Millersville University of Pennsylvania

# Impact of Light Cycles on Behavior of Marine Fish

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Abstract

Behavior changes in response to light and dark cycles have been shown in many different organisms. To examine the effect of 24-hour light cycles on aquatic animals mummichogs (*Fundulus heteroclitus*) were exposed to a 24-hour light cycle. The controls for this experiment were held under normal long- and short-day light cycles. Behavior and body mass were evaluated for each group. It is hypothesized that there will be a difference in behaviors and body mass in the treatment group compared to the control groups. Fish exposed to 24-hour light cycles did exhibit different exploratory behavior but showed no difference in social behavior. Body mass reflected normal growth in all three treatments, suggesting no impact of stress.

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Introduction

Circadian rhythm is the natural clock that is based on the day-night cycles that affect physiological and behavioral traits in many organisms and most animals have innate responses to changes in light cycles. Feeding, reproduction, spawning, and migration are among the many behaviors that occur rhythmically and for many animals are triggered by changes in daylight cycles. Light cycles act on animals by triggering the release of melatonin, which is the key hormone that drives circadian rhythms in animals. During the day, there is little melatonin released, with a large amount being released at night (Meissl & Ekström, 1988). These changes in melatonin are crucial to circadian rhythms and the timing of different behaviors and are tied to light and dark cycles which are an important component of circadian rhythm.

Natural changes in light cycles, such as seasonal changes, provide natural triggers for animal behaviors. Photoperiodism is referred to as an organism's ability to calculate the amount of daylight in the environment (Chen et. al., 2020). When this period is disrupted, there is the potential for disrupting natural behaviors essential for survival.  For example, research on oysters in oyster farms has shown that when exposed to light, the oysters responded by slowly opening the mantle gap, in contrast to no open gap during the dark cycles (Wu et al., 2015). This indicates that feeding behavior in oysters is determined by the amount of light present, and constant light could result in constant feeding. Reproduction also relies on seasonal changes. Japanese quail breed in the spring making them long-day breeders and sheep breed in the fall making them short-day breeders, each having gestation periods to ensure optimal birthing times during early summer (Nicholls et. al., 1983; Legan & Winans, 1981).

Studies have shown that photoperiod can affect feeding behaviors and predator-prey interactions (Zhdaova & Reebs, 2006). Rainbow trout demonstrate a circadian rhythm when feeding. Feeding activity occurred only during light hours when exposed to 45-minute cycles between light and dark. Light for 24-hours resulted in significantly more feeding activity and movement (Sánchez-Vázquez & Tabata, 1998). The increased movement during light periods shows how the trout rely on light in order to forage. Other studies of circadian rhythms in fish have shown that feeding activity habits do not rely on the time of day, instead relying on prey availability (Sánchez-Vázquez et al., 1997, Sánchez-Vázquez et al., 2001). A study of inangas (*Galaxias maculatus*) found that predation risk resulted in decreased motion but did not change the natural circadian rhythm (Reebs, 1999). Additionally, research done on predation risk factors in fish in relation to circadian rhythms found that fish will not change their circadian clocks to avoid predators (Pettersson et al., 2001). The fact that these fish maintain circadian rhythm even in the face of predation indicates that circadian rhythms are crucial for survival resulting in the evolution of tactics to avoid predation while maintaining circadian rhythm behaviors. While light cycles may not be a factor in avoiding predation or influencing feeding activity in fish, research on zebrafish found that spawning rates are influenced by light with 74% of spawning occurring during 14 hours of light and peaking 3 hours after the light was turned on. Blanco-Vives & Sánchez-Vázquez (2009) found that when darknesses was applied to the peak spawning hour, the fish shifted to spawning 7 hours after initial light exposure. Studies of the circadian rhythm in mummichogs showed that spawning is triggered by moonlight instead of daylight (Taylor et al., 1979). These studies show that organisms rely on circadian rhythms in order to spawn at the right time for offspring survival.

Although it’s clear that light cycles are important to animals, and changes to light cycles can affect behavior, there are some animals that do not exhibit circadian rhythms (Bloch et al. 2013). The eyeless cave Mexican tetra (*Astyanax mexicanus*) maintains constant oxygen consumption throughout day and night, compared to the surface tetra which varies consumption throughout the day (Moran et. al. 2014). The cave tetra has lost the ability to detect light and has adapted to no longer rely on circadian rhythms to regulate its physiology. A lot of herbivores also exhibit no circadian rhythm. For example, voles show an ultradian cycle during feeding, which is a 90 minute on, 20 minute off period throughout the day (Bloch et al. 2013). Animals that do not rely on light cycles to trigger behaviors may have an adaptive advantage; however, for animals that have behaviors that are tightly tied to light cycles, removing the natural light cycle may have detrimental effects.

The mummichog, *Fundulus heteroclitus*, is a common intertidal species with a native range along the mid-Atlantic coast in bays, estuaries and tidal creeks (Hardy, 1978). They range in size from 51 to 102 mm and feed on algae, *Spartina,* and small crustaceans such as fiddler crabs and copepods (Armstrong & Child, 1965; Fritz, 1974; Hughes & Sherr, 1983). Mummichogs can breathe and move on land and a study done by Bressman et. al. (2016) found that mummichogs rely on light and visual cues to navigate towards water after terrestrial docking. Fish held under light moved towards the water basin and the fish that were exposed to a tank with foil on top of the water, reflecting light, also moved towards the basin. However, fish held in darkness showed no movement to the water basin, indicating that the light helps them navigate to water. One of their most characteristic behaviors is the formation of large schools and the common name, mummichog, is a Native American word that loosely translates as “travels in groups”, a reference to this schooling behavior (Nichols & Breder, 1927). Because this species adapts well to captivity and it is easy to keep, they are ideal subjects for research. For this study, *Fundulus heteroclitus*, will be used to compare behavioral changes when exposed to a 24-hour light cycle compared to normal short- and long-day light cycles.

The distinction between shoaling and schooling has only recently been clearly defined; however, these terms are often used interchangeably. A shoals is an aggregation of individuals, whereas a school is defined as a coordinated swimming group (Pitcher, 1983; Pitcher, 1998; Miller & Gerlai, 2012). Previous research on schooling behavior in mummichogs considering all groups of fishes as schools as the distinction between shoals and schools had not yet been clearly defined. References to mummichog schooling did not distinguish between coordinated and uncoordinated groups and although schooling is widely recognized in mummichogs, it's important to note that their aggregations are actually more like shoals rather than schools.

Mummichogs are known for their schooling behavior with groups that can consist of 2 to 3 individuals up to a hundred individuals (Hildebrand & Schroeder, 1928). In terms of schooling distance, Symons (1971) found that mummichogs regularly space themselves with the nearest neighbor one fish length away, but larger distances between fish resulted in irregular spacing. Another study found that across a three-day cycle of monitoring in a lab, mummichog schooling behavior decreased throughout time (Salierno et. al., 2008). At the beginning of the experiment tight schooling behaviors were exhibited, but as time went on schooling distance loosened, and this was interpreted as an adaptation to the new environment. These studies suggest that mummichogs respond to changes in their environment by exhibiting different schooling behaviors and studies suggest that tighter schooling is associated with novel environments, fearfulness and the presence of stressors.  Furthermore, circadian rhythms in mummichogs are impacted by school size. Single individuals and large schools of 25 or more demonstrated more movement in darkness compared to 24-hour light but schools of 5 demonstrated a varied cycle of movement, with no differences in movement in the dark compared to the light (Kavaliers, 1980). This indicates that individuals and large schools move around more at night due to lower predation factors but the schools of 5 feel threatened all the time. Mummichogs are also shown to engage in intra-season migrations due to prey availability (Haas et. al., 2009). This suggests that mummichogs are often on the move and regularly explore their environment in order to forage.

One non-invasive means of assessing whether or not a treatment has an effect on an organism is to conduct behavioral assays, which can be used to understand whether individual behaviors are influenced by exposure treatments. Social behavior tests done in zebrafish (*Danio rerio*) which school heavily, found that schools of fish learned how to avoid and escape predators more compared to individual fish (Nunes et. al., 2017). In brown trout (*Salmo trutta*), fish were placed under cups in order to test exploratory activity. Individuals who were more active left the cups more often, which suggests those individuals that move around their environment may fare better in the wild, perhaps having an advantage in terms of ability to forage and escape when faced with predation or environmental challenges (Lothian & Lucas, 2021). The use of a behavioral assay in this study will provide a non-invasive and non-lethal means of testing the impacts of treatments on individuals.

When first built, light timers were installed in the aquatic rooms at Millersville University. Many of these have since fallen into disrepair or become obsolete, and the result is that the lights in some of the aquatics rooms remain on continuously. Professors and student-researchers keep a variety of marine and freshwater animals in these rooms and as a result these creatures are removed from a natural light-dark cycle to a 24-hour light cycle. The impact on these animals has not been assessed but given the importance of light cycles to most animals this is likely to have some detrimental impact on the animals. For this study, changes in body mass and behavior will be assessed in the estuarine fish, *Fundulus heteroclitus* to determine if there are differences between animals kept in 24-hour light versus controls kept at natural long and short-day light cycles. It is predicted that changes will occur and that the animals kept in a 24-hour light cycle will exhibit signs of stress both in terms of change in body mass and behavior. If the 24-hour light cycle is stressful for the fish we would predict lower fitness as measured by lower body mass, and perhaps less exploratory and social behavior compared to the control fish.

## Methods

*Fundulus heteroclitus* were collected from tidal creeks and the intertidal region around Chincoteague Bay, Virginia, using standard minnow traps. Fish were transported to Millersville University and housed in groups of six in 10-gallon tanks. Each group of fish was weighed before entering the treatment period. During the entire experiment, *F. heteroclitus* were in one of three treatment groups. The first treatment group (treatment one) consisted of 12 fish, in 2 separate tanks, exposed to a natural long-day light cycle, which for Chincoteague Bay is 14 hours and 50 minutes. The second treatment group (treatment two) consisted of 12 fish, in 2 separate tanks, exposed to a natural short-day light cycle of 9 hours and 34 minutes, consistent with the shortest light cycle at Chincoteague Bay. The third treatment group (treatment three) was 12 fish, in 2 separate tanks, exposed to a 24-hour light cycle which is representative of the light cycle in some aquatic rooms at MU. Tanks for treatments one and two had lights on timers suspended over the tank with the tanks wrapped in black plastic to eliminate any additional light. The two tanks for the 24-hour light treatment had an overhead light and were held in a room with the lights kept on. Treatments lasted for six weeks. Fish care consisted of daily feeding and water testing twice each week. Water changes were made as needed to support a healthy tank environment.

After the six-week treatment period *F. heteroclitus* underwent a suite of behavioral tests to understand whether group behaviors were influenced by different light cycles. Each test was conducted in 40 x 50 cm plastic basins with a 5 cm grid on the floor (Figure 1). Each test consisted of a 5-minute habituation period and a 15-minute observation period that was recorded on a digital camera. All six fish were added to the basin at the same time. The tests examined exploratory behavior and sociality. Exploratory behavior was measured by counting the total number of times each fish crossed one of the lines on the arena. The fish had to be completely over the line to count and an immediate cross back over equaled two lines crossed. Pictures were taken every 30 seconds during the 15-minute period and distance was measured between each fish at each time slot using ImageJ. After treatment, all fish were once again weighed. Body mass for the three treatment groups was compared as a possible indicator of fitness before and after treatment.

Models were run for dependent variables: lines crossed and schooling distance. Model 1 used treatment as a fixed effect, with tank as the random effect. Model 2 removed treatment and used 1 as the fixed value. A general linear mixed model was used to determine if treatment is the cause of differences between tanks. Data were analyzed using Excel. Linear mixed-effect models, ANOVA, chi-squared tests, and Student's t-test were used on RStudio.

**Model 1:** Dependent variable ~ treatment + (1|tank)

**Model 2:** Dependent variable ~ 1 + (1|tank)

Fish were collected under Virginia Scientific Collect Permit 23-016. All care and treatment was approved by the Millersville University Institutional Animal Care and Use Committee (IACUC) (ref. 24-4).

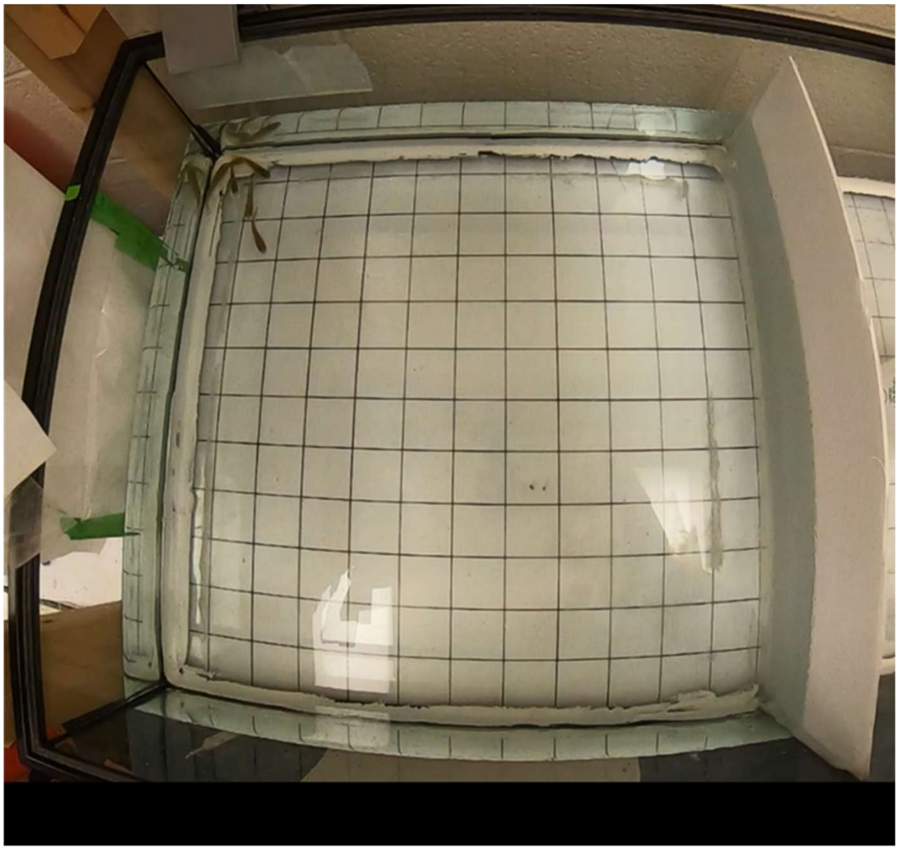

**Figure 1.** Arena (40x50 cm) used for behavior trials with a 5 cm grind. Fish were placed into the arena and given a 5-minute habituation period. Video was then recorded for 15 minutes.

Results

We focused on the impacts of light cycles on exploratory and social behaviors. There was no significant difference in schooling behavior ($X^2$= 0.0456, df= 2, p-value= 0.9774, Figure 2). However, there was a significant difference in the number of lines crossed ($X^2$= 6.144, df=2, p-value= 0.046; Figure 3). Fish in the 24-hour light cycle crossed fewer lines compared to the short and long light cycles.

In terms of body mass, weights did differ significantly before and after the treatment, which is expected due to daily feedings (t=3.41, df=10, p-value=0.006; Figure 4). Weights between treatments did not differ significantly ($X^2$= 10.765, df=5, p-value=0.056).

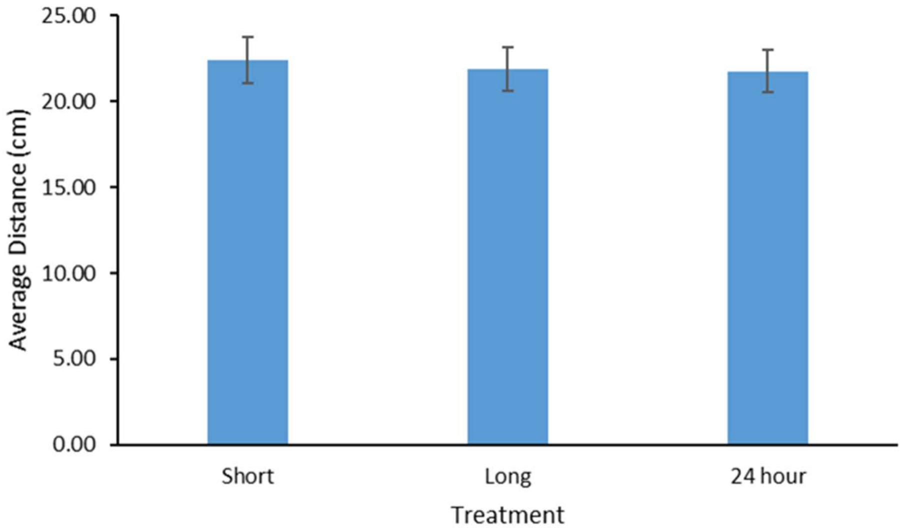


**Figure 2.** Average distance between each fish over a 15-minute period (n=12 per treatment). There was no significant difference in schooling distances between treatments. Error bars denote SE.

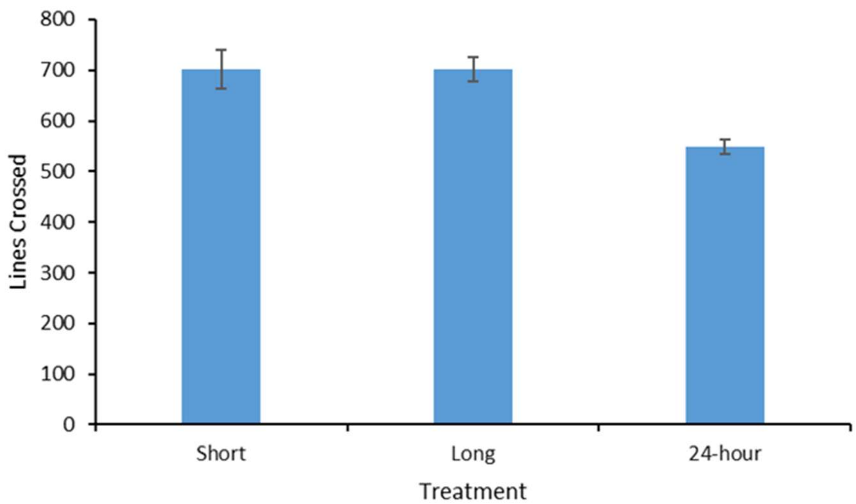


**Figure 3.** Total number of lines crossed per fish over a 15-minute period (n=12 per treatment). Significantly fewer lines were crossed in the 24-hour group. Error bars denote SE.

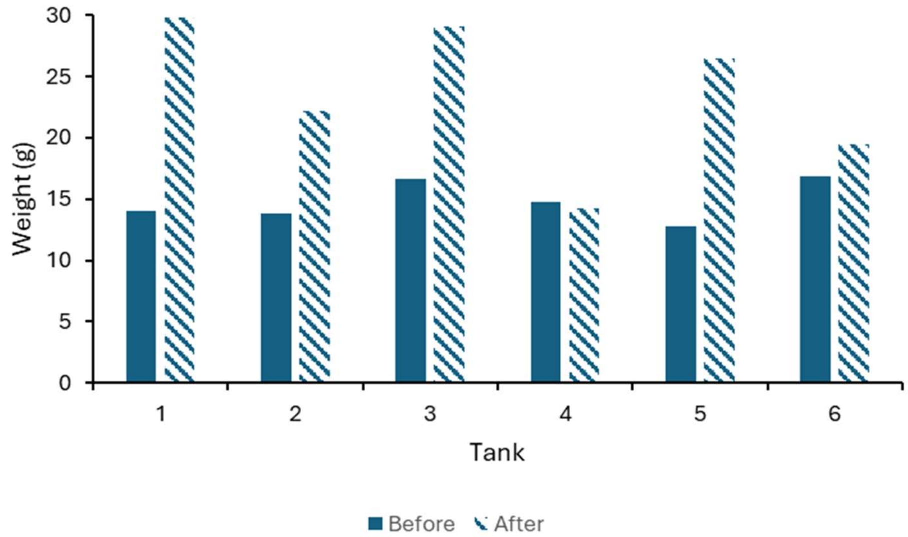


**Figure 4.** Total body mass of fish per tank before and after treatments (n= 6 per tank). There was a significant difference in mass after the treatment but there was no difference in weights between treatments. Solid bars represent weights before treatment. Dashed bars represent weights after treatments.

Discussion

Many studies have addressed the impact of circadian rhythm on physiological and behavioral traits in many organisms, with most organisms experiencing innate responses to changes in light cycles. However, little is known about the impact of 24-hour light cycles on the behavior of captive fish.

Daily observations of the fish in the 24-hour light cycle indicated differences in behavior with these fish staying towards the top of the tank and being more skittish when the tank was approached. Data from the behavioral assay showed that these fish crossed significantly fewer lines, suggesting less exploration among fish exposed to a 24-hour light cycle. Naturally, mummichogs perform intra-season migrations due to prey changes, meaning there is constant movement towards or away from the shore (Haas et. al., 2009). In their natural environment mummichogs would typically engage in high activity levels. However, exposure to a 24-hour light cycle reduced activity level, measured as fewer lines crossed. Lower activity levels are correlated with exploratory behavior, and it can be predicted that if constant light limits exploration, it will, in turn, likely limit foraging and mate seeking as well and this does have implications for captive fish by altering their natural behaviors.

Exposure to a 24-hour light cycle did not affect schooling behavior and fish in all three treatments exhibited the same social behavior with all groups spaced evenly throughout the time trial. During the trials it was observed that Only one to two of the bigger fish left the group at a time and often came back into the group within the 30 second increment So while the results indicate no significant difference in distance between fish, this analysis did not adequately capture individual variation exhibited by some fish that moved away from the school. While these results indicate no difference in schooling between the three groups, another possible explanation for the same grouping patterns may be the limiting range for spreading. In the 40 x 50 cm arena, the fish may have been limited by space and could not separate from the group. Symons (1971) found that in smaller areas with fish spaced at an average distance of one fish length, schooling occurred, but when placed in larger areas there was more spacing between fish. In the arena, the fish were evenly spaced with only one or two fish leaving the group at a time. It was also observed during the video trials that the presence of someone in the room caused the group to tightly school towards the back wall so human interaction was limited.

Body mass was calculated for the whole group of 6 per tank and individual variation was not measured. The increase in body mass from beginning to end reflected a weight gain from normal daily feedings. There was no difference in weight between treatments. However, tank 4 (long-day) did see a slight decrease in weight and tank 6 (24-hour) did not gain as much weight as the other four (Figure 4). Stress is well known to impact or shunt growth in many organisms (Canosa & Bertucci, 2023). Because there was no difference in weights between treatments, we can predict that the increased light exposure did not cause enough stress to impact growth. It is worth noting that the fish in the 24-hour light cycle may have been stressed, with impacts to feeding, but regular feeding in a captive environment ensured sufficient nutrition and is not comparable to feeding in the wild. It's possible that body mass may have been impacted if the captive fish had to hunt and feed as they would in their natural environment. Weights may have also been impacted by gonadal development. Mummichogs' gonadal development is shown to be accelerated by water temperature, with warmer temperatures in the spring and lower temperatures in the fall (Shimizu, 2003). However, when they are held under constant temperature, photoperiods of 16 hours of light cause the normal circa-annual rhythm of gonadal development in spring and fall (Shimizu, 2003). Since we collected fish in early September, and moved them to an environment of warmer temperatures and different light cycles than they would have experienced in the wild at this time of year, they may have started gonadal development which may in turn have impacted their weight This was not assessed but there was no morphological evidence of the fish entering a breeding phase since no change in coloration occurred.

Overall, the goal was to determine if 24-hour light cycles had an impact on behavior of mummichogs. It was found that constant light does result in less exploratory behavior but does not affect social behavior. Decreased exploration has implications for reduced foraging and mate-seeking and highlights the need for proper light conditions in aquatic rooms at Millersville.

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