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Viability of affordable animated lures for animal behavior mimetic field experiments in *Gambusia*

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ABSTRACT

Robotic fish have emerged as an alternative to live animals in behavioural studies of individuals and responses to group schooling. However, few studies have utilized affordable alternatives to some of the more extensively engineered and expensive robotic fish, which include more recently developed animated lures as a proxy for live fish behavioural studies as predators. These lures offer a new tool to study fish behaviour, and even the potential to aid in mimicking predator fish swimming for fish reared in aquaria or for captive propagation as a behavior stimulus. Here, we investigated the efficacy of robotic fish as potential predator stimuli in Mosquito fish, *Gambusia holbrooki*. Behavioural trials conducted in the field in the spring of 2021 indicate animated lures elicit anti-predatory behaviours in both individual and schooling trials, including avoidance and altering of fish depth in the water column, as we found a significant difference in sudden movements among size of lure stimuli, and time spent in the middle of testing chambers, and overall a difference in the frequency of out of water leaps of schooling fish in the presence of predator animated lure. We conclude these affordable animated lures offer promise for future fish behaviour and mimetic studies.

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1. Introduction

Animal behavioural studies have increasingly become important components of fisheries research, investigating predator and prey relationships across many species. Moreover, there is a growing need for newer, more affordable methods to conduct ethological experiments in natural environments, outside of laboratories, which minimize the need for housing live fish. The idea of using robots to test animal behaviour has increased in recent years (Klein *et al*., 2012), especially those such as robotic fish (Abaid *et al*., 2012; Landgraf *et al*., 2016) with some using a robotic fish as a predator (Spinello *et al*., 2019) or to elicit aggressive behaviour (Romano *et al*., 2017). Many of these studies have been introduced and center around more expensive and engineered robotic fish with custom programming installed or controlled by a mixture of a magnetic field below or user-controlled joystick to mimic natural movement (Landgraf *et al*., 2013; Bonnet *et al*., 2017). Interactions of fish with potential predatory robotic fish rely largely on the speed and size of the robot to properly elicit a response (Kruusmaa *et al*., 2016). Responses can include attraction to the robotic replica (Ruberto *et al*., 2016) or aggression towards it. Robotic fish have also been implemented to study fish schooling behavior and duration (Swain *et al*., 2011; Romano *et al*., 2020). Subsequently, these robotic fish have the potential for further studies and application to many species, as they may in the future become increasingly affordable as lures developed for anglers, utilizing technology while still mimicking natural fish movement.

Mosquitofish have been utilized in behavioural studies to study their interactions with predators (Goodyear, 1973; Rehage *et al*., 2005). Responses of *Gambusia* to *Lepomis* (sunfish) may rely on various visual and chemical cues leading to avoidance behaviour with the risk of predation perceived from a single cue, including predator size (Smith and Belk, 2001). *Gambusia* moves readily in schools or groups (Pyke, 2005) and are typically found along shallow edges of bodies of water (Moyle and Nicols, 1973), frequenting urban areas (Lloyd *et al*., 1986). Predators of *Gambusia* include water snakes and other larger fish (Pyke, 2008), making anti-predatory behaviours likely selected for in the presence of these predators. Indeed, small schools of *Gambusia* may move continuously in the presence of predators or inspect predators in smaller groups (Bisazza *et al*. 1999). Moreover, *Gambusia* is documented to respond to *Lepomis* predators which often consume them where they co-occur (Hubbs *et al*., 1991) by moving away from predator stimulus (Blake *et al*., 2015). While *Gambusia* has been successfully utilized as a study species to assess the feasibility of robotic fish on behaviour (Polverino and Porfiri, 2013), no previous studies have incorporated readily available and affordable animated lures instead of more traditional costly designed robotic fish.

This study aimed to test the efficacy of affordable animated lures in conducting predator/prey experiments in the field under semi-natural conditions. Specifically, we assessed individual behavioural responses of *Gambusia holbrooki* to a predatory and a smaller control robotic fish, as well as the behaviour of schooling in the presence of predator robotic fish. This research adds to our knowledge of behavioural interactions between fish and robotic fish to answer fundamental questions in ethology. We also discuss the potential for using these robotic fish for future research in fisheries as animated lures, which look and move like live fish, are developed for anglers, and can be incorporated into preliminary behavioural fisheries research.

2. Materials and Methods

2.1 Behavior Experiment 1

This study was conducted February 23 to April 1 2021 on the University of Wingate campus at Campus Lake, a small pond. Mosquitofish, *Gambusia holbrooki*, were collected using a dip net and stored temporarily just before experiments were conducted during each trial day. Trials were conducted in a clear plastic container with 30 cm x 39 cm x 35.5 cm under a shelter to minimize the potential impact of weather (sun, wind, etc.) on experiments. We placed the white lid directly below the plastic container to divide the testing chamber into 4 quadrants and allow for increased contrast for experimental fish in the water. We filled containers with lake water filtered through sieves to decrease the presence of sediment and increase visibility for filming. We only conducted experiments when turbidity of lake water was low (no rain previous 48 hours).

Each individual was tested in separate containers, hereafter referred to as a testing chamber. Each fish was carefully netted into the testing chamber, then allowed to acclimate for 5 minutes. Treatments of either predator or control were randomly assigned. We then placed either a control or predator robotic fish (animated lure, available from www.animatedlure.com) into the testing chamber (Fig. 1 A, B) directly in the middle and the video was recorded on GoPro5 action cameras for 5 minutes. The video was recorded at 720 dpi. One camera was placed directly above the testing chamber for an aerial view which allowed a clear view of individual movement and behavior, while another camera was placed on the side of the testing chamber (to assess individual fish depth in the water column relative to the animated lure (predator or control). This allowed for the quantification of fish behaviour in the testing chamber. Robotic fish or animated lures measured 13.5 x 4.5 cm for predators, and 6.35 x 2.5 cm for control. The predator animated lure resembled bluegill, *Lepomis*, which has been observed in the same pond and is a likely predator of *Gambusia*, whereas the control animated lure was a rainbow trout, *Oncorhynchus mykiss*, chosen primarily for its smaller size and availability at the time of the experiment (Fig. 1 D). We placed a bobber on both lures (4.5 diameter cm for predator, diameter 3.8 cm for control) to ensure lure swimming behaviour mimicked natural fish swimming behaviour and to position robotic fish directly in the middle of the testing chamber depth. The distance between bobber and the robotic lure was 11.4 cm for both lures to ensure the distance was similar across behavioural trials. Chambers were rinsed following 5 trials. Water temperature was taken during trials to ensure similarities in movement behaviour across experimental trials and dates. Authors periodically checked on safety and well-being of fish throughout the experiment, but were mostly distant to remove the potential for added stress on fish or to prevent bias during experimental trials. Following behavioural trials, individuals were removed from the testing chamber, measured, weighed, and sexed, then released unharmed and returned to their exact capture site.

2.2 Behavioral Experiment 2

This experiment was conducted on April 6 and April 13 2021 using a similar protocol for experiment 1, however, for experiment 2, 5 fish were placed into the testing chamber to investigate the potential for our setup to document group schooling behaviour and record any antipredator behavioural responses to large predator lure (Fig. 1. C). Experiment 2 consisted of only the predator robotic fish, as this experiment tested schooling behaviour in response to the presence of a predator, and thus represented preliminary

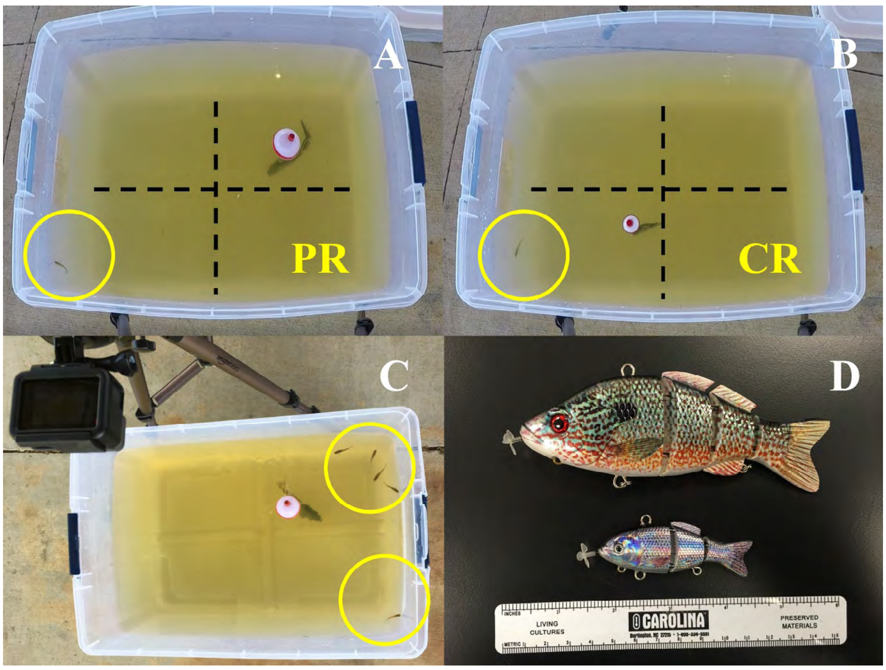

Fig. 1. Experimental setup showing field trials conducted and video set up. Top left (A) shows experiment 1 with predator robot (PR), top right (B) shows experiment 1 control robot (CR), bottom left (C) shows experiment 2 with 4 fish schooling, 1 not schooling in the presence of PR, and bottom right (D) shows PR and CR size comparison. Quadrants (A, B) of the testing chamber are shown (A, B), represented on top left and right as separated by dashed lines. Yellow circles denote fish in the testing chamber

data on schooling. Antipredatory behavior was recorded during this experiment. For this experiment we did not collect biological information from individual *Gambusia*. All fish were returned to the point of capture immediately following the experiment. For both experiments, fish were temporarily kept in buckets or containers for ~1–2 hours total.

2.3 Behavioral Analysis

Following field trials, SD cards were removed from cameras, backed up, and organized by date and treatment (control or predator) for both experiments. For behavioural analysis, we reviewed video collected during trials using standard Microsoft video player. For experiment 1, using the top camera, we quantified the amount of time a robotic fish (predator or control) was in the same quadrat as a trial fish. We also quantified the total amount of quadrants mosquito fish swam in (4 sections of testing chamber). We also enumerated the frequency of sudden movements using the top camera. Using the side video camera, we quantified the depth of the water time of *Gambusia* during trials (top, middle, bottom). For experiment 2, we noted the frequency of "out of water leaps" in response to animated lure and the amount of time schooling. We defined schooling whenever 3 or more fish were swimming in close proximity.

2.4 Statistical Analysis

Descriptive statistics were reported for behaviours. We ran a Man Whitney U test for time spent in the middle of the test chamber, frequency of fast moves, time spent fast moves, and total number of quadrants swum in. Data from trials was analyzed using R version 3.3.1. Alpha levels for all tests was 0.05. We also report descriptive statistics on biological data collected for individual fish for experiment 1 and ran a t test to compare the mass and total length of fish for both control and predator trials to ensure similarity among the overall size of fish used in trials. We report descriptive statistics for experiment 2, since it consisted of only predator robotic fish as a stimulus for schooling fish.

3. Results

3.1 Experiment 1

A total of 20 individuals were successfully tested during field trials (10 control and 10 predator), consisting of adult 9 males, 7 females, and 4 unknown sex fish. Control (CR) and predator (PR) treatments were not significantly different for size in both mass (mean = 0.45 g ± 0.027 S.E. g) or total length (33.2 mm ± 0.767 S.E.), t value = –0.382, p = 0.353, and t value = –1.252, p = 0.113, respectively. Both control (CR) and predator robots (PR) remained charged for ~10 trials per charge, as we observed no decrease in the swimming ability of fully charged animated lures. We observed approximately one "fast movement" per second with varying trials. There was a significant difference between predator and control robotic fish in frequency of fast movements, U = 4, $p < 0.001$, CR mean frequency = 4.7 ± 1.66 SE, PR mean frequency = 28.8 ± 3.60 (Fig. 2). There was a significant difference between time spent in the middle for CR and PR, U = 20, $p = 0.0129$, with mean time spent in middle (seconds) for CR and PR, 40.2 ± 6.5 S.E. and 21.3 ± 2.6 S.E., respectively (Fig. 3). Time spent in other areas of the testing chamber (top and bottom) were similar across trials, with mean CR top and bottom = 90.3 s and 169.5 s, and mean PR top and bottom = 85.8 s and 192.9 s. Water temperature for both experiments was similar across trials, 14.3–15.1 °C.

3.2 Experiment 2

Interestingly, no fish elicited an "out of water leap" behaviour for experiment 1 that we observed in experiment 2. We observed a total of 111 out of water leaps across 20 trials (5 fish per trial, n = 100). Mean out of water leaps were 5.55 ±0.57 S.E. and ranged from a minimum of 2 to a maximum of 10 per trial. Mean time spent schooling for experiment 2 was 137.6 s, versus time spent not schooling mean = 162.4 s. Time spent schooling for experiment 2 ranged from 23 to 239 s, median = 150.5.

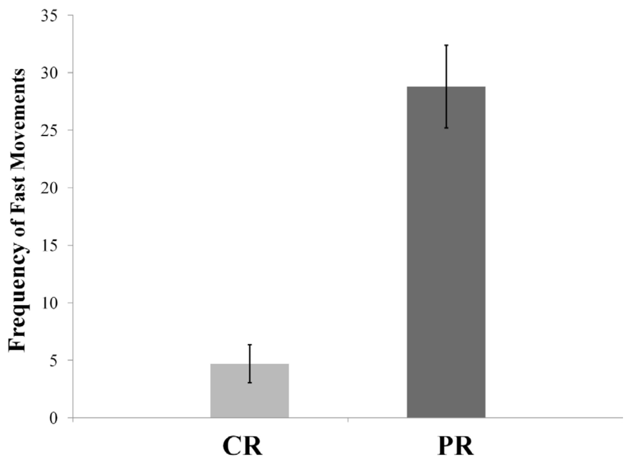

Fig. 2. Frequency of fast movements in presence of control versus predator-animated lures of *Gambusia* in experiment 1 (n = 20). Error bars denote standard error, CR denotes control robot, and PR denotes predator robot

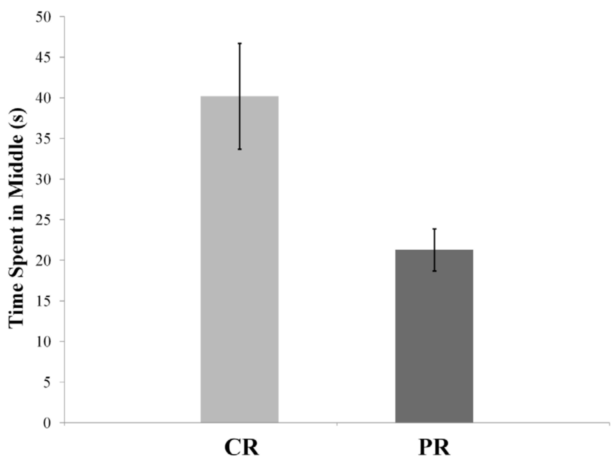

Fig. 3. Time spent in the middle of the testing chamber in seconds of *Gambusia* in experiment 1 (n = 20). Error bars denote standard error, CR denotes control robot, and PR denotes predator robot.

4. Discussion

Animated lures appear to provide great potential for use in behavioural experiments in fish, albeit with some caveats for researchers. We noted that the container size may have affected the swimming distance and turning radius of the larger (predatory) animated lure in the first testing designs and size of containers before conducting this study. However, given the affordability of these lures (~$35–45 US) and wide assortment of "skins" or covers which resemble live fish, researchers can modify the experimental containers to accommodate different sizes and likely numbers of fish, in both a lab or natural setting. We anticipate these lures becoming smaller and more affordable, highlighting the potential for their use in various ecosystems (i.e., streams, ponds, tidal zones, etc.). Moreover, using GoPro action cameras allowed ease of field recording from multiple angles (top and side views) to calculate the distance of lure to individual fish using our methodology. Among other caveats to our short-term preliminary study included the random swim pattern of animated lures, which may more accurately mimic fish movement, only allowed a subset of our experimental observation time to include both predator and control animated lures in the same proximity as experimental fish. Quantifying the behavior of experimental fish to robotic fish required further exploration, as there is variation among individual responses. For example, Abaid *et al*., 2013 found behaviour among individual shiners, *Notemigonus*, was modulated by either the "bold" or "shy" movements of robotic fish. However, based on our observations, animated lures show great potential for behavioural studies.

Using affordable, robotic fish with realistic movement offers many avenues for use, outside of antipredatory behavioural research. Aquarium personnel can also use animated lures to stimulate fish reared in tanks for enrichment purposes, and even for conservation purposes related to captive propagation to train animals to respond to predators. Robotic fish could even reduce the potential for zoonotic disease in observational studies as they could potentially minimize the accidental spread of unknown diseases within or across laboratory studies using live fish. While there is no substitute for live animals in research, we recommend further study using robotic fish alongside chemical stimuli as an alternative to live animals for enrichment in aquaria, conditioning young fish to predators reared for captive propagation and release or exploratory behavioural studies where obtaining live fish presents challenges. For example, predator fish can be kept in tanks, with water from live predators frozen, and combined in future experiments to elucidate the effect of chemical stimuli versus physical or visual stimuli if used alongside robotic fish. These experiments can be designed in combination with other factors that may impact fish behaviour.

Individual fish behaviour varies significantly across species and factors associated with habitat or the presence of predators. Many fish engage in inspection behaviour when encountering a predator (Brown and Magnavacca, 2003), which may have been a factor in our experimental design. However, we did not directly observe individual fish inspecting predators over controlled animated lures. As we observed a high incidence of individual fish leaping out of water for experiment 2, future studies could investigate whether this behaviour is altruistic or an antipredatory response to the presence of potential predators. Other predators of *Gambusia*, including chain pickerel, *Esox niger*, can alter *Gambusia* habitat use in the presence of one or multiple predators (Winkelman and Aho, 1993). Therefore, we recommend further studies using either different species of animated lure predators or potentially conducting experiments in larger tanks with more than one animated predator lure. Moreover, as we utilized animated lures to study schooling behavior successfully, we recommend future camera studies can incorporate tracking software to study individual grouping patterns, as previous research has documented varying individual leadership behaviors in shoals of *Gambusia* (Burns *et al*., 2012).

5. Conclusion

Our results indicate that affordable animated lures can be successfully utilized for fish antipredatory and schooling experiments, with minimal financial investment as they morphologically resemble common fish species and realistic movement. Moreover, researchers can purchase more than one animated lure to ensure charge and movement are similar across trials or to increase their use in behavioral studies in aquaria, labs, or in the field of more natural aquatic ecosystems. If researchers are limited by access to live animals or adequate laboratory space, our method for using robotic fish and video in the field offers an alternative to maintaining live animals in aquaria for fish behaviour experiments. Additionally, we show that field video trials using animated lures can pave the way for researchers to conduct exploratory behavioural studies in local pond systems which potentially decrease the need to house large numbers of experimental, control, or predatory fish, decreasing risks of unknown diseases all while obtaining baseline information on fish behaviour.

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