

Anti-predator response and neophobia in juvenile bluegill sunfish (*Lepomis macrochirus*) across an environmental gradient.

Annick Singh

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By: Annick Singh

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Signed by the final examining committee:

_____ Chair
Patrick Gulick

_____ Examiner
Dylan Fraser

_____ Examiner
Emma Despland

_____ External Examiner
Selvadurai Dayanandan

_____ Thesis Supervisor
Grant Brown

Approved by _____
Robert Weladji

_____ 2020 _____
André G. Roy

Abstract

Anti-predator response and neophobia in juvenile bluegill sunfish (*Lepomis macrochirus*) across an environmental gradient.

Annick Singh

Microhabitats with high structural complexity can impede visual information of local predation threats. This can shape individual behaviour, resulting in more fearful behavioural phenotypes within complex microhabitats. In this experiment, I explored the impact of environmental complexity on shaping individual predator avoidance behaviour. I observed the behaviour of bluegill sunfish (*Lepomis macrochirus*) along an environmental gradient. I predicted that as visual complexity of the environment increases, antipredator and neophobic response also increases. I conducted *in situ* observations at Lake Opinicon, where I presented bluegill sunfish shoals with a novel object and one of three different chemosensory stimuli (water, alarm cue or novel odour). Multiple regression analysis found that the strongest predictor, in shaping predator avoidance behaviour, is vegetation diversity. Bluegill sunfish exposed to conspecific alarm cues exhibited stronger avoidance responses under higher diversity patches. Additionally, bluegill sunfish were not neophobic to neither the novel object nor the novel stimuli. Other environmental factors, such as the ratio of open area to visually obscured area, height of vegetation, and depth of the water were not significant predictors of behavioural response. Bluegill sunfish, under elevated risk of predation, have previously been observed to seek refuge in areas of high vegetation, avoiding open, low complexity patches. Instead, I found that in this population of bluegill sunfish, response was not associated with the amount of vegetation within a patch, but more so with an increase in vegetation diversity. Areas with higher vegetation diversity can contain plant species of different spatial structures (broad leaved plants vs thin leaves), potentially obscuring more visual information to the individual. This high diversity may increase visual obscurity in this system, leading prey to respond to conspecific alarm cues with a strong antipredator response.

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Table of Contents

List of Figures.....	vi
List of Tables.....	vii
Introduction.....	1
Methods.....	5
<i>Study Site</i>	5
<i>Chemical Cues</i>	6
<i>Novel Model</i>	6
<i>Behaviour Recordings</i>	7
<i>Environmental Data</i>	7
<i>Behaviour Assay</i>	8
<i>Statistical Analysis</i>	8
Results.....	9
Discussion.....	12
References.....	29
Appendix.....	36

List of Figures

Figure 1 : Number of individuals present around a novel object (square root transformed), before a cue was presented as a function of open score.....	19
Figure 2 : Number of individuals present around a novel object (square root transformed), before a cue was presented as a function of Shannon diversity index of the surrounding vegetation.....	20
Figure 3 : Difference of individuals present around a novel object (Yeo-Johnson transformation after a cue was presented as a function of Shannon diversity index of the surrounding vegetation.....	21
Figure 4 : The difference in individuals present near a novel object (Yeo-Johnson transformation) as a factor of open score, Shannon index, cue and the interaction between open score and cue.....	22

List of Tables

Table 1 : Formula of regression slopes for figure 4.....	23
Table 2 : ANOVA output of the number bluegill sunfish present around the novel object (square root transformed) before cue was presented as a function of the open score.....	24
Table 3 : ANOVA output of the number bluegill sunfish present around the novel object (square root transformed) before cue was presented as a function of Shannon index.....	25
Table 4 : Regression output of the difference in number of bluegill sunfish present around the novel object (Yeo-Johnson transformed) as a dependant variable to Shannon index, cue, open score, depth, average height of vegetation and AIC scores.....	26
Table 5 : The relationship between the difference in the number of individuals present around the novel object (Yeo-Johnson transformed) with Shannon index and stimuli presented.....	27
Table 6 : The relationship between number of individuals present around the novel object (Yeo-Johnson transformed) with Shannon index and the interaction between cue and open score.....	28

Introduction

Prey animals are continually faced with the often-conflicting demands of predator avoidance and other fitness related activities such as foraging (Lima & Dill 1990). Choosing appropriate microhabitats (defined as the fine-scale variation in habitats), can impact these fitness related trade-offs (Godin 1997). This entails choosing adequate foraging sites, areas of shelter, and refuge from predators (Godvik et al 2009). Choosing optimal habitats can be costly, due to the large variation in spatial structure (Newton 1991, Valencia et al. 2004, Lima et al. 1987), foraging opportunities (Ehlinger 1989, Zeifel-Schielly et al. 2009), and predation risks (Valeix et al. 2009, Andruskiw et al. 2008). Additionally, habitats can vary temporally in both abiotic and biotic conditions including vegetation (Fryxell et al. 2005), light (Gilbert & Boutin 1991), and predators (Creel & Winnie Jr 2005), all of which can shift daily or seasonally. Such variation in microhabitat structure and complexity is expected to shape the behavioural decisions of prey (Crane et al. 2020). Depending on their choice of habitat types, prey may be faced with additional cost–reward trade-offs. Elk (*Cervus elaphus*), for example, choose different foraging grounds, depending on perceived predation risk. In the presence of predators, or high perceived predation risk, they have been observed to reduce foraging in productive grassy meadows and shift to the protective cover of less productive forests, trading foraging opportunities to minimize mortality (Creel & Winnie Jr 2005).

In addition to shaping foraging tactics across broad habitat scales, predation risk also affects prey microhabitat selection (Miner & Stein 1996, Jordan et al 1995). Predation risk assessment, within fish communities, is commonly achieved through a combination of chemosensory and visual cues (Brown 2003, Meager et al. 2006, Kelley & Magurran 2003, Canfield & Rose 1996, Fay & Tavalga 2012). Juvenile common roach (*Rutilus rutilus*), for example, have been observed to choose different microhabitats depending on the type of cue they receive. Juvenile roach respond differently to the visual

cue and olfactory cue of the same predator. They respond to the olfactory cue of an ambush predator, the Northern pike (*Esox lucius*), by selecting open-water habitat, but choose to enter structured habitat when presented with a visual cue of the same predator (Martin et al. 2010). Similarly, when exposed to the olfactory cues of a pursuit predator (yellow perch; *Perca fluviatilis*), the common roach chooses structured habitats. However, when presented with a perch visual cue, they switch to open water (Martin et al. 2010). Juvenile roach's habitat choice depends, not only on predator presence, but also on that specific predator's foraging strategies and the sensory modality by which the threat is perceived. This example shows that both information and sensory channels affect microhabitat choice. Similarly, bottlenose dolphin (*Tursiops aduncus*), have been observed to avoid foraging in productive shallow waters during warm months of the year, when predator density is highest. Echolocation efficiency decreases in shallow water, and visual detection of predators is impaired by the seagrass present in shallower waters (Heithaus & Dill 2002). Dolphins may perceive shallow waters as a high-quality foraging site, but recognize that the structure and nature of this environmental characteristic carries a level of risk. This risk is generated by impeding predator detection, through the blocking of information channels, causing dolphins to trade a productive foraging site in favor of reducing mortality risk (Heithaus & Dill 2002). Therefore, habitat choice is affected by both predation risk and the types of information channels available for exploitation in each microhabitat.

Prey species integrate information received from multiple channels. The information they receive from these cues, shapes the prey's behaviour and allows them to respond appropriately to predation threats (Brown 2003). Guppies (*Poecilia reticulata*), after being exposed to alarm cue, become more attentive to visual cues. This increases their information acquisition through visual modalities, and improves the accuracy of their overall risk assessment (Stephenson 2016). Therefore, environments rich with visually obscuring structures, lead to stronger responses to olfactory stimuli, than less structured environments. An example of this can be found in the damselfish (*Pomacentrus amboinensis*), which

display stronger antipredator avoidance, in the presence of conspecific damage-released alarm cues, a known indicator of risk, while in a dense microhabitat than in an open microhabitat (McCormick & Lönnstedt 2013). Similar response patterns have been observed, in a natural setting, in pumpkinseed sunfish (*Lepomis gibbosus*; Golub et al. 2005), showing that microhabitat complexity limits available visual cues, and that there is an adaptive behavioural response from individuals to compensate for this potential loss of information. Such heightened fear responses in structured microhabitats, indicate that prey consider certain microhabitat characteristics to be situations of heightened risk, especially when those characteristics increase uncertainty, due to restricted information acquisition and reduced predator detection (Feyten and Brown 2018; Crane et al. 2020).

In such situations of increased uncertainty, generalized neophobia (broadly defined as the avoidance of novel stimuli; Crane & Ferrari 2017) can be an adaptive strategy to reduce fitness costs for individuals. Prey are exposed to information that varies greatly in reliability, ranging from known (such as conspecific alarm cues or learned information; Ferrari et al. 2010) to unknown (novel) cues (Brown et al. 2013). Responding to novel information can lead individuals to novel foraging patches, allowing them to explore for beneficial resources in low risk conditions. Contrarily, novel stimuli may also, in the context of high risk, signal novel predators (Ferrari et al. 2015) or toxic food sources (Greenberg & Mettke-Hofmann 2001, Mettler & Shivik 2007). Neophobia is expected to aid in reducing the costs associated with having to directly acquire information about the potential value of new microhabitats (Greenberg 2003, Visalbergi & Addessi 2000). Neophobia is a phenotypically plastic response, (Joyce et al. 2016, Webster & Lefebvre 2000) and is observed in multiple species including cane toads (*Rhinella marina*; Candler & Bernal 2014), warblers (*Dendroica pensylvanica*; Greenberg 1983), corvids (*Corvus frugilegus*; Greggor et al. 2016), Nile tilapia (*Oreochromis niloticus*; Champneys et al. 2018), daffodil cichlids (*Neolamprologus pulcher*; Bannier et al. 2017), and capuchins (*Cebus apella*; Visalberghi et al. 2003). Recently, Feyten & Brown (unpublished data) have observed that Trinidadian guppies exhibit

strong neophobic predator avoidance and foraging patterns, as substrate complexity within discrete habitat pools increases. Additionally, Crane et al. (2020) have observed neophobic behaviour in fathead minnows (*Pimephales promelas*) as a response to environmental complexity. Minnows exhibit a neophobic response to odours in visually complex environments, when previously conditioned with high background risk. Both low-risk and high-risk conditioned minnows, however, show a neophobic response in simple environments. Crane et al. (2020) hypothesized that visually obscuring vegetation may be perceived as beneficial protection from predators by minnows. Open environments are then considered risky, shaping minnow's behaviour to reflect the situation. The neophobic response to novel odour in low background risk, in simple environments, indicates that simple environments may be deemed as situations of acute risk. Simple environments, that do not confer protection against predation, shift the individual's perception of low background risk to a situation of acute risk, meriting a neophobic response to a novel odor. Therefore, background risk, and environmental complexity both shape individual neophobic behaviour in minnows. The observed effects of microhabitat complexity, which can potentially restrict information (i.e. increased uncertainty) and resulting patterns of neophobia, may be difficult to predict.

In this experiment, I test the effects of microhabitat complexity on the ability of juvenile bluegill sunfish (*Lepomis macrochirus*) to assess acute predation risks, based on chemosensory cues. Microhabitat complexity holds the potential to limit information acquisition, preventing individuals from relying on visual cues when attempting to assess local acute predation threats, or to consolidate information from multiple sensory modalities, when examining novel stimuli. Exploring the combination of information acquisition, under the pretence of risk and antipredator response in complex environments, I test the hypothesis that increasing habitat complexity increases antipredator (response to alarm cue odour) and neophobic (response to a novel odour) responses in prey species. I predict an increased antipredator response to alarm cue as visual information decreases with increasing

environmental complexity. Similarly, I predict an increase in neophobic response as complexity increases, due to the reduced ability of prey to detect acute threats. I observed bluegill sunfish in Lake Opinicon at the Queen's University Biological station (QUBS) and quantified the response intensity towards known and novel chemical cues (paired with a novel visual cue). And quantified the microhabitat complexity at the observation point, to test the predicted links between habitat complexity and the strength of neophobic and antipredator responses. With the increasing impact of anthropogenic effects on ecological systems, neophobia can be a beneficial behaviour trait. Potentially allowing prey to circumvent costly interactions within uncertain conditions. Additionally, with the rise of invasive species, understanding how prey behave in the presence of novel individuals or predators, is of imperative interest to conservation practitioners (Ferrari et al. 2015). Understanding the role of neophobia in predator-prey interactions can allow future conservation efforts to employ neophobia to condition prey for potential invasions, which may minimize the effect novel predators have on native prey species.

Methods

Study Site

Experimental trials on bluegill sunfish were performed at Queen's University Biological Station at Lake Opinicon. Behavioural assays were conducted along two shorelines: Keast Beach and Stumpy Bay. Keast Beach is characterized by its more open habitat and a population of Eel grass (*Zostera marina*). Stumpy Bay, conversely, contained a higher density of vegetation and a substantially higher variety species present including Eel grass (*Vallisneria americana*), Common waterweed (*Elodea canadensis*), Broad-leaved pondweed (*Potamogeton amplifolius*), Milfoil (*Myriophyllum sibiricum*), Reeds, Sago (*Stuckenia pectinate*), Slender Water-Nymph (*Najas flexilis*), and floating leaved pondweed (*Potamogeton natans*). Additionally, the substrate was siltier at Keast Beach, compared to Stumpy Bay. Lake Opinicon contains several species of predators, that are expected to forage on juvenile bluegill

including largemouth bass (*Micropterus salmoides*), smallmouth bass (*Micropterus dolomieu*), northern pike (*Esox lucius*), yellow perch (*Perca flavescens*) and brown bullhead (*Ameiurus nebulosus*).

Behavioural trials were performed from May 23, 2018 to July 16, 2018.

Chemical Cues

Alarm cue was obtained from captured juvenile bluegill sunfish. Bluegill sunfish were captured using Gee's minnow trap baited with 'No Name'TM dog food. Captured bluegill sunfish were taken from the minnow trap and placed into a 19-liter bucket of lake water. Individuals were euthanized with a quick blow to the head in accordance with Concordia University Animal Care Protocol (AREC 30000255). Skin fillets were obtained from both sides of each euthanized individual. Filets were held in a beaker on ice. Using a hand blender, the filets were homogenized with lake water. The mixture was filtered using polyester floss to remove large debris and subsequently diluted with lake water to a concentration of $\sim 0.15 \text{ cm}^2 \text{ ml}^{-1}$, which has previously shown to elicit an antipredator response (Chivers et al. 2012, Brown et al. 2014, Golub et al. 2005). A total 92.4 cm^2 of bluegill sunfish skin extract was obtained and combined with lake water to create 616 ml of alarm cue solution. Novel odour was generated by combining 10 ml of 'No Name' lemon extract and 500 ml of lake water. Chemical cues were frozen and stored in 40 ml aliquots. Previous studies with Trinidadian guppies have shown reliable neophobic responses to lemon odour at these concentrations (Elvidge et al. 2016). Samples were taken to the field site on ice and only thawed immediately before dispensing in a behaviour trial.

Novel Model

I created a novel model using 6.35 mm diameter dowels. The dowels were cut into two pieces, one was a meter long while the other was 30 cm long. The dowels crossed at the 50 cm mark of the meter-long dowel and at 10 cm of the shorter dowel. Both sides of the 30 cm dowel were sharpened to a point, the shorter end was used to penetrate the substrate during experimental trials. When placed in

the ground, the longer dowel was parallel to the substrate and the shorter one was perpendicular. The other sharpened point was used to place a generic orange hockey ball at the end. Dowels were also marked at 2.5 cm intervals using a black sharpie to measure the length of individuals interacting with the object.

Behaviour Recordings

Observations took place between 9am to 6pm during sunny days. No observations took place in the rain, which creates a situation of higher than normal turbidity. I would slowly swim around the shoreline to locate shoals of the focal species. Once a shoal of bluegill sunfish was located, the novel model was placed into the substrate ([Image 1](#) and [Image 2](#)). I placed a GoPro Hero 4 one meter away from the model. To discourage the shoal from interacting with the camera instead of the novel object I was positioned right above the camera. After the introduction of the novel object and camera, a three-minute acclimation period elapsed before behaviour observations occurred. Following this, the camera was turned on and behaviour was recorded for 10 minutes. After the initial five minutes of the trial elapsed, I introduced 20 ml of the stimulus through an airline tubing that runs through to the novel object, and subsequently flushed it with 60 ml of lake water.

Environmental Data

Once the trial ended, I began collecting environmental data. The trial area was a 1 m² subdivided into 10 cm squares. Each square was defined by its predominant environmental characteristic. A grid was constructed with 100 squares representing each 10 cm area. The squares were defined as either: silt, cobble, algae, vegetation taller than 5 cm or vegetation shorter than 5 cm. The species of vegetation was also recorded for each quadrant where vegetation is present. Using this grid, an open score was given to each trial. The open score ranges from 0 to 100 where 0 represents an environment with vegetation in each square. A score of 100 represents an area with no vegetation in each square.

Additionally, vegetation present in each square was recorded, allowing to extrapolate percent cover of each species, as well as Shannon index.

To identify the vegetation in the lake, samples were taken post trial of unidentified vegetation. They were uprooted and placed in a plant press. Finally, a Shannon index score was calculated for every trial using the identified species and the relative frequency of each. Shannon index scores were calculated by the proportion the species covering the test area multiplied by the natural log of that proportion. Calculated as follows: $H = -\sum(p_i \times \ln p_i)$. Where p_i represents the proportion of the area the vegetation was found within the trial area.

Behaviour Assay

Videos were later analyzed by a single viewer. All cue and environmental data were kept blind to prevent viewer bias. Over the 10-minute period the number of individuals interacting with the constructed model was recorded. When an individual interacts with the model, the part of the model is recorded (object, vertical or horizontal dowel) and the approximate size of the individual. Additionally, the number of individuals within the frame were counted in 15 second intervals during a two-minute period both pre and post stimulus injection.

Statistical Analysis

I analyzed the data using R version 3.6.1 and Rstudio 1.1.442. Final analysis consisted of 60 total observations, 20 observations for each cue. The average number of individuals present within a two-minute period was calculated using the data obtained from behavioural observations. From this the difference between post and the pre-stimulus period was used as the dependant variable in a multiple regression analysis. To meet the assumptions of a regression analysis, the difference in the number of bluegill sunfish present (post-stimulus – before stimulus was introduced) underwent a Yeo-Johnson

transformation. After transformation, data were analysed with a global procedure described by Pena & Slate (2006), and was not skewed ($\hat{S}_1^2 = 0.39$, $df = 4$, $p = 0.53$), met the assumptions of kurtosis ($\hat{S}_2^2 = 1.16$, $df = 4$, $p = 0.28$) for a normal distribution, did not violate link function assumptions ($\hat{S}_3^2 = 1.94$, $df = 4$, $p = 0.16$), and was homoscedastic ($\hat{S}_4^2 = 0.22$, $df = 4$, $p = 0.64$).

I performed a linear multiple regression, with the transformed difference of individuals as the dependant variable to vegetation height, depth, open score, Shannon index, cue and possible interactions between environmental variables.

I generated two single linear regressions, the first observing open score and cue as predictors, the second with Shannon index and cue as predictors. Shannon index, cue and open score were found to be notable predictors to change bluegill sunfish behaviour after cue introduction and were analyzed with a linear model to see if individual behaviour were affected by these variables.

Results

I tested baseline differences (pre-stimulus) in number of bluegill sunfish present with open score and cue as predictors. No environmental predictor was found to significantly affect bluegill sunfish behaviour prior to the introduction of stimulus. Neither cue ($F = 0.89$, $df = (2, 54)$, $p = 0.42$) nor open score ($F = 0.0048$, $df = (1, 54)$, $p = 0.95$) was significant ([Figure 1, Table 2](#)), suggesting there was no difference in number of sunfish present prior to stimulus injection, across the gradient of habitats based on open score. I found similar patterns with regards to the vegetation diversity as a predictor to number of bluegill sunfish present before stimulus was introduced ($F = 1.42$, $df = (1, 54)$, $p = 0.24$) and cue ($F = 0.53$, $df = (2, 54)$, $p = 0.59$) ([Figure 2, Table 3](#)). My analysis suggests that bluegill sunfish behaviour was similar in each treatment prior to introduction of any cue.

Following that, a multiple regression analysis (Multiple R-squared = 0.37, adjusted R-squared = 0.29, df = 53, $p < 0.01$) found that the open score ($p = 0.36$), height of vegetation ($p = 0.20$), and depth ($p = 0.94$) were not significant predictors in describing the difference in the number of bluegill sunfish present ([Table 4](#)). I performed a step wise AIC reduction which removed vegetation height, open score and depth as predictors (multiple R-squared = 0.37, adjusted R-squared = 0.22, p-value < 0.01) ([Table 4](#), [Table 5](#)). Shannon index ($p < 0.001$) and cue ($p < 0.001$) were found to be significant predictors after model reduction. Of all the models created, including all combinations of independent variables, and possible interactions between diversity, cue, depth, vegetation height and open score, Shannon index and cue were always significant predictors. All models created underwent a step wise AIC reduction, and found that Shannon index and cue were the two predictors that explained bluegill sunfish behaviour. The response to water and novel odour appear significantly different, from the response to alarm cue ($p < 0.001$ for both) ([Figure 3](#), [Table 5](#)). My results indicate that bluegill sunfish were avoiding areas where alarm cue was present, but not where water or lemon odour were present. Contrary to my predictions, bluegill sunfish do not respond to the amount of vegetation, but instead to the diversity of vegetation in the area. Additionally, bluegill sunfish exhibit the same behaviour in the presence of novel odour as they do to our control, lake water. This indicates the absence of neophobic behaviour in this population when responding to environmental complexity. I found that Shannon index before step wise reduction was significant ($p = 0.04$, [Table 4](#)) and remained significant after reduction of non-explanatory variables ($p < 0.01$, [Table 5](#)). Overall, the general trend was a decrease in number of bluegill sunfish present as vegetation diversity increased ([Figure 3](#)). The slopes for all curves were equivalent but differing in their intercepts ([Figure 1 caption](#)). This visualises the decrease in number of bluegill sunfish, which was greatest in response to alarm cue (compared to novel odour and water), and the y-intercept for novel odour and water were similar to one another. Given this, difference in individuals between post- and

pre-stimulus decreases as microenvironment diversity increases. As the Shannon diversity index increased around the novel object, fewer fish were present after the introduction of a cue.

I found the interaction between open score and cue were trending towards significance ($p = 0.07$) during analysis. To visualize this trend, an additional multiple regression model was created (multiple R-squared = 0.36, adjusted R-squared = 0.24, p -value < 0.01) with open score, diversity, cue and the interaction between open score and cue as predictors ([Figure 4](#), [Table 6](#)). In this model, novel odor was not considered significantly different to alarm cue ($p = 0.94$), nor was water significantly different to alarm cue ($p = 0.58$). The interaction between open score and novel odor was not significantly different to the interaction of open score and alarm cue ($p = 0.25$), and neither is the interaction between water and open score ($p = 0.07$). Finally, open score is not significant ($p = 0.29$). Shannon index, however, was still a significant predictor in affecting the number of bluegill sunfish present around the novel object ($p < 0.01$, [Table 6](#)). Four regression curves were generated for each cue when observing the difference in individuals as a dependant variable, to open score as a predictor ([Figure 4](#)). Each curve represents a different Shannon index to visualize the relationship between cue, open score and Shannon index to the difference in individuals present (pre- post stimulus observations). In general, all slopes were relatively neutral, and the number of bluegill sunfish present after alarm cue was introduced decreased as the environment became more open. Additionally, the number of bluegill sunfish decreased, as Shannon index increased after alarm cue was introduced. The same trends towards Shannon index was observed with water and novel odour, indicating that bluegill sunfish have an aversion to diversity of vegetation present around the object. However, the number of bluegill sunfish present after introduction of water or novel odour increased as open score increased, opposite of the trend found when alarm cue was introduced. Overall, as diversity around the novel object increased, fewer fish were present around the novel object. Trends were observed with open score and cue, but they were not considered significant predictors in this interaction plot ([Table 1](#), [Table 6](#)).

Discussion

My results suggest that bluegill sunfish in Lake Opinicon, show no signs of neophobia, regardless of the environmental gradient of the shoreline. The number of bluegill sunfish present near the novel object did not change in response to either the novel odour or a water control. However, when conspecific alarm cue was introduced, there was a significant decrease in the number of bluegill sunfish present. Bluegill sunfish tended to avoid areas in which they detected alarm cues, consistent with previous reports with centrarchids (Golub et al. 2005, Marcus & Brown 2003), brook charr (*Salvelinus fontinalis*), brown trout (*Salmo trutta*), rainbow trout (*Oncorhynchus mykiss*; Mirza & Chivers 2001), zebra danios (*Danio rerio*; Korpi & Wisenden 2001), Trinidadian guppies (Brown & Godin 1999), convict cichlids (*Amatitlania nigrofasciata*; Pollock et al. 2005) and other aquatic species (Friesen & Chivers 2006).

The number of individuals present, after exposure to alarm cue, decreased with increasing diversity in vegetation. This suggests that prey have a stronger antipredator response to alarm cue when aquatic plant diversity is high (i.e. higher microhabitat complexity). The amount of the vegetation around the object did not have a significant effect as a predictor, contrary to my original hypothesis. Previously, Gotceitas and Colgan (1990) have observed that juvenile bluegills prefer dense vegetation, when a predator is nearby. Similarly, convict cichlids choose complex habitats in the presence of a predator (Church and Grant 2018). My results add to this body of research, suggesting that diversity of vegetation within a microhabitat patch may also contribute to its perceived complexity by prey. If patch quality is heterogenous within this system, then individual bluegill sunfish might benefit from foraging in areas with diverse vegetation because of the added protection from predation events.

Many prey species prefer more complex habitats, or areas with cover during predation events. Additionally, lowered success rate of predators finding prey, is observed across a variety of taxa in

complex habitats (Mattila 1991, Almany 2004, Gotceitas & Brown 1992, Martin et al. 2010, Werner & Hall 1988, Russo 1987, Hovel & Lipcius 2001, Warfe & Barmuta 2004). Lesser treefrog tadpoles (*Dendropsophus minutus*) and lanceback treefrog tadpoles (*Scinax curicica*) for example, are under a lower risk of predation in environments containing vegetation, than in environments with no vegetation (Kopp et al. 2006). Additionally, McCormick and Lonnstedt (2013) have found a similar proportional trend to increasing complexity. When complexity increases, antipredation response is still apparent but reduced compared to low complexity patches. Bluegill sunfish in this lake system may consider less open environments (i.e. more structured or complex) as areas with lower acute risk, that confer a level of protection against a predation event. This runs contrary to previous work in sunfish. For example, Golub et al. (2005) found that pumpkinseed sunfish have an equivalent response to conspecific alarm cue across all environmental contexts. However direct comparisons of these results to establish a trend in sunfish behaviour is difficult, as I found methodological differences between this experiment and the Golub et al. (2005) study. In that study, habitat was measured categorically (defined as low, intermediate, or high complexity), instead of the continuous measurement used in this study. Additionally, the study site was described to contain only four dominant plant species. Additionally, two species of sunfish predators were recorded, it is likely that pumpkinseed sunfish are under a stronger and more stable predation pressure than the bluegill sunfish in Lake Opinicon. This could explain the difference in individual behaviour between study sites given the large differences in overall environmental structure recorded in both sites.

Responses among prey to chemosensory information and environmental contexts may be shaped differently between taxa. Moreover, this can extend to predator communities as well (Ferrari et al. 2010). I speculate that predators may also be impacted by microhabitat complexity. The majority of studies have focussed on prey responses, but it is likely that predators will also be influenced by microhabitat complexity. Spiny damselfish (*Acanthochromis polyacanthus*) exposed to increased

turbidity, reduce their foraging significantly compared to patches with low turbidity (Leahy et al. 2011). Additionally, spiny damselfish reduced their overall activity and exhibited heightened antipredator response to conspecific alarm cue as turbidity increased. Spiny damselfish may then view increased turbidity as a risky environmental structure and are more fearful to situations of predation. Complexity and sensory deprivation can affect both predators and prey, if they rely on the same sensory information. Predator-prey interactions can shift, depending on how heavily either relies on vision for detection. Trends observed may then be one-sided, showing that prey either rely on complex environments to avoid detection, or to detect threats. This threshold at which the loss of information affects both the predator and prey has been observed in Black-axil chromis (*Chromis atripectoralis*) avoiding predation from the Brown dottyback (*Pseudochromis fuscus*) in turbid environments. Mortality rates increases along the turbidity gradient, and peak mortality was found in the intermediate turbidity environment. At the highest turbidity level however, mortality rate decreased to a value equivalent to the control treatment (Wenger et al. 2013). Predators may also be affected by environmental conditions, even if prey are not seeking specific habitat conditions as refuge. In non-experimental settings prey and predator behaviour, can be shaped by the use or avoidance of environmental structure. Seeking refuge and foraging in potentially less productive structure can be beneficial for prey in high risk scenarios, if predators require equivalent sensory modalities to seek prey, or are incapable of maneuvering the structure. Structured environments, or environmental conditions that restrict information acquisition can then shape how prey and predators view environmental conditions and their responses. Understanding predator-prey interactions within environmental gradients is a complex web to disentangle and can be limited by experimental design.

Neophobia as a behavioural tactic is beneficial to prey species, when background risk is high or in situations of high ecological uncertainty (Dall 2010). Unreliable risk assessment cues can lead to prey with maladaptive behavioural tactics, neophobia is expected to counteract this by minimizing the costs

of responding to an uncertain cue (Feyten & Brown 2018). This is not observed when novel odor is presented to this population of bluegill sunfish. Individuals did not respond to the structure of the environment but responded instead to the diversity of vegetation when novel odor was present. Bluegill sunfish avoid areas that are more open in situations of known risk and remain in the area when the open score is lower. The difference remains positive, when the environment is described as complex (low open score) and with low diversity. The difference decreases as either of these predictors increases, inferring that the bluegill sunfish in this system prefer more complex areas with low flora diversity (low open score, low Shannon index) when under situations of risk, and avoid open areas when risk is present. Counter to our original hypothesis, bluegill sunfish in Lake Opinicon show no signs of neophobia.

Neophobia is commonly observed in populations experiencing elevated risk (Brown et al. 2016, Crane et al. 2017, Ferrari et al. 2018). This study system contains several species of predators that are expected to forage on juvenile bluegill sunfish, which I expect would categorize the system as a high predation site. Absence of neophobic behaviour in this prey species while residing in a system with high predation risk runs counter to previous observations (Chivers et al. 2014, Abudayah & Mathis 2016). However, in addition to certain elevated risk, uncertain ecological conditions (i.e. unknown or variable risks) may also induce neophobic behaviour (Feyten & Brown 2018). Bluegill sunfish in this lake system may be exposed to high but consistent predation risks (i.e. high risk from a consistent predator guild). As a result, juvenile sunfish, under these conditions of low uncertainty, may not benefit from exhibiting strong neophobic behavioural responses (Feyten & Brown 2018).

My findings that microhabitat complexity does not impact the presence and/or intensity of neophobia in this population of bluegill sunfish is contrary to recent findings of neophobic behaviour and environmental complexity with Trinidadian guppies, which exhibited neophobic behaviour in

complex environments (Feyten & Brown unpublished). However, Crane & Brown (2020) have found that complexity does not increase uncertainty with fathead minnows. Inducement of neophobia relies on background risk, consistent exposure and environmental cues of uncertainty (Crane et al. 2017, Brown et al. 2015). Juvenile coral reef fish (*Pomacentrus amboinensis*), for example, residing in an environment with degraded coral habitats show no signs of induced neophobia when background risk is high. Neophobia was successfully induced in individuals residing in healthy coral habitats, however (McCormick et al. 2017). Therefore, habitat variation can have a species-specific effect in inducing neophobia by shifting uncertainty and risk assessment. Additionally, areas with higher vegetation are not situations of acute risk or increased uncertainty for bluegill sunfish, given their reliance on using more structured areas for safety. Finally, given their aversion to open spaces across all contexts, being in open space represents increased risk for bluegill sunfish, but not increased uncertainty. My study suggests that bluegill sunfish treat open spaces as situations of high risk, seeking complex environments as shelter. And that environment has no effect in neophobic behaviour in juvenile bluegill sunfish.

The results indicate that flora diversity around the novel object is the strongest predictor for antipredator behaviour. Open score and the interaction between open score, diversity and cue were not significant but suggest some trends. Bluegill sunfish show a tendency to avoid areas with low amounts of vegetation after encountering alarm cue, preferring vegetation in these situations of acute risk. Areas with vegetation were considered refuges from risk, but this behavioural pattern shifts as diversity increased. Bluegill sunfish considered diversity of vegetation as unsuitable in situations of acute risk under the same open score. I speculate this pattern is a product of sensory deprivation. The most common species of plant found in the trials was Eel Grass (41 of the 60 recorded trials contained eel grass), its structure consists of having long thin leaves that extend up the water column from the substrate. In these low diversity, low open score trials, bluegill sunfish may prefer this type of foliage as refuge from risk but still allow them to visually scan for predators. Additionally, other low diversity trials

may contain types of vegetation, that only cover one part of the water column, which still leave the possibility of bluegill sunfish to seek visual affirmation of predation while using the vegetation as shelter. With increasing diversity, vegetation that cover a specific part of the water column in part, now cumulatively cover the water column with foliage. This combination of different plant morphologies may restrict visual assessment of risk, after bluegill sunfish detect alarm cue. Further study is required to understand this shift in antipredator behaviour between vegetation density and diversity.

Future research in this system can be performed by isolating predictors. The shoreline I explored houses 8 species of plants, each of which has a different morphology, which can shift risk assessment in bluegill sunfish. By providing shelter, visually obscuring predators or housing resources to forage upon, individual behaviour can be explored along this gradient of vegetation. Eel grass has long thin leaves that may not provide as much shelter as broader leaved plants. Isolating and exploring individual behaviour, with different flora morphology, would grant more insight on individual antipredator behaviour. Bluegill sunfish behaviour in complex environments were observed to differ from other taxa. Trinidadian guppies, for example were neophobic when residing in complex pools. The environment in Trinidad differs from Lake Opinicon. Trinidadian guppies reside in riffles and pools in the dry season that are heterogenous in complexity. Additionally, the pools are isolated from one another, with little or no water flow in between. This prevents guppies from travelling between environments. Bluegill sunfish however are not under a similar environmental condition in Lake Opinicon. If residing in a complex environment can induce neophobic behaviour, it would be difficult to observe this in an open lake system. Bluegill sunfish can travel between microhabitats with different overall structure. This ability to disperse prevents the option of observing the inducement of neophobia in bluegill sunfish within this lake system. An experiment using seine nets to isolate sections of the lake with high or low complexity would allow the exploration of individual behaviour when constrained in an environment.

Figures and Tables

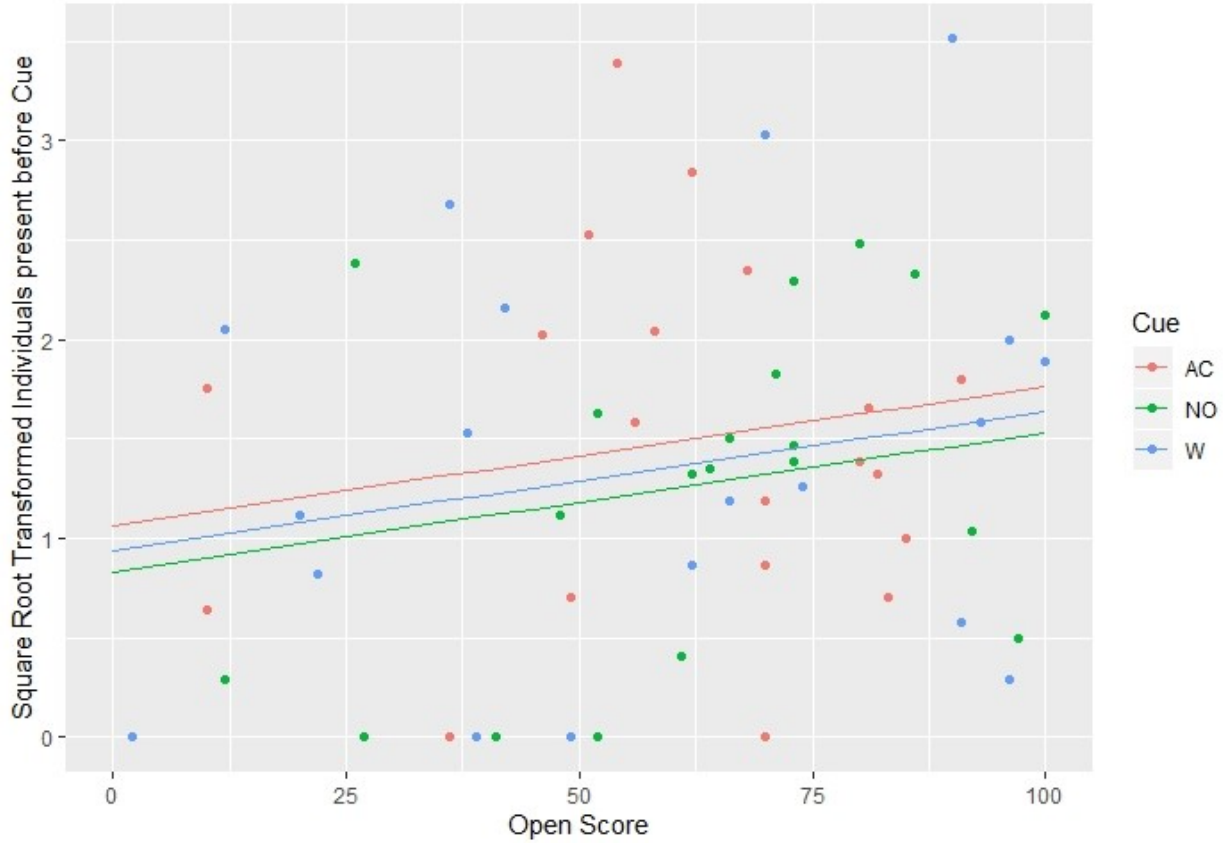


Figure 1: Number of individuals present around a novel object (square root transformed), before a cue was presented as a function of open score. The orange slope represents alarm cue (AC), the blue line represents the slope for water (W) and the green line represents the slope of novel odour. The slopes for each curve: alarm cue $y = 0.01x + 1.07$, novel odor $y = 0.01x + 0.83$, and water $y = 0.01x + 0.94$. Response to open score each cue was not found to be significant ([Table 2](#)).

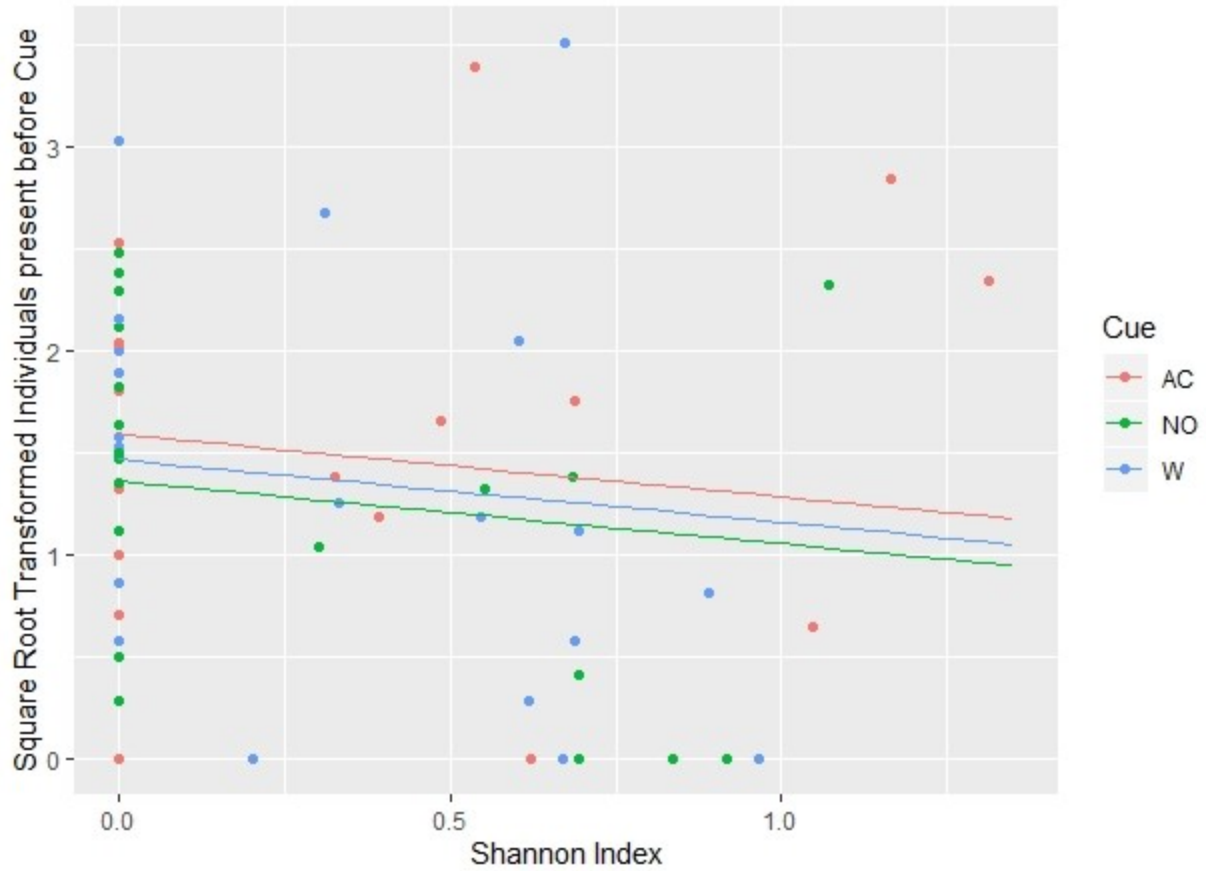


Figure 2: Number of individuals present around a novel object (square root transformed), before a cue was presented as a function of Shannon diversity index of the surrounding vegetation. The orange slope represents alarm cue (AC), the blue line represents the slope for water (W) and the green line represents the slope of novel odour. The slopes for each curve: alarm cue $y = -0.31x + 1.59$, novel odour $y = -0.31x + 1.36$, and water $y = -0.31x + 1.47$. Response to diversity and each cue was not found to be significant ([Table 3](#)).



Figure 3: Difference of individuals present around a novel object (Yeo-Johnson transformation after a cue was presented). The orange slope represents alarm cue (AC), the blue line represents the slope for water (W) and the green line represents the slope of novel odor (NO). Positive values in the transformed difference in individuals indicates a higher number of bluegill sunfish after the introduction of stimulus. Shannon index and cue are significant predictors of bluegill behaviour. Response to alarm cue was significantly different to water and lemon odour ([Table 5](#)). The slopes for each curve: alarm cue $y = -0.35x + 0.12$, novel odor $y = -0.35x + 0.47$, and water $y = -0.35x + 0.46$.

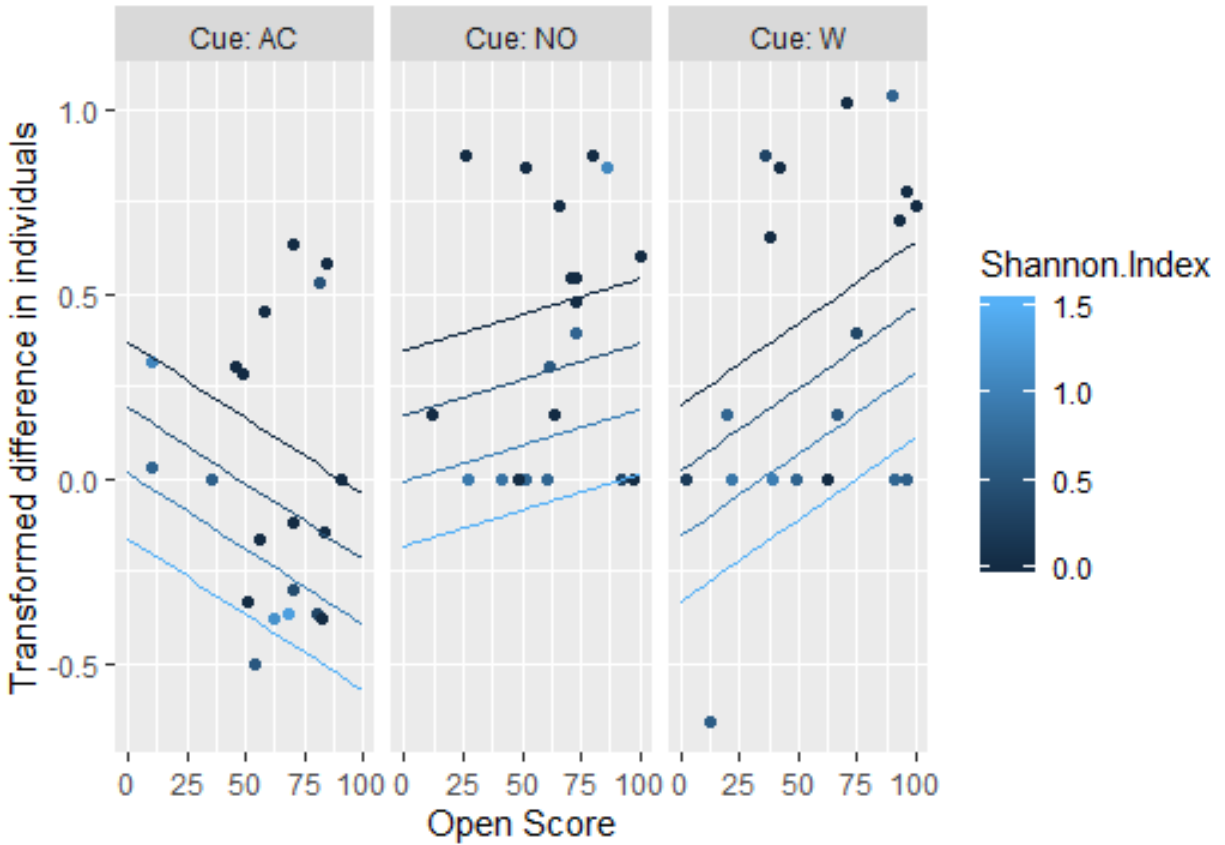


Figure 4: The difference in individuals present near a novel object (Yeo-Johnson transformation) as a factor of open score, Shannon index, cue and the interaction between open score and cue. Shannon index is a significant predictor in effecting the difference in the number of bluegill present. Cue, open score and the interaction between cue and open score are not significant predictors. Regression formulas can be found in [Table 1](#) and statistical output can be found in [Table 6](#).

Table 1: Formula of regression slopes for [figure 4](#). Slopes differ based on cue but remain the same within cues. Constants differ as Shannon index changes.

Shannon Index:	Alarm Cue	Novel Odor	Water
0	$y = -.004x + 0.37$	$y = 0.006x + 0.35$	$y = 0.008x + 0.2$
0.5	$y = -.004x + 0.19$	$y = 0.006x + 0.17$	$y = 0.008x + 0.02$
1.0	$y = -.004x + 0.02$	$y = 0.006x - 0.01$	$y = 0.008x - 0.15$
1.5	$y = -.004x - 0.16$	$y = 0.006 - 0.18$	$y = 0.008x - 0.33$

Table 2: ANOVA output of the number bluegill sunfish present around the novel object (square root transformed) before cue was presented as a function of the open score.

	Sum of Squares	DF	F value	Pr(>F)
Intercept	4.97	1, 54	5.95	0.02
Open	0.004	1, 54	0.0048	0.95
Cue	1.48	2, 54	0.88	0.42
Open*Cue	1.066	2, 54	0.64	0.53
Residuals	45.13	54		

Table 3: ANOVA output of the number bluegill sunfish present around the novel object (square root transformed) before cue was presented as a function of Shannon index.

	Sum of Squares	DF	F value	Pr(>F)
Intercept	21.502	1, 54	27.2112	< 0.01
Shannon Index	1.12	1, 54	1.42	0.24
Cue	0.84	2, 54	0.53	0.59
Shannon Index*Cue	4.634	2, 54	2.93	0.06
Residuals	42.67	54		

Table 4: Regression output of the difference in number of bluegill sunfish present around the novel object (Yeo-Johnson transformed) as a dependant variable to Shannon index, cue, open score, depth, average height of vegetation. AIC scores prior to stepwise reduction of the model is included for environmental predictors. Post reduction p-values can be found in Table 5.

	Estimate	Std. Error	t value	Pr(> t)	DF	Sum of Squares	RSS	AIC
Intercept	-0.17	0.25	-0.68	0.50				
Shannon Index	-0.29	0.13	-2.16	0.04	1	0.65	8.0440	-108.56
Cue					2	1.61	9.00	-103.81
Novel odour	0.36	0.12	3.04	< 0.01				
Water	0.33	0.12	2.79	< 0.01				
Open Score	0.002	0.002	0.929	0.36	1	0.12	7.52	-112.64
Depth	-0.00027	0.0039	-0.071	0.94	1	0.00071	7.40	-113.60
Average Height of the Vegetation	0.0037	0.0028	1.31	0.20	1	0.24	7.64	-111.69
Degrees of freedom	53							
Residual standard error	0.37							
Multiple R-squared	0.30							
Adjusted R-squared	0.22							
F-statistic	3.72							
p-value	0.003681							

Table 5: Regression output of [figure 3](#). The relationship between difference in the number of individuals present around the novel object (Yeo-Johnson transformed) with Shannon index and stimuli presented. Novel odour and water are compared against alarm cue in the analysis.

	Estimate	Std. Error	t value	Pr(> t)
Intercept	0.12	0.093	1.31	0.20
Shannon Index	-0.35	0.13	-2.82	0.0066
Novel odour	0.350	0.12	2.97	0.0044
Water	0.34	0.12	2.91	0.0052
Degrees of freedom	56			
Residual standard error	0.37			
Multiple R-squared	0.26			
Adjusted R-squared	0.22			
F-statistic	6.58			
p-value	0.00070			

Table 6: Regression output of [figure 4](#). The relationship between number of individuals present around the novel object (Yeo-Johnson transformed) with Shannon index and the interaction between cue and open score. Novel odour and water are compared against alarm cue in the analysis.

	Estimate	Std. Error	t value	Pr(> t)
Intercept	0.37	0.26	1.42	0.16
Open	-0.0041	0.0038	-1.07	0.29
Shannon Index	-0.35	0.13	-2.72	0.0088
Novel odour	-0.022	0.34	-0.063	0.95
Water	-0.17	0.30	-0.56	0.58
Open Score*Novel Odour	0.0061	0.0052	1.17	0.25
Open Score*Water	0.0085	0.0046	1.85	0.070
Degrees of freedom	53			
Residual standard error	0.37			
Multiple R-squared	0.32			
Adjusted R-squared	0.24			
F-statistic	4.08			
p-value	0.0020			

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Appendix



Image 1: Trial conducted with a Shannon index of 0.62 and open score of 36. July 12th, 2018; trial 4.

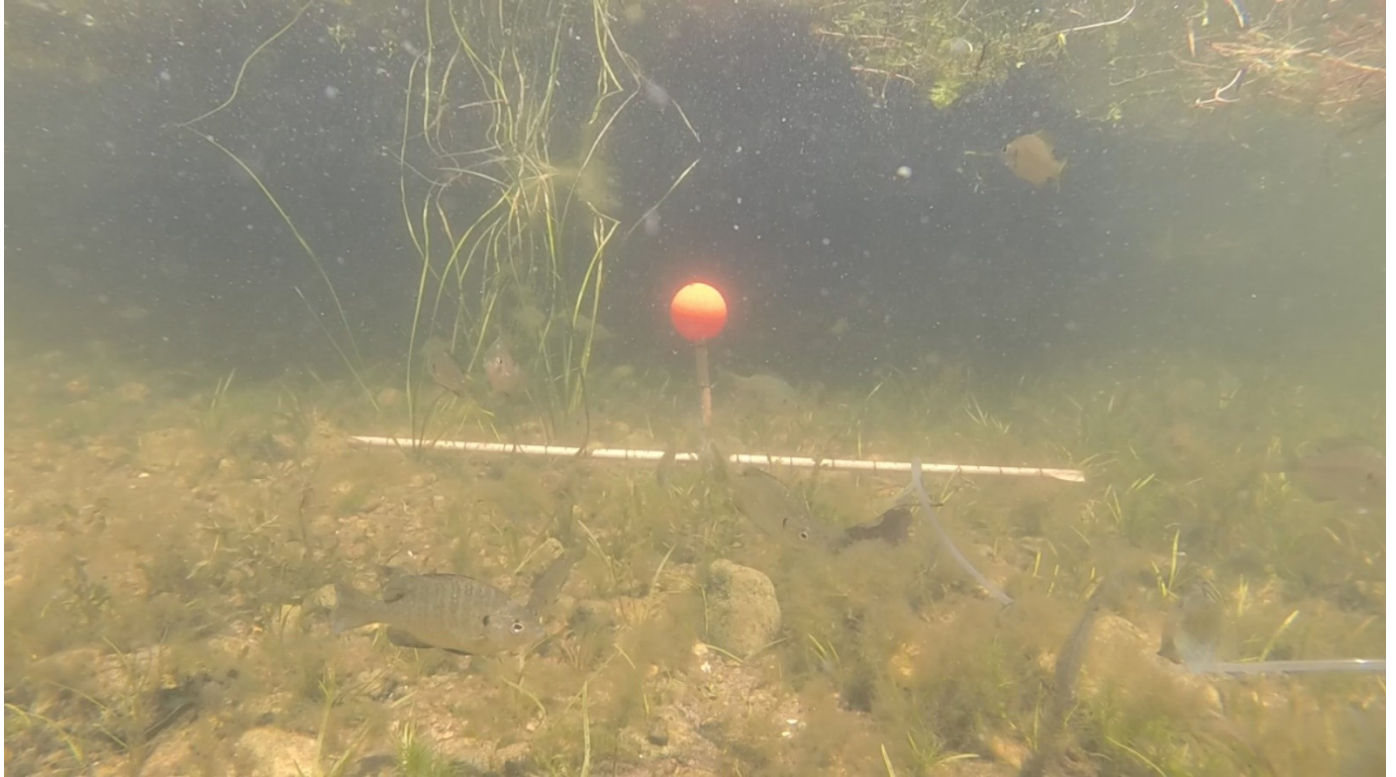


Image 2: Trial conducted with a Shannon index of 0 and open score of 96. July 3rd, 2018; trial 5.