Different Wavelengths of Light Have No Effect on Zebrafish Fecundity

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Abstract

Danio rerio (zebrafish) have rapidly gained popularity in the study of vertebrate development and gene function. Zebrafish are an excellent model for performing large-scale genetic and drug screens to be performed coupled with the visualization of downstream perturbations on live, developing organisms. Due to the fact that large numbers of embryos are required to perform these experiments, we investigated ways to efficiently increase zebrafish embryo production while not being overly invasive or using chemicals that might affect the developmental processes. To perform these experiments, we mated wild type (wt) zebrafish while exposing them to the different wavelengths of light. Fish were exposed to 12 h of dark, followed by 4 h with the different colored light treatments. Once this light cycle was over, embryo production was enumerated. Results indicated that exposing zebrafish to the different wavelengths of fluorescent and LED light had little effect on embryo production. Further research may elucidate other methods to increase embryo production to further studies of developmental processes, making zebrafish an even better model organism for studying a multitude of biological processes at a large-scale.

Introduction

Danio rerio (zebrafish) have become an increasingly popular model system to study vertebrate development, gene function, and molecular pathways involved in human diseases and disorders. One major reason that zebrafish are utilized as a developmental model system is their fecundity; the ability to generate hundreds of offspring per mating is advantageous to performing large-scale genetic screens [1] [2] as well as drug screens to test compounds of therapeutic interests [3] [4] [5]. Drug screens in zebrafish have identified compounds currently in clinical trials for treating human disease [6] and many researchers are developing high throughput technologies to perform the automated, large-scale drug compound screens for the toxicology and human cancer research [7].

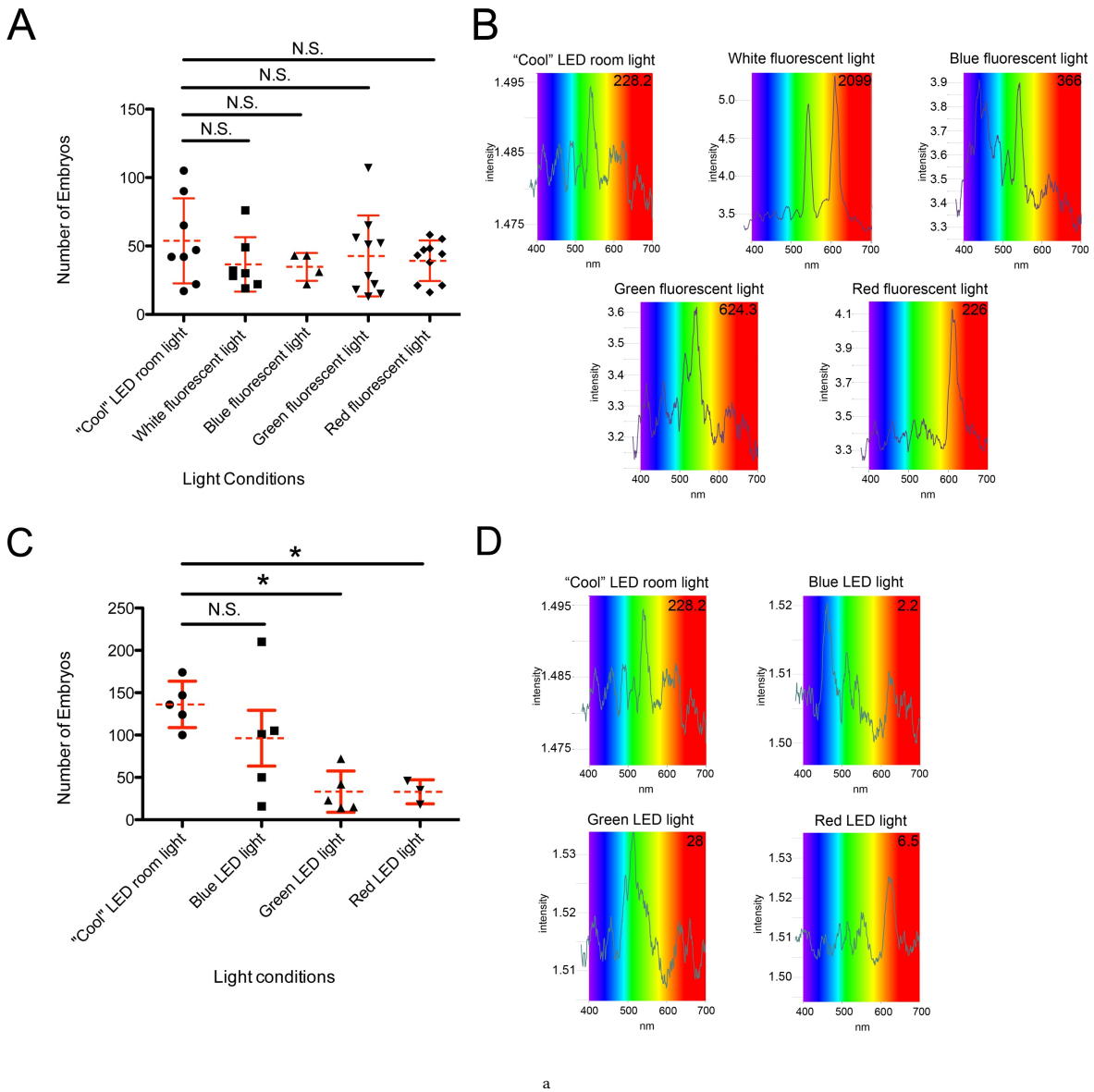
Advances in zebrafish spawning technology have greatly increased embryo collection. Capitalizing on the tendency of zebrafish to mate in shallow water [8] [9] influenced the creation of a spawning apparatus to harvest thousands of developmentally staged zebrafish embryos [10]. Other factors influence spawning, including the temperature of the water and the atmosphere [8] [11], the pH of the water [12], and the diet of the zebrafish [13] [14]. Another essential environmental factor for efficient spawning is based on the zebrafish's diurnal nature; their highest level of activity is during daylight hours, particularly in the morning [15], and they sleep most frequently during the night [16]. The circadian rhythm of fish is essential for many physiological and behavioral processes, and this can be regulated by establishing a regular light/dark cycle [17]. For example, interruptions in the light/dark cycle can negatively affect the zebrafish reproductive cycle; ovarian follicle maturation in female fish is dependent on the time of the day [18]. The shift from a dark period to a light period triggers spawning in captive zebrafish, likely due to their natural mating occurring predominately within the first hours of the sunlight [8]. Research indicates that the disruption of the circadian rhythm, even by low-level red and green lighting from "EXIT" signs in a vivarium, is detrimental to the breeding [19].

Since low-level green and red light negatively affected breeding in a zebrafish vivarium [19], we sought to determine if this was an intrinsic function of the light itself,

or merely because it disrupted the fish's normal circadian rhythm, as these lights were constantly on. To avoid negatively impacting their light-dark rhythm, we exposed fish to the different wavelengths of light while they were spawning. As we normally spawn fish in the laboratory over a span of 4 h (between 09:00 and 13:00), we utilized this time frame for our experiments. In this way, our research study took a different approach to optimize spawning; we hypothesized that exposing mating zebrafish to the different wavelengths of light during their optimal mating time period would affect clutch sizes. Further experiments could then be performed to not only maximize mating productivity but also to further understand the role of the circadian rhythm and the regulation of light sensing involved in fish fecundity.

Objective

The objective of this experiment was to modulate the different wavelength of lights that zebrafish were exposed to during mating to increase zebrafish fecundity.



a

Figure Legend

Different wavelengths of visible light do not increase embryo production when compared to room lights.

(A) Numbers of embryos generated from light box experiment. The dashed red line denotes mean, and red error bars represent standard deviation. "Cool" LED room light (black circles), white fluorescent light (black squares), blue fluorescent light (black triangles), green fluorescent light (inverted black triangles), and red fluorescent light (black diamonds). N.S., not significant.

(B) Wavelengths of light used in the light box experiment. Light intensity is shown in the top right corner of plots (presented in lumens/meter^2). nm, nanometer.

(C) Numbers of embryos generated from the iSpawn-S experiment. The dashed red line denotes mean, and red error bars represent standard deviation. "Cool" LED room light (black circles), blue LED light (black squares), the green LED light (black triangles), and red LED light (inverted black triangles). N.S., not significant; $^*p$ <0.005, measured with two-tailed Student's t-test.

(D) Wavelengths of light used in the iSpawn-S experiment. Light intensity is shown in the top right corner of plots (presented in lumens/meter^2). nm, nanometer.

Results & Discussion

To examine if different light conditions would affect the spawning of zebrafish, we first created an isolation chamber that allowed us to modulate 4 different wavelengths of the light simultaneously during zebrafish spawning (see Methods). To ensure that the isolation box had no detrimental effects on the mating of zebrafish itself, we monitored the physical environment within the isolation chamber before adding fish. During this time, we measured the humidity, water temperature, and the temperature of the air under the box. Even when the lights were left on for 5 h in the isolation box, there were no significant changes in these parameters (data not shown).

As we measured no negative parameters within the isolation box, we set up controlled crosses of wt AB strain zebrafish (2 females to 1 male per tank, 10 tanks in total), exposing fish to 4 different lighting conditions simultaneously for 4 h (see Methods); this experiment was repeated over the course of 16 weeks. As a control, 2 tanks were also set up next to the isolation box. The isolation box was then removed, embryos were collected, and the fish were placed back in their respective tanks. While we hypothesized that there would be a difference in the number of embryos produced under different lighting conditions, we saw no significant difference in embryo production between the control facility lighting and exposure in the isolation box to white, green, red, or blue light (Fig. 1A). All the environmental factors and the water conditions were monitored at the initial setup of each treatment for the entire study, and no differences were observed (data not shown). Importantly, none of these light treatments had any effect on embryo survival when assessed 24 h later. Overall, these data indicated that the wavelength and the intensity of light (Fig. 1B) that zebrafish were exposed during the mating time did not appreciably increase the numbers of embryos produced during mating.

We also examined the likelihood that fish would spawn when exposed to the different colored lights. When fish were mated in room light they spawned 17.1% of the time, when exposed to white light they spawned 14.3% of the time, when exposed to red light they spawned 22.9% of the time, and when exposed to green light they spawned 20% of the time. The only major difference was the blue light, which only spawned 5.7% of the time, with roughly 33% less successful matings than the facility room lights. Overall, these data indicated that the wavelength and the intensity of light that zebrafish were exposed during the mating time did not appreciably increase the rate of successful matings and the blue light actually had a negative effect.

To confirm that lighting conditions did not increase fecundity, we approached the experiment in a different way, utilizing an iSpawn-S system covered with a black plastic bag with lights inserted into the top of the device. We set up controlled crosses of wt AB

strain zebrafish (4 females to 4 males) the night before, where males and females were physically separated. In the morning, the divider was removed and a lighting condition was applied during 4 h of mating (see Methods). The color was cycled randomly every week so that fish were exposed to a specific color 5 times over the course of 20 weeks. The black bag was then removed, embryos were collected, and the fish were placed back in their respective tanks. We observed no significant difference in embryo production between the control facility lighting and exposure to blue light, but there was a reduction in embryos produced in exposure to green and red light (Fig. 1C). Again, the environmental factors and water conditions were monitored for the entire study, and no differences were observed (data not shown). While none of these light treatments had any effect on embryo survival when assessed 24 h later, these data indicated that the wavelength, and the intensity of light (Fig. 1D) that zebrafish were exposed during the mating time did not increase the numbers of embryos produced during mating. In the case of red and green LED lighting, embryo production was decreased, as seen in previous studies by others [19].

Again, we examined the likelihood that fish would spawn when exposed to the different colored lights. When fish were mated in room light they spawned 46.7% of the time, when exposed to red light they spawned 71.4% of the time when exposed to green light they spawned 62.5% of the time, and when exposed to blue light they spawned 54.6% of the time. Overall, these data indicated that the wavelength and the intensity of light that zebrafish were exposed during the mating time increased the rate of successful matings.

Conclusions

We hypothesized that we could maximize zebrafish embryo production by modifying environmental factors such as the color of light that fish were exposed during mating. However, this modulation did not increase the number of embryos produced. In fact, in the iSpawn-S experiments, red and green light had a negative overall effect on the embryo production.

We also found that light had some effect on the number of successful matings. In the light box experiments, there was little difference in successful matings under different light conditions, except for the blue light, which generated significantly less successful matings. In the iSpawn-S experiments, exposing the fish to any color besides the room lights increased the chances of obtaining embryos.

Limitations

One limitation of this study was that the light intensities varied in the treatment groups. However, we found that embryo production did not directly correlate with the intensity of light were the fish exposed during the treatment. For the isolation box experiment, the numbers of embryos generated by the room light (228.2 lumens/m^2) compared to white fluorescent light in the isolation chamber (2099 lumens/m^2) were not statistically different, even though the light intensity was almost 9× less in the room. Red (226 lumens/m^2), blue (366 lumens/m^2), and green fluorescent light (624.3 lumens/m^2) were also much lower than the white fluorescent light, but this had little effect on the fecundity. In the iSpawn-S experiments, the LED intensities were much lower; red light (6.5 lumens/m^2), blue light (2.2 lumens/m^2), and green light (28 lumens/m^2) was lower than the white fluorescent light by between 75–955×. While the optimal intensity of light for zebrafish mating has not been experimentally determined, it is recommended to be between 53.82–322.92 lumens/m^2 when measured at the surface of the water [20]. However, previous studies had shown no correlation in spawning efficiency when fish were exposed to light intensities ranging from 161.46–1829.86 lumens/m^2 [21]. Our LED lights were far below this range, and the white fluorescent light in the isolation chamber was above it. Regardless, the light intensity did not appear to correlate with the number of embryos produced. Research into this in the future might be warranted, however, to see if there are optimal intensities for stimulating embryo production.

Alternative Explanations

Zebrafish spawn in clear, shallow floodplains [22] at dawn [23] [24] during the rainy season [22]. We hypothesized that preference for such area relied on preferential sites to leave developing eggs [24] and also on the lighting conditions. The intensity of light as it hits the surface of water varies by the time of day and seasons. At dawn, sunlight hits the water surface at a low angle and from a long atmospheric path, resulting in a reduced intensity and shorter wavelengths [25]. Additionally, the rainy monsoon season is rich in UV and blue light [26]. In essence, zebrafish spawn preferentially at times when blue light is the highest. It was plausible that blue light might affect the mating behavior of zebrafish by functioning as a signal for time of day and seasonality. However, blue light did not positively affect zebrafish embryo production and reduced the chances of successful mating encounters. One explanation for this is that our facility lights have blue wavelengths and that increasing the blue light during mating had little effect. It could also be because our blue lights also had detectable levels of the green light presence; they are not just giving off light in the blue spectrum.

Water also acts as a chromatic filter by absorbing different wavelengths of light; the intensity and spectral characteristics of the light altered at different depths. Light at wavelengths within the IR, red, violet and UV range is absorbed more strongly, while light in the middle of the visible range is not [26]. In this way, wavelengths of the light filtered through water may also instruct zebrafish the proper depth for successful mating, controlling the fecundity.

Zebrafish have color vision [27]; studies training zebrafish to respond to appetitive stimuli indicate that adult fish prefer swimming towards green, red, and UV stimuli [27]. Importantly, fish can adapt their photoreceptor sensitivity [28]; teleosts shift opsin expression in their cones to respond to different light environments [29]. Zebrafish can aggregate or disperse melanophores in response to various stimuli such as aggression [30] and light intensity [30] [31]. This ability to sense and respond preferentially to different colors, adapt photosensitivity, and change color in environmental situations may explain the results of our study.

Conjectures

Our data indicate that light on its own does not increase the number of embryos produced during mating; red and green light actually decreased the number of embryos produced in the iSpawn-S experiments. While it is hard to control for every variable in these types of experiments, future research might focus on modulating lighting in conjunction with water or room temperature, pH, water depth, stress hormone production, and diet. Future studies might also focus on regulating the timing of the light exposure as well as controlling the potential of fish to adjust in a physiological or behavioral manner to the lights they are being exposed.

Additional Information

Methods

Fish husbandry and care

AB wild type fish were utilized for this study. Zebrafish were housed in a 700 L recirculating zebrafish aquarium system (Aquatic Enterprises, Seattle, WA) regulated by a Profilux 3 Outdoor module that regulated salinity, pH, and temperature (GHL International, Kaiserslautern, Germany) 24 h a day. The facility was illuminated on a 12 h light/12 h dark cycle. Zebrafish were fed once a day with hatched brine shrimp (Brine Shrimp Direct, Ogden, UT) and once a day with Gemma micro 300 (Skretting, Westbrook, ME).

Isolation box experiments

An isolation box was constructed from ¾" plywood and placed over the breeding tanks. The box was designed to accommodate 8 mating chambers total and hold fluorescent

light bulbs on its ceiling to create specific lighting conditions, as well as exclude lighting from the surrounding area. The box was divided into 4 equal chambers so that 2 tanks were exposed to red light, 2 tanks were exposed to blue light, 2 tanks were exposed to green light, and 2 tanks were exposed to soft white light simultaneously. Inside the top of the box was a standard E26 light bulb socket wired to a timer. Treatment lights were fluorescent 13 W bulbs in red (BPESL13T/R, Feit Electric), blue (T3 Twister, Phillips Lighting), green (BPESL13T/G, Feit Electric), and soft white (13 W, Feit Electric) coloring. Facility lights were used as a control (see Fig. 1B for wavelengths). The isolation box lighting was synchronized with the facility lights, which allowed fish to maintain their 12 h light/ 12 h dark cycle. Light wavelengths for the fluorescent bulbs were assessed inside the isolation box with a spectrometer (USB2000, Ocean Optics, Dunedin, FL), and light intensity was assessed in the same location with a light meter (401027, Extech Instruments, Nashua, NH).

Experimental design: breeding tank setup

Three 9 L tanks (Tanks A, B, and C) housing 30 6-month-old wt zebrafish each (10 males and 20 females) were utilized. Every week, 10 total crosses (2 females to 1 male) were set up in 10 separate DuraCross zebrafish breeding tanks (Laboratory Products Sales, Rochester, NY). All breeding tank inserts were inclined to create a depth gradient within the chamber. Every Monday, all the fish in Tank A were mated to each other. This was repeated for Tank B on Wednesday and Tank C on Friday. In this way, every week each tank of fish was mated. These matings were subjected to our modulated lighting conditions. This pairing continued for 16 weeks.

Experimental design: lighting

Treatment began at the onset of the dark-cycle (21:00 h). At this time, the isolation box was placed over 8 breeding chambers and left there overnight. The next morning at the transition from the dark-light cycle (09:00 h), the timer illuminated treatment lights inside the box. This treatment lasted for 4 h. At the end of the treatment, embryos were collected, placed in embryo media, and counted the same day. A control treatment (with room lights) was carried out at the same time; 2 mating chambers were left on the counter next to the isolation chamber, exposing the fish to the facility lighting for 4 h. After completion of treatment, individual fish were put back into their designated housing tanks. Room temperature, humidity, water temperature, water conductivity, and water pH was also recorded every week. The embryos were placed in a 28℃ incubator and counted later that afternoon. Viability of the embryos was assessed the next day.

iSpawn-S experiment

An iSpawn-S system (Techniplast, Westchester, PA) with minor modification was utilized for breeding; LED light bulbs were placed into the top of the plastic lid. Large dark bags were placed over the iSpawn-S to exclude lighting from the surrounding area. Treatment lights were adjustable LED deck lights in red, blue, and green (Paradise lighting, Model GL34001SS6). Facility lights were used as a control (see Fig. 1D for wavelengths). The lighting was synchronized with the facility lights, which allowed fish to maintain their 12 h light/ 12 h dark cycle. Light wavelengths for LED lights were assessed inside the iSpawn-S with a spectrometer (USB2000, Ocean Optics, Dunedin, FL), and light intensity was assessed in the same location with a light meter (401027, Extech Instruments, Nashua, NH).

Experimental design: breeding setup

Two 9 L tanks (Tanks D and E) housing 8 6-month-old wt zebrafish each (4 males and 4 females) were utilized. Every week 2 females were removed from Tank D and 2 females were removed from Tank E, and placed in the bottom section of the iSpawn-S. 2 males were then removed from Tank D and Tank E, and placed in the top section of the iSpawn-S. In this way, there were 4 females and 4 males per breeding. Every week each tank of fish was mated. These matings were subjected to our modulated lighting conditions. This pairing continued for 20 weeks.

Experimental design: lighting

Treatment began at the onset of the dark cycle (21:00 h). At this time, bags were placed over the iSpawn-S and left there for overnight. The next morning at the transition from the dark light cycle (09:00 h), the timer illuminated treatment lights inside the device, and the separator between males and females was removed. This treatment lasted for 4 h. At the end of the treatment, embryos were collected, placed in embryo media, and counted the same day. Control treatments (with room lights) were also performed. After mating, individual fish were put back into their designated housing tanks. Room temperature, humidity, water temperature, water conductivity, and water pH was also recorded every week. The embryos were placed in a 28℃ incubator and counted later that afternoon. Viability of the embryos was assessed the next day.

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Ethics Statement

Wildtype (wt) zebrafish used in these studies were raised and maintained in accordance with the California State University, Chico Institutional Animal Care and Use Committee (IACUC) guidelines. The IACUC committee approved all the experiments before they were performed.

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