Effect of light on aggregational behavior of planarian *Dugesia tigrina*

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Introduction. Planarians are renowned for their regenerative ability due to pluripotent stem cells, as well as their peculiar photophobic response. However, few facts are known about their aggregational behavior. This study aims to reveal the effect of light on aggregational behavior. Reynierse (1966) suggested that light has a negative effect on the formation of aggregations. However, one of his objectives for aggregational behavior was inappropriate. This study reevaluated the effect of existence of light on aggregational behavior, as well as ascertained the effect of wavelength on the formation of aggregations.

Methods. In this study, the ratio of individuals participating in aggregations was measured as a criterion to determine aggregational behavior. Aggregational behavior was measured after two hours from the initial exposure to different light sources. The behaviors under white LED light and under shade were compared, as well as the behaviors under five different light sources: infrared lamp, red, green, blue LED, and ultraviolet lamp.

Results. The existence of light interfered the formation of aggregations (t-test, $p < 0.0001$), which supports the former study of Reynierse. Also, aggregational behavior differed under different wavelengths (ANOVA, $p < 0.0001$). Except for the infrared light which emitted a wide range of wavelengths, the behavior showed hierarchy: decreasing aggregational behavior in accordance with decreasing wavelength. UV light has the most significant negative effect on the formation of aggregations.

Discussion. Exposure to light caused negative effects on performing aggregational behavior. Participation in aggregations appears to be influenced by photophobic response, especially under lights of short wavelength. Disintegrating aggregations under exposure to lights can potentially bring evolutionary benefit. This behavior possibly makes the aggregating planarians altogether exposed to a higher risk or predation, considering that they lack defense mechanisms. Planarians can lower the risk and continue the populations by disintegrating the aggregational behavior under the existence of UV and lights of higher wavelength, which are indicatives of daytime. Understanding aggregational behavior of animals of a lower order would give better insight on general herding behavior, and potentially help interpreting more complex behaviors of higher animals.

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Introduction

Grouping behaviors are widely observed in a wide range of animals, in which a number of individuals participate in movements to form a group. It includes herds of livestock, schools of fish, groups of birds (e.g. broods of hens, colonies of penguins, and gaggles of geese), and swarms of insects. Participating in groups helps protect populations from predators and parasites by causing dilution effect (Mooring & Hart, 1992), also known as 'selfish herd'; this describes the spatial dynamics of individuals within a group whereby an animal moves toward the center of the group to pass on the risk of predation and parasitism to other group mates (Hamilton, 1971). Grouping also increases the net energy intake and reproductive opportunities (Bertram, 1978; Pulliam & Caraco, 1984).

Freshwater macroinvertebrates are known to aggregate (Elliott, 1977; Downing, 1979; Murphy *et al.*, 1998). The formation of aggregations could be due to the direct benefits that are mentioned above, as well as the influence of extrinsic factors such as velocity and depth of water, compositions of substrates, the amount of detritus, and interactions such as competition and predation (Allan, 1975; Minshall & Minshall, 1977; Orth & Maughan, 1983; Erman & Erman, 1984; McAuliffe, 1984; Lancaster *et al.*, 1991; Kohler, 1992).

Planarians are a group of mostly free-living flatworms of the Class Turbellaria (Phylum Plathyhelminthes). The term 'Planaria' refers to a genus. However, the name 'planarian' is not designated to specific enclosed group, but to any member of the family Planariidae and sometimes of related families (Gremigni, 1979; Vries & Sluys, 1991; Campbell & Reece, 2005). Planarians are generally aquatic, and they have crossed eyespots that can detect light.

Freshwater planarians are usually observed in small flocks in both natural and captive environments. In nature, they can be easily found on ground-facing rock surfaces in slowly flowing streams. In many cases, planarians allocate themselves close to the other individuals and form aggregations; the group size ranges from two up to ten. In captivity, they are found in flocks, where, on the walls or at the corners of water tanks, the group size can reach up to 25 individuals. Pearl (1903) noted that the size of the group ranges from six to twenty individuals, again supporting the observations. According to several studies (Reynierse, 1967; Reynierse *et al.*, 1969), the behavior seems to be general among planarians, including *Phagocata gracilis*, *Cura foreman*, *Dugesia tigrina*, and *D. japonica*.

Despite its prominence, the herding behavior of planarians are barely studied. Researches have used different terms: 'the formation of collection (Pearl, 1903)', 'formation of aggregation (Reynierse, 1966)', 'grouping (Cash *et al.*, 1993)'. In this study, this peculiar behavior will be defined as 'aggregational behavior'. Pearl (1903) noted that the aggregational behavior of planarians is a result of either photokinesis or chemokinesis, or possibly both. Reynierse (1966) suggested that the formation of the aggregations is to be interfered with under bright environments. However, one of the objectives he used to determine the aggregational behavior was inappropriate: the ratio of free swimming individuals. Planarians without participating in aggregations are generally static, not motile. Therefore, the criterion was irrelevant to the aggregational behavior, suggesting the needs for revaluation. Cash *et al.* (1993) revealed that *D. tigrina* gains trophic benefits from group living, but it is not clear whether the benefit is an evolutionary cause or a consequence of group living, especially considering that group foraging is generally considered to be a result rather than a cause of gregariousness (Packer & Ruttan, 1988; Vickery *et al.*, 1991).

There are a few possible mechanisms that explain the relationship between the existence of light and the aggregational behavior. It is plausible that the aggregational behavior might be a type of photosensory organ-protecting mechanisms that are widely selected by diverse groups of the Kingdom Animalia (Hendler, 1984; Griffiths, 1994; Fernald, 2000). In this case, light will positively affect the formation of aggregations, as well as possibly make the individuals show the 'selfish herd'. This will cause a positive relationship between the existence of light and the aggregational behavior. Otherwise, the existence of light may not be the cause of the aggregational behavior, but the behavior is interfered with by photophobic response. Photophobic response is a negative phototaxis that is widely observed in planarians (Walter, 1907; Arees, 1986). Formation of static aggregations can be interrupted by light-triggered movement, which leads to negative relationship between the existence of light and the aggregational behavior. The latter will be consistent to the study of Reynierse (1966) that suggested the formation of aggregations is interfered by light.

This study aims to reevaluate the effect of the existence of light on the aggregational behavior. Also, the aggregational behavior may differ under different wavelength if the negative phototaxis affects the formation of the aggregations. Paskin *et al.* (2014) suggested that planarians perform hierarchical photophobic responses under different light sources; light of the shortest wavelength (UV) caused the most intense response, and IR and red light produced no significant reaction. Another objective of this study is to check the aggregational behavior under different sources of light. Experiments were conducted with *Dugesia tigrina*, a free-living freshwater planarian species which is widely distributed across America.

Methods

Individuals of *Dugesia tigrina* were collected from Strawberry Creek, Berkeley, California, USA (GPS coordinates of two sampling sites are N 37˚ 52.223' W 122˚ 15.582' and N 37˚ 52.317' W 122˚ 15.842'). Stream water was collected from the sampling sites and used for experiments. Planarians generally eat small animals such as worms and snails. In this experiment, dried boiled-yolk was provided once every two weeks.

Aggregational behavior was quantified by measuring the percentage of individuals participating in aggregations. Aggregational behavior cannot be affirmed if a significant number of individuals turn out not to be participating in aggregations. Therefore, the ratio of individuals participating in the aggregations was used as a criterion to determine aggregational behavior.

Aggregations with two or more individuals were included, and the distance between two individuals engaged in an aggregation was less than 7 mm, which was the average body size.

The experiments were conducted in 20 × 10 × 10 cm^3 transparent cuboidal water tank, and the depth of water was 8 cm. Former studies (Pearl, 1903; Reynierse, 1967; Reynierse & Gleason, 1975) suggest the possibility of the existence of chemotactants, which are thought to induce aggregational behavior. Therefore, stream water was changed after every experiment was done in order to remove possibly-remaining chemotactants secreted by planarians. Planarians were evenly distributed on the bottom of the water tank by manipulative locating.

Planarians were exposed to white LED light to check the effect of the existence of light on aggregational behavior. The lamp was placed 50 cm above the water tank, and the intensity of light was 920 ± 10 lx. For comparison, planarians were located in a dark room with less than 3 lx of light intensity. The percentage of individuals participating in the aggregations was measured after two hours. The experiments were repeated 30 times, and t-test was applied to compare the aggregational behavior under light and shade by using R (R Core Development Team, 2013).

Five different light sources were used to test the effect of different wavelength on the aggregational behavior. Light sources similar to the ones used for the study of Paskin *et al*. (2014) were used: infrared (650 – 1000 nm), red LED (620 – 680 nm), green LED (510 – 550 nm), blue LED (450 – 480 nm) and ultraviolet (315 – 400 nm) lamp. Bulbs were placed 50 cm above from the water tank, and the intensities of lights were controlled as 960 ± 20 lx. The experiments were repeated 30 times, and the results were compared with an ANOVA test, and then a Tukey post hoc comparisons by using R (R Core Development Team, 2013).

Results

The mean percentages of individuals participating in the aggregations differed under the existence of light and under shade (t-test, $t = -11.90$, $df = 56.61$, $p < 0.0001$). The mean percentage under white LED light was 55.47%, much lower than the percentage under shade, which was 77.67% (Fig. 1).

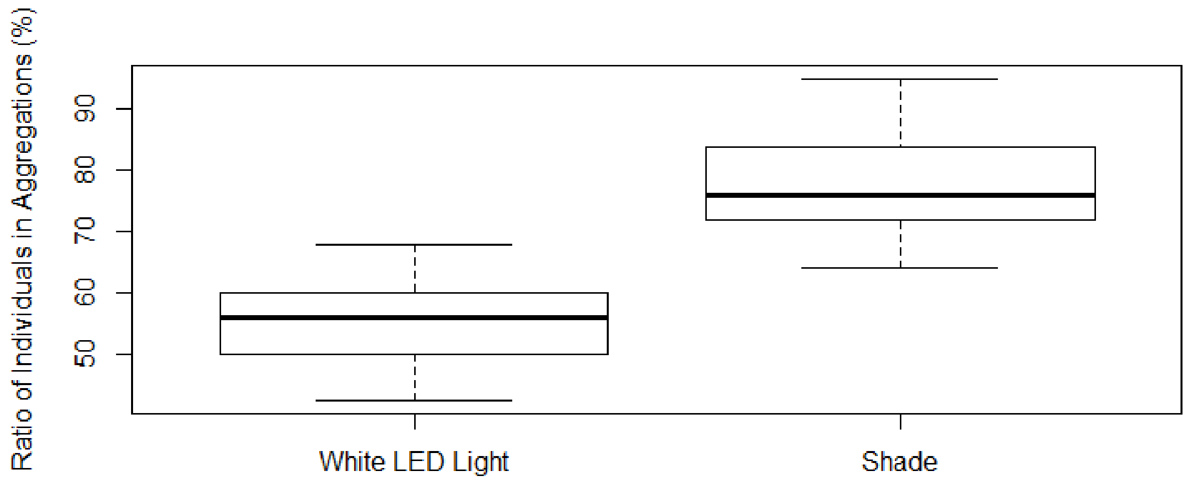

Figure 1. The percentage of individuals participating in aggregations under white LED light and under shade

The percentage of individuals engaged in aggregations under different wavelength differed (ANOVA, $F = 26.02$, $p < 0.0001$). Aggregational behavior was the least intense under the exposure of UV, significantly different to other light sources. The behavior under blue light differed from that under IR and red light, and there was no difference between the reaction under IR and red, IR and green, red and green, and green and blue (p-values of Tukey post-hoc comparisons are summarized in Table 1). The mean percentages of individuals participating in aggregations under IR, red, green, blue, and UV light were 73.82, 77.45, 70.43, 64.83, and 52.70 %, respectively (Fig. 2).

Table 1. Summary of Tukey post-hoc comparisons between five different light sources

	Infrared	Red	Green	Blue	Ultraviolet
Infrared		0.658	0.716	< 0.01	< 0.0001
Red			0.073	< 0.0001	< 0.0001
Green				0.231	< 0.0001
Blue					< 0.0005
Ultraviolet					

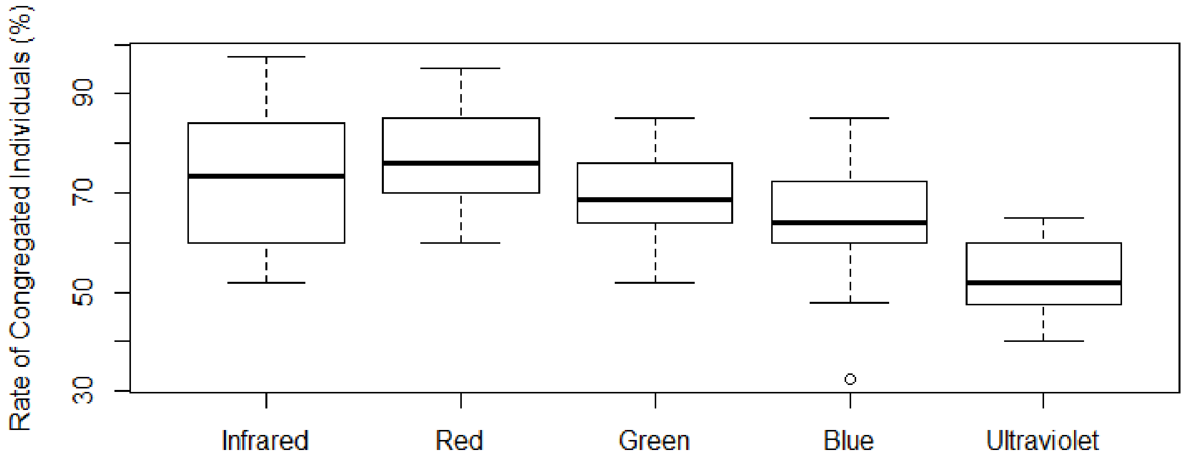

Figure 2. The percentage of individuals participating in aggregations under five different light sources: infrared (650 – 1000 nm), red LED (620 – 680 nm), green LED (510 – 550 nm), blue LED (450 – 480 nm) and ultraviolet (315 – 400 nm) lamp

Discussion

The percentages of the individuals participating in the aggregations under different conditions show that the existence of light is creating a negative effect on performing aggregational behavior. A much higher number of individuals were engaged in aggregations under shade compare to under exposure to white LED light. Also, light of lower wavelengths (which have higher energy) interferes with aggregational behavior more than others. Almost half of the individuals were motile and not participating in aggregations under exposure to ultraviolet light. Blue light reduced the percentage of individuals engaged in the aggregations as well.

These differential aggregational behaviors under different sources of light seem to be due to hierarchical photophobic response based on wavelength (Paskin *et al.*, 2014). According to the study, the strongest response was performed under exposure to UV, and the intensity of photophobic response decreased with the increase of wavelength. And the study noted that IR even caused opposite effects. It seems to be that light is negatively affecting aggregational behavior by making individuals to perform negative phototaxis. Planarians form aggregations spontaneously under shade. However, exposure to light forces planarians to show photophobic responses, makes them motile, and disturbs static aggregational behavior. Therefore, it is plausible to conclude that exposure to light is interfering with aggregational behavior by triggering planarians to perform photophobic response.

The negative effect of photophobic response on aggregational behavior could possibly bring evolutionary advantages. Planarians do not possess especially defensive mechanisms such as a rigid exoskeleton, venom, or a quick runaway response. Aggregational behavior would be hazardous for maintaining the populations of planarians with tender bodies by exposing the whole individuals in the aggregations and making them vulnerable under the presence of predators. Performing sensitive photophobic response toward ultraviolet and low-wavelength light, which is daylight-related, can be decisive for their survival. Disintegrating the aggregations under exposure to UV and blue light, which are indicative of daytime, will reduce the risk of being predated at the same time altogether, and possibly lead to better continuation of the population.

It is still not known what triggers the formation of aggregations. As proposed in several studies (Pearl, 1903; Reynierse, 1967; Reynierse & Gleason, 1975), the formation of aggregations seems to be a result of chemotaxis, especially considering that planarians perform significant chemotaxis (Mayamoto & Shimozawa, 1985). Contrasting to the study of Reynierse (1975) which suggested that planarians use both chemical and visual cues to perform aggregational behavior, it is more relevant to suppose that planarians are attracted to certain chemotactants since planarians' eyespots work for detecting the existence of light, not distinguishing figures or colors (Carpenter *et al.*, 1974). These certain chemotactants might possibly be secreted by other individuals, as mentioned in some studies, suggesting that planarians recognize conspecifics by chemical cues (Reynierse, 1967; Wisenden & Millard, 2001). Further study is needed to identify these aggregational behavior-inducing chemotactants.

According to a pilot study, planarians generally did not switch the position within their aggregation. Without specific stimulation such as unintended contacts from other wandering individuals, each individual in aggregation kept their initial position, which is unlikely to be interpreted by selfish herd theory (King *et al.*, 2012). Even though *Dugesia tigrina* is known to obtain nutritional benefits from group living (Cash *et al.*, 1993), group foraging is generally thought to be a consequence rather than a causation of gregariousness (Packer & Ruttan, 1988; Vickery *et al.*, 1991); hence, it is not likely to be a cause of aggregational behavior. Further investigation is required to figure out what caused the evolution of aggregational behavior.

Although it is not known what makes planarians aggregate and what caused the evolution, planarians still do aggregate somehow. Studying aggregational behavior of the animals of a lower order such as freshwater planarians would give better understanding for general herding behavior, which may be helpful to interpret more complex behaviors of higher animals.

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