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June 27th,11

Conditioned Alarm Behavior in Fathead Minnows (*Pimephales promelas*) and Test Their Ability of Differentiate Between Different Visual Stimulus (i.e. Red Light, Green Light and Blue Light)

Abstract

Fathead minnows (*Pimephales promelas*) were tested for their ability to associate predation risk with novel visual stimuli after visual stimulus was presented simultaneously with chemical alarm cues. Minnows gave a fright response when exposed to skin extract (chemical alarm cue) and an artificial visual light stimulus. When they were retested with light stimulus alone, the minnows that had previously been conditioned with alarm cues and light exhibited anti-predator behavior in response to the visual cue. To carry out this experiment, we hypothesized that fathead minnows would learn to associate predator risk stimulus with visual stimulus, and they would be capable to differentiate between the three different colors by showing associate response to the red color and no response to the green and blue lights. The results of this experiment have far-reaching implications because they provide important information on the role of visual stimuli in the ecological environment of fishes.

Introduction

There have been numerous assessments of predation risk through chemical cues among many aquatic organisms (Kats and Dill (1998) and Wisenden (2003)). Chivers and Smith (1998) report that most of the intense studies have been conducted in superorder Ostariophysi. It is easy to reliably identify the presence of an active predator in the environment because chemical alarm cues released during injury can only be released after a predation event (Wisenden and Chivers 2006, p.263). Mathis and Smith (1993) suggest that the reduced activity in response to these cues also reduces the probability of predation.

The prey species are able to use injury-released cues to associate predation risk with the stimulus that leads to the release of alarm cues (Brown and Chivers 2005, p.45). It is important to note that learned recognition of foreign stimulus of risk requires only one chance for association otherwise called release-induced recognition learning (Suboski 1990).

Release-induced recognition learning allows fathead minnows as well as other fishes to learn to associate risk with kairomones- predator's gut dietary-based chemical cues or even the chemistry of water from a certain habitat (Chivers and Smith 1998).

In addition to chemical alarm cues, organisms acquire recognition and learn to respond to other forms of novel stimuli such as light and sound. Visual stimuli have been reported to be associated with risk in minnows in the presence of a predator, the social behavior of other frightened minnows, non-biological stimuli (e.g. a flashing red light, or moving objects) (Yunker et al. 1999).

The Ostariophysans consists of minnows, catfish, characins, and suckers, which are approximately 64% of all fish species in freshwater (Nelson, 1994, p.600). It is reported that the minnows and most members of Otophysi possess Weberian ossicles connected to the swim bladder, which is also the resonating for sensing sound in the range of 50 to 2000 Hz with extremely low detection thresholds. These specialized structures, used to detect sound in these organisms, strongly indicate both ecological and evolutionary importance of auditory stimuli in aquatic habitats. Popper, Fay, Platt, and Sand (2003, p.27) suggested that fish use auditory stimuli in intraspecific interactions during courtship and territorial marking or defense.

Minnows can be conditioned to respond to light alarm cues with anti-predatory behaviors by first associating the light stimuli with chemical cues indicating predation risk. This type of conditioning/learning occurs in fathead minnows when light stimuli become associated with predation. We hypothesized that fathead minnows response to associate light alarm cues with anti-predatory behaviors and distinguish the differences between the three visual stimulus (red, green and blue light); no response to the green and blue light. This paper reports the results from simple tests conducted for learning association of predation risk with visual stimuli, using fathead minnows, *Pimephales promelas*, as test species.

Method

- **Test stimuli**

Conspecific alarm cue was prepared from the skin extract of 5*7 centimeters of fathead minnows' skin. The skin extract (alarm cue) was prepared by making superficial cuts on each side in the presence of red light before rinsing the fish with water and collecting the solution in a syringe. The extract was used within one hour to preserve the cue potency. The fathead minnows (43 fathead minnows) were conditioned by skin extraction and expose at same time with red light (100 watt) for about 15 minutes. The same fish were exposed to red light after the tank had been cleaned (24 hours) and then separated into a different tank to count their line cross. The tank consisted with horizontal and vertical lines about 25 squares with $5.0cm^2$ size.

- **Data analysis**

It was predicted that if the test minnows use chemical alarm cue to associate predation risk with visual stimuli, then the minnows in the alarm cue treatment would respond to the visual stimuli in the test trials with anti-predator behavior (for one minute). The Wilcoxon Mann–Whitney tests were used to compare the magnitude of the change in anti-predator behavior or reduced activity between various treatment groups. The test statistics were then interpreted by two-tailed probability distributions.

Results

The fathead minnows generally developed anti-predator behavior when the red light stimulus accompanied the introduction of skin extract. Performing a t-test shows that the magnitude of the after conditioning (AC) – (p=0.0002) activity of the fathead minnows in alarm tests was significantly different from the before conditioning (BC) – (p- 0.0001) activity. The probability of this result assuming the null hypothesis is 0.001. The mean values of both the BC and AC activities show that the BC activity increased four times to approximately 29.125 movements from the initial mean of approximately 7.313. Using a statistical analysis of paired sample t-test two-tail p-value of .000038 to compare the two mean before and after conditioning.

The results of a similar experiment using green light on 10 fathead minnows indicate that the fishes developed reduced activity when the green light stimulus was introduced to the red light conditioned fish. Analyses of the results suggest that the magnitude of the AC with green light – (*p*= 0.5687) activity of the fathead minnows in alarm tests was significantly different from the BC with green light – (*p*= 0.2843) activity and two-tail p value of 0.10. A t-test analysis shows that the probability of this result assuming the null hypothesis is 0.320. The mean values of both the BC and AC activities show that the AC movements almost doubled from 7.4 to 12.6.

When a blue light was used as a stimulus on 10 fathead minnows, it was observed that the fishes developed changes in anti-predatory behavior to the conditioned fish. Analyses of the results suggest that the magnitude of the AC with blue light – (*p*= 0.0025) activity of the fathead minnows in alarm tests was significantly different from the BC with blue light – (*p*= 0.0013) activity, and two -tail p value of 0.00063. A t-test analysis shows that the probability of this result assuming the null hypothesis is 0.002. The mean values of both the BC and AC activities show that the AC movements almost tripled from 9.3 to 26.9 (figure 3).

Comparative studies show that both red light and blue light had the highest effect on the anti-predatory behavior of fathead minnows. The green light had on the other hand minimal effect but still increased the movements of the fish (figure 4). A t-test analysis gave a two -tail p value of 0.24 for mean number of line crosses between the red light and the green light. And for the blue light and red light, t-test gave two-tail p-value of 0.75.

Figures:

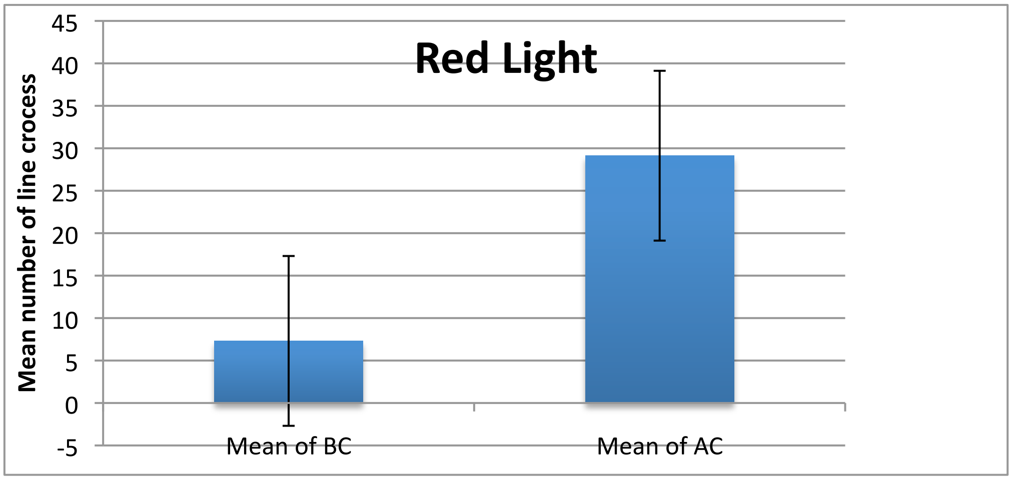

Figure1: The error bar (+/-e.s) shows no significant correlation in the mean differences in movement of fathead minnows, *Pimephales promelas*, between before conditioning movements and after conditioning movements with respect to red light. P(T<=t) two-tail = p-value of .000038 shows the significant difference between the numbers of line crosses before and after exposure to the red light.

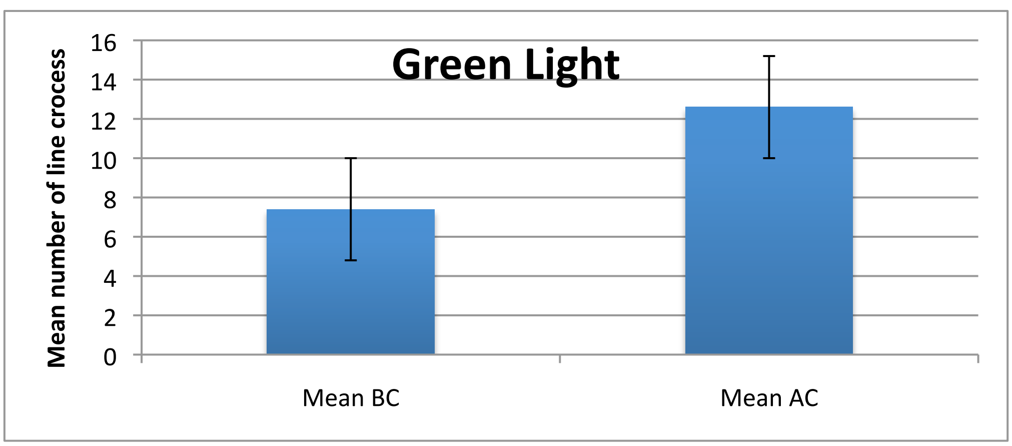

Figure2: The error bar (+/-e.s) shows no significant correlation in the mean differences in movement of fathead minnows, *Pimephales promelas*, between the before conditioning movements and after conditioning movements with respect to green light. P(T<=t) two-tail p value of 0.10, shows no statistically significantly differences.

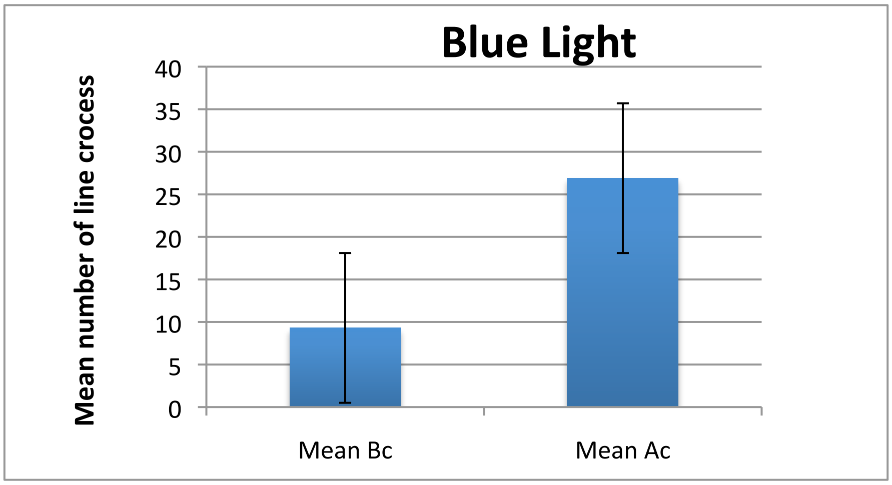

Figure 3: The error bar (+/-e.s) shows no significant correlation in the mean differences in movement of fathead minnows, *Pimephales promelas*, between the before conditioning movements and after conditioning movements with respect to blue light. P(T<=t) two-tail p-value of .75 indicating no significant difference between the two means.

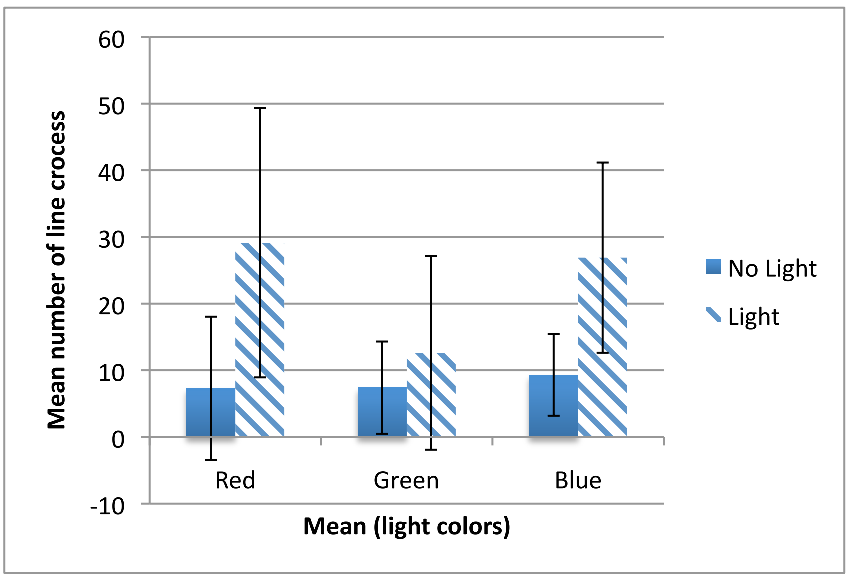

Figure4:. Comparison of response to red, blue, and green light stimuli fathead minnows, *Pimephales promelas*, between the Before Conditioning movements and After Conditioning movements. The error bar (+/-e.s) shows no significant correlation in the mean differences in movement of fathead minnows. P(T<=t) two-tail p value of 0.75 for blue and red lights and p value of 0.024 for red and green lights.

Discussion

The experiments conducted in this study provide evidence that minnows associate novel light stimuli with predation risk after simultaneous exposure to light and conspecific chemical alarm cue. Different conditions were used for this experiment; different light frequencies. The response was measured as reduced activity, which is an anti-predator behavior that reduces predation probability in organisms (Mathis and Smith). This study is very important because no other study has extended the visual sensory modalities with which organisms assess predation risk. Although previous works have focused on red light as the visual stimulus, no other study has assessed this aspect in comparison with other light frequencies.

Many studies such as those of Wisenden and Chivers (2006) that demonstrate single event acquired recognition of new stimuli of risk are shows the ecological and evolutionary advantages of early attendance to information. Various groups of fishes such as the minnows use light for competition over food resources, territorial dominance, and or courtship (Popper, Fay, Platt and Sand, 2003). There is limited literature on the role of light in the context of predator-prey interactions. The investigation of the role of red, blue, and green light in these predator-prey interactions opens an avenue for testing the classical learning paradigm for fish interactions.

This study reports high movement induced by both red light and blue light with the red light recording the highest mean values. However, the green light reported the least mean values. This phenomenon is hard to explain because the green light frequency (wavelength) comes between the red and blue light spectrum. How the fathead minnows are sensitive to low and high frequency light spectra is subject that requires further investigation. In general, it can be concluded the fathead minnows respond to the light with the lowest frequency (red) more sensitively than higher frequency light (blue and green).

Conclusion

It is clear that more information is required on the types of light stimuli generated by predators or restless prey in order to fully explore the role of visual stimuli in mediating predator-prey interactions. The present experiment used a highly artificial conditioning stimulus as an arbitrary light. For this reason, future studies should focus on the frequencies and durations of light produced during the predation event in order to develop ecologically realistic experiments. In spite of the ecological role of light in predation risk assessment, the results of this study show that induced recognition learning for acquiring new recognition ways for the presence of predation risk is sufficiently artificial to accommodate sensory input from visual stimuli.

Work Cited

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