Bosiger et al., 2012; Brown et al., 2020; Ferrari et al., 2008a; Reeb, 1999). However, there is a lack of consensus regarding the ability of prey to learn to associate risk with a particular time of day. One reason could be attributed to experimental setup conditions. Because the temporal variability of predation risk is influenced by day-night oscillations of many environmental variables, investigating the effects of temporal predictability of risk on prey decision-making under constant laboratory conditions may disrupt their ability to learn temporal patterns of risk. For example, multiple laboratory studies found weak or no evidence that the temporal predictability of risk affects the behavioural response of prey, raising the question of whether predation risk can be predictable (Brown et al., 2020; Ferrari et al., 2008a). In contrast, some field studies showed that prey can learn temporal patterns of risk and adjust their antipredator behaviours based on conditions of elevated risk at a specific time of day (Bosiger et al., 2012; Crane et al., 2019). While little is known about the temporal patterns of predation that cichlids naturally encounter, we know that the circadian clock is heavily regulated by ecologically relevant environmental cues such as sunlight, food availability and temperature

cycles. Honeybees were shown to rely on sun location for nest guarding, foraging, sun-compass orientation, and dance communication (Beer & Bloch, 2020). In these experiments, the calm foraging behaviour of cichlids differed during the morning and afternoon testing. For both risk schedule treatments, cichlids exposed to a high-risk background responded with a greater intensity to the test cues in the morning than in the afternoon, and vice versa for low-risk fish. The lack of congruency in antipredator response throughout the day could be the result of the absence of temporal variation in environmental variables. Because they were fed in the morning 30 minutes prior to background cue exposure, cichlids were likely referring to food allocation for predicting risk rather than predation risk per se. Reeb's (1999) found that inangas raised in laboratory conditions were unable to associate time and place with predation risk but did so with food. While the behaviour of convict cichlids has been extensively studied in the laboratory, mesocosm and field studies seem to be more appropriate for understanding how prey compensate for increased uncertainty mediated by the temporal predictability of predation risk.

Neophobia has been postulated as an adaptive response to increased uncertainty in predation risk experienced among prey populations. When the costs of responding to uncertain information as if it were risky outweigh the costs of missing foraging or mating opportunities, prey should err on the side of caution. This model of asymmetry of costs highlights how uncertainty plays a crucial role in inducing neophobia. If prey cannot accurately assess risks due to uncertainty, they may compensate by overestimating risk and maintaining prolonged predator avoidance. Although this series of experiments failed to elucidate how the temporal predictability of risk affects neophobia, the results shed light on several factors that influence the perception of prey about temporal patterns of risk, particularly under the uniform environment of an aquarium. Indeed, an animal's environment shapes the availability and accessibility of information, hence fear acquisition may heavily depend on habitat complexity. For example, wild Trinidadian guppies from complex habitats were described as more neophobic than their counterparts from pools of lower complexity (Feyten et al., 2023). However, Crane et al. (2020b) found the opposite trend in fathead minnows raised and tested in the laboratory. Future research should consider how these factors interact and contribute to the asymmetry of costs associated with prey decision-making.

## Chapter 4: General Discussion

### 4.1. Uncertainty in predation risk as a driver of neophobia

Combined, my thesis work tested the hypothesis that uncertain predation risk drives the expression of neophobia in a well-studied model prey species. In Chapter 2, convict cichlids maintained the expression of neophobia after being exposed to the uncertainty within chemical risk cues that were less reliable than conspecific alarm cues. In Chapter 3, I failed to find an effect of temporal predictability of risk on neophobia in cichlids exposed to high background risk. However, I found that cichlids in a low background risk environment developed neophobia when exposed to a random risk schedule but not to a fixed schedule. Moreover, low-risk cichlids that experienced a fixed risk schedule showed a stronger antipredator response when a threat is unexpected versus when it is expected. Conversely, high-risk cichlids showed a generalized fear of disturbance regardless of risk predictability but elicited a weaker antipredator response when a threat is unexpected versus expected. These results highlight the complexities of investigating the question of how prey cope with increasing uncertainty and the sophisticated nature of antipredator strategies. Moreover, it offers valuable insights into future experimental design and protocols for investigating uncertainty in prey decision-making in an ecologically relevant manner.

To test the uncertainty hypothesis and its effect on the expression of neophobia, I first investigated how exposure to chemical risk cues with differing reliabilities shapes the intensity of neophobic responses. In Experiment 1, the data showed that cichlids maintained induced neophobia when exposed to conspecific alarm cues followed by heterospecific alarm cues, both from closely related and unrelated species. To date, most of the research on the onset of neophobia has been induced by highly reliable alarm cues from conspecifics. However, prey are exposed to an array of chemical risk cues either from conspecifics or heterospecifics that inform them about the level of risk in the environment; some of which are unreliable and unknown public information. My results demonstrate that prey acquire information about risk from heterospecific alarm cues. Unsurprisingly, alarm cues from a closely related species can induce the maintenance of neophobia due to the taxonomic conservation of these cues. Conversely, the lack of association between unrelated species may result in prey treating alarm cues from such species as a novel odour. Cichlids also developed a generalized fear of disturbance after being exposed to conspecific alarm cues followed by conspecific disturbance cues. This response could

be explained by two reasons. First, subsequent exposures to disturbance cues may have primed individuals to be highly sensitive to any type of disturbance as these cues serve as an early warning signal to potential threats. Alternatively, these cues are inherently ambiguous as it does not provide information about the nature and source of a threat; thus, a heightened vigilance to non-threatening cues is likely necessary to verify the credibility of the risk information. Future research should expand on these results by comparing the duration at which neophobia is maintained to confirm the reliability of these cues and, by extension, measure how uncertain prey were about the information contained within these cues. It would also be fruitful to investigate whether prey develop neophobia upon exposure to heterospecific alarm cues only.

Another factor that shapes uncertain predation risk is its predictability across temporal scales. In Chapter 3, I attempted to tease apart the effects of temporal predictability of risk on the intensity of neophobia. However, my experiments did not yield strong evidence that temporal predictability of risk affected neophobia due to the water control unintentionally creating a low-risk environment and other methodological issues. Nonetheless, Experiment 2.1 showed that low-risk cichlids developed a marked neophobic response after exposure to a random risk schedule but not when exposed to a fixed schedule, whereas high-risk fish showed a fear response regardless of risk schedule. Moreover, as shown in Experiment 2.2, low-risk fish showed a marked antipredator response against disturbance when a threat is unexpected. On the other hand, high-risk fish engaged in antipredator behaviour regardless of risk predictability but showed a weaker response when risk was unexpected versus expected. Overall, I failed to show an effect of predictability in high-risk cichlids, but predictability affected the behavioural response of low-risk fish. These results suggested that risk intensity plays a role in how prey cope with the uncertainty associated with the temporal variability of risk, influencing the effect of risk predictability on neophobia and antipredator behaviour in general. Prey experiencing low predation risk have the luxury of being more selective of when to invest effort into predator avoidance, whereas those under high-risk conditions remain vigilant as any disturbance or cue could potentially be a threat. While it is difficult to conclude the implications of these experiments on the uncertainty hypothesis, future researchers are encouraged to test the effect of temporal predictability of risk in semi-natural conditions to ensure that abiotic environmental cues that shape how prey perceive temporality are present.

Studying the effect of temporal predictability of risk on neophobia to understand uncertain predation risk proved to be extremely complex. These experiments were initially designed for semi-natural conditions with wild-caught prey but were conducted under laboratory conditions due to unprecedented circumstances. As a result, abiotic environmental variables that shape the circadian rhythm of several prey species and, by extension, their ability to predict temporal patterns of risk were largely overlooked. The lack of variability in water temperature, food availability and light positioning in laboratory settings may be a crucial factor in explaining the conflicting evidence associated with studying the temporality of risk. Moreover, this highlights the idea that the uniformity of laboratory conditions may not be appropriate for investigating this issue. However, it is undeniable that laboratory studies are critical for testing the breadth and feasibility of a theory. If this experiment were to be replicated under laboratory conditions, prey must be given a longer background exposure period to allow for adequate acclimation to the temporal patterns of risk and to ensure that the test species can predict these patterns. Additionally, it is important to note that the experiments were conducted in the same room. Prey may have associated external disturbances with risk and incorporated them into how they perceived risk temporality. This may explain the effect of time of testing on cichlids that received risk under a random schedule. Consequently, it likely dampened the effect of the background cues on risk predictability. Thus, separate experimental rooms during the background exposure phase should be considered to minimize external disturbances, especially for the 'random' risk group. That said, researchers should ensure a similar setup and room conditions (e.g., lighting, room temperature) to avoid potential confounds.

Throughout an individual's life history, prey may encounter several novel environments in which risk information is not readily available. They must deal with the uncertainty inherent in these environments to perform an optimal antipredator strategy (Dall, 2010; Schmidt et al., 2010). While they can sample information to learn more about the environment, the asymmetry in the costs of engaging in learning is biased towards injury or death (Crane et al., 2020a; Dall & Johnstone, 2002). Moreover, increased uncertainty about risk can lead to more decision errors (Trimmer et al., 2011). Consequently, prey may exhibit neophobia as a short-term adaptive strategy to uncertain predation risk. My results suggest that uncertainty-induced neophobia may have underlying mechanisms that must be addressed to isolate the effects of chemical risk cue reliability and the temporal predictability of risk. Future researchers should assess the interacting

effects of these factors on the maintenance and retention of neophobia. Feyten & Brown (2018) postulated that the predictability of risk and reliability of risk cues decouple the physiological correlates of fear (i.e., stress hormone response) from vigilance behaviour, resulting in variable and context-dependent antipredator strategies to cope with uncertainty of risk. For example, prey may increase vigilance but maintain a tempered stress response upon encountering known or reliable risk cues in a predictable but high-risk predation environment. Indeed, a low-stress response in the face of uncertain predation risk may encourage information gathering to identify potential risks and rewards (Brown & Godin, 2023; Parsons et al., 2018). Hence, changes in physiological correlates of antipredator behaviour induced by uncertain predation risk may regulate the capacity of prey to mount prolonged vigilance to novel stimuli. This thesis highlights the complexity of studying uncertainty and the sophistication of prey decision-making within the context of neophobia, marking it as ripe for further investigation.

#### **4.2. Conservation applications of the effects of uncertainty on antipredator decisions**

As ecological uncertainty is predicted to increase from the combined effects of climate change, anthropogenic disturbances, and invasive species, understanding the drivers and consequences of uncertain predation risk becomes an increasingly pressing issue for ecologists and conservation biologists. In the age of the Anthropocene, disturbances such as urbanisation and traffic noise, habitat destruction, episodic turbidity and acidity, and light and chemical pollution can contribute to the loss of public information (Crane et al., 2023b). These anthropogenic changes can exacerbate the degree of uncertainty that animals experience. Consequently, it can alter the decision rules and outcomes related to risk assessment and avoidance wherein behavioural errors are more likely to occur and may rapidly lead to extirpation.

An important outcome of anthropogenic change is the increased exposure to novelty. Recent models predict that hundreds of thousands of new and first encounters between species are likely to occur because of climate change (Carlson et al., 2022; Thornton et al., 2014); many of which can become invasive predators of native prey. Thus, the ability of prey to mount an antipredator response to novel predation threats has importance in the context of invasive species biology. As habitat loss/fragmentation and habitat shifts become more rampant, the issue of invasive species becomes more widespread. Understanding the behavioural context and

antipredator strategies between invasive and native species provides useful insight into mitigation efforts to control this issue. It is known that invasive species tend to have lower levels of neophobia than native species. However, invasive prey are tasked to navigate increased uncertainty as they march through the process of invasion and establishment within a novel environment. For example, the success of invasive Italian wall lizard can be attributed to their higher capacity for behavioural plasticity in exploratory behaviour, boldness and neophobia compared to their native counterparts, the green Iberian wall lizard (Damas-Moreira et al., 2019). Thus, it is worth investigating the drivers of neophobia in the face of uncertainty to improve our understanding and mitigation of the success of invasive species.

Although predicting such outcomes is often difficult, incorporating how uncertainty, information reliability and intensity of predation pressure impact behavioural decisions into models of population dynamics is essential in producing models that reflect the variability and stochasticity of predator-prey relationships (Brown & Godin, 2023). It will shed light on how multiple anthropogenic stressors interact and determine the cognitive and physical limitations of prey decision-making and, in turn, allow us to develop informed conservation and management policies. For example, re-introduction efforts may benefit from inducing neophobia in captive animals before their release (Crane et al., 2020a). Prey that will be exposed to a diverse set of predators across multiple time periods may have better chances of surviving if they were neophobic at the time of release. Moreover, prey may benefit from mounting a neophobic phenotype as a short-term strategy as they navigate their way through establishment within a certain region. Investigating the role of neophobia in a changing world can help us comprehend how it shapes the ecology of predator-prey interactions and allows us to enact effective solutions for the effects of human-induced rapid environmental change.



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