Phototactic behavior of the marine harpacticoid copepod *Tigriopus japonicus* related to developmental stages under various light conditions

Hee-Jin Kim[1]*, Takahiro Yamade[1], Keisuke Iwasaki[1], Helen S. Marcial[1] and Atsushi Hagiwara[1, 2]

[1]Graduate School of Fisheries and Environmental Sciences, Nagasaki University, Bunkyo 1-14, Nagasaki 852-8521, Japan

[2]Organization for Marine Science and Technology, Nagasaki University, Bunkyo 1-14, Nagasaki 852-8521, Japan.

*Corresponding author.

Tel.: +81-95-819-2829

E-mail address: heejin@nagasaki-u.ac.jp (H.-J. Kim)

Abstract

Marine harpacticoid copepod *Tigriopus japonicus* is commonly distributed in the tide-pools and shows benthic behavior. To determine its phototactic behavior, the movement pattern was investigated with different light wavelengths (white, peaks at 460 and 570nm; blue at 470 nm; green at 525 nm; and red at 660 nm) and intensities (0.5, 2.0, 3.5, 5.0, 15.0 W/m^2) related to developmental stages i.e., nauplius and adult. The eyespot of the two developmental groups efficiently absorbed the light wavelength from 400 to 550 nm, while the level of absorbance was different. For the horizontal phototactic behavior, nauplii showed negative phototaxis with the all tested light wavelengths and intensities ranging 0.5-5.0 W/m^2, while they lost phototactic movement at 15 W/m^2 of all conducted light wavelengths except with the red light shown negative phototaxis. The adults showed negative phototaxis at 0.5 and 3.5 W/m^2, while positive phototaxis at 2.0 W/m^2 regardless of light wavelengths. The vertical phototactic movement was only monitored with adults. At 2.0 and 3.0 W/m^2, more than 40 % of adults showed planktonic behavior with the blue light. The results elucidate that *T. japonicus* has different patterns of phototaxis related to developmental stages which can be used to manipulate its distribution for dispersal.

Keywords: Copepod; Eyespot; Light wavelength; Light intensity; Phototaxis

1. Introduction

The marine harpacticoid *Tigriopus japonicus* is a widespread species in intertidal and supralittoral rock pools along the coast in the Western Pacific including Japan, South Korea, Taiwan, and Hong Kong (Ito, 1970; Dethier, 1980; Jung et al., 2006). Most the intertidal zone is exposured to harsh physiological stresses such as high light intensity compared to other oceanic zone. That is why their light as well as physiological endurances are stronger than other planktonic copepods (Davenport et al., 1997; Raisuddin et al., 2007). However, there has been no information on the movement patterns of intertidal copepods related to external factors, so far. It is only known that the planktonic copepods show diel vertical migration synchronously affected by abiotic and biotic factors. Representative biotic factors are the followings: predator, competition, and food availability biotic factors (Dahms et al., 2004; Glazier and Deptola, 2011), and abiotic factors include temperature, salinity, light, oxygen content and wave action (Hicks and Coull, 1983; Miliou, 1992; Zengling et al., 2010; Miljeteig et al., 2014). Among these factors, light is generally known as the utmost influencer on vertical migration of copepods. This is because copepods are known to possess light sensor that is complex frontal eyes which are thought to have evolved for predator avoidance, increase success in foraging, navigation and mating (Cronin et al., 2003). Current studies on the behavioral responses of copepods to different light wavelengths and light intensities are gaining interests; most of these publications point out copepods' diel migration in relation to feeding rhythm and escape from predator and environmental hazards (Cohen and Forward Tr., 2002; Manor et al., 2009; Elofsson, 2006; Martynova and Gordeeva, 2010). The benthic copepod *T. japonicus* also has an eyespot and it was hypothesized that the light condition affect the movement pattern of copepods related to their physiological conditions.

Most of copepods constitute of a major part of the diet of larval animals in the pelagic area because of the following reasons: (1) nutritional value that matches with the nutritional requirements of larval animals,

(2) wide size distribution related to developmental stages. Among copepods, calanoids are preferred in rearing fish larvae because of their planktonic behavior, while other species including harpacticoids are not typically used because of their benthic behavior (reviewed by Stottrup, 2000). Among the copepod species, the harpacticoid copepod *T. japonicus* has high nutrient value (Hagiwara et al., 2016), and also wide size distribution (0.1-0.7 mm) through 6 naupliar, 5 copepodid, and adult stages (Ito, 1970). In addition, *T. japonicus* yields higher population density compared to calanoid copepods (Fukusho, 1980; Hagiwara et al., 1995; Cutts, 2003; Ribeiro and Souza-Santos, 2011) which makes it a better species for intensive mass culture. In fact, a large-scale and high yielding (2-3kg wet weight harvested daily in 200 m^2 tank) *T. japonicus* culture technology had been developed in Japan in the early 1980's (Fukusho, 1980). However, harpacticoid *T. japonicus* usually remain near the bottom of the culture container which make them unsuitable food source for fish larvae that usually hunt up in the water column.

The light plays an important role in the migration of copepods. This study was established to understand the phototactic behavior as well as exploring the effect of different light wavelengths and intensities on the planktonic behavior of *T. japonicus*. *T. japonicus* develops into adults through 11 developmental stages, and experiences significant morphological and physiological variations (Ito, 1970). Therefore, their phototactic behavior was investigated with two developmental stages i.e., nauplii and adults under different light conditions which were regulated with light wavelengths and intensities. The observation was performed in horizontal and vertical dimensions with aims of (1) determining the phototactic behavior of nauplii and adults under various light conditions regulated with light wavelengths and intensities and (2) modifying vertical distribution of *T. japonicus* in fish larval rearing tanks with light irradiation.

2. Materials and Methods

2.1. Experimental specimens

The target species *T. japonicus* was collected from an outdoor pond of the Fisheries Laboratory, University of Tokyo, near Lake Hamana (Japan) and has been continuously maintained in our laboratory for over 25 years (Hagiwara et al., 1995). They were cultured in sterilized natural seawater (about 34 salinity) at 25 °C and fed on *Tetraselmis tetrathele ad libitum*, in total darkness. One month before the start of experiments, the copepods were randomly collected from the stock culture, and transferred into 1000 mL of screw-capped bottle with 700 mL of culture medium (at 34 salinity) and kept at 25 °C in total darkness to prepare experimental specimens nauplii. They were fed on *T. tetrahele* at 2.5×10^6 cells/mL every three days. The cultures for the experiments on phototactic behavior of adult individuals were maintained at 22 salinity, while the other culture conditions were adjusted at the same as those for nauplii.

2.2. The characteristics of eyespot

The single frontal eye (eyespot, Fig. 1) of copepod individuals obtained from the culture kept at 34 salinity for nauplii and at 22 salinity for adults, was characterized by obtaining the area and relative light absorbance. The pigment area in eyespot was estimated using the average from 5 individuals in each developmental group with formalin fixation. The eyespot area was regarded as ellipse, and both major and minor axes which were measured with digital imaging software (Axio Vision Rel. 4.8, ZEISS). For the light absorbance of eyespot, 10 individuals in each developmental stage were randomly selected and treated on a slide glass without anesthesia and fixation. The light absorbance was individually measured using a microscope spectrophotometer system which consisted of spectrophotometry (308 PV TM, CRAIC Technologies TM) mounted on optical microscope (BX 61, Olympus). Absorbance of eyespot was calculated using the following equation: log (l_0 / l), where l_0 is the intensity of radiant energy striking the sample (i.e. emitted from the light source of microscope) and l is the intensity of energy emerging from sample. The net absorbance of eyespot was automatically calculated in the spectrophotometer system as the following methods: the reference absorbance (carapace only) was subtracted from the measured eyespot absorbance. The results were sequentially obtained with a range of light wavelengths. The eyespot absorbance of an individual was estimated with the mean of 5 measurements and the mean of 10 individuals was used to characterize the eyespot of each target group; nauplius, female and male.

2.3. Horizontal phototactic movement relative to light wavelength and intensity

Phototaxis on a horizontal dimension was observed with experimental container made of plexiglass (15.6 cm long × 3.0 cm wide × 3.0 cm high for nauplii; 15.6 cm long × 6.2 cm wide × 3.0 cm high for adults) modified from Kim et al., 2014a (Fig. 2A); four black side walls and a transparent bottom. The container was divided into three compartments of equal size using two sliding partitions. To prevent vertical movement of target organisms, the water depth was regulated under 1 cm with sterilized culture medium i.e., 20 mL for nauplii and 100 mL for adults. The employed individuals were continuously fed until 2 h before the experiment to maintain their activities during experiment set up with no diets. Fifteen individuals comprising of copepodites and adults (males and females) for the adults group, and 15 nauplii among 6 stages for the nauplius group, were randomly pipetted out from the stock cultures and inoculated into the center compartment of the experimental container. The copepods were first conditioned to dark condition by keeping the experimental container in a dark room for 5 min (dark adaptation). This was then followed by the sliding partitions being lifted out and the container exposed to unidirectional light irradiation for 10 min (Fig. 2A). We used LEDs (light emitting diodes, CCS, Inc., Japan) with different light wavelengths (white, with peaks at 460 and 750 nm; blue at 470 nm; green at 525 nm; and red at 660 nm). The light intensity was adjusted to the following levels: 0.5, 2.0, 3.5, 5.0, 15.0 W/m^2 for nauplii and 0.5, 2.0, 3.5 W/m^2 for adults, using a light meter (LI-1400, LI-COR Inc., Japan). After irradiation, the two sliding partitions were put back into the initial locations and the number of individuals in each compartment was counted under a stereomicroscope. Each experimental series was repeated 3-5 times with different individuals. The trials were performed in the dark room to avoid other source of light except irradiation. The phototaxis was estimated as the percentage of the total number of individuals found in each compartment to compare with the patterns of distribution in total darkness (control).

2.4. Vertical phototactic movement relative to light wavelength and intensity

The vertical phototactic movement was investigated with adult group comprising of copepodites and adults (males and females). The experimental container made of transparent flexiglass (3.6 cm long x 5.6 cm wide x 15.6 cm high) was used (Fig. 2B). The container was filled with 200 ml of sterilized seawater (at 22 salinity), resulting in 135 mm of water depth. For the experiment, 20 individuals (copepodites, males and females) which had previously been kept in total darkness, were irradiated for 15 min with bottom light (Fig. 2B). The light sources (i.e., white, blue, green and red LEDs) were adjusted at two intensities (2.0 and 3.0 W/m^2). The vertical distribution of each copepod were recorded every 3 minutes during light irradiation. Each experimental series was repeated 3-5 times with different individuals. The animals were fed 4 hours before the experiment to maintain their activities under no diet condition in the experimental container.

2.5. Statistical analysis

The characteristics of eyespot (i.e., area and light absorbance) were compared with the analysis of variance (ANOVA) followed by Tukey-Kramer post doc test ($P<0.05$). The different patterns of phototaxis associated with light wavelengths and intensities were transformed to arcsine square-root for the analysis of variance (one-way ANOVA) followed by Tukey-Kramer post doc test ($P<0.05$). All statistical analyses were performed with Statview version 5.0 software (SAS Institute, Inc., USA).

3. Results

3.1. The characteristics of eyespot

The area of eyespot was observed in two developmental groups, nauplius and adult (female and male) (Table 1). The eyespot area was larger in the adult group (1806.6-1918.3 μm^2, $n=5$) than that in the nauplius group (247.6 μm^2, $n=5$) regardless of the gender. The light absorbance of eyespot was 1.4- 1.5 times higher at shorter light wavelength (450-540 nm) compared to longer wavelength (> 660 nm) in all developmental groups (Fig. 3). On the other hand, the level of relative absorbance was different between the two groups. The adults (both sexes) showed the higher absorbance than nauplii in all light wavelength ranges observed ($n=10$).

3.2. Horizontal phototactic movement relative to light wavelength and intensity

The distribution of 15 individuals found in the three compartments of the experimental container is shown in Fig. 4 (for nauplius) and in Fig. 5 (for adult). The nauplii were equally distributed in the three compartments without light irradiation for control ($n=4$, Fig. 4). In the light irradiation trials, the nauplii showed the pattern of negative phototaxis under all the tested light conditions ($n=4$, Fig. 4).

Copepod adults stayed in the center compartment with about 75% of distribution rate in total darkness for control (n=3, Fig. 5A). At 0.5 W/m^2 of low light intensity, adults did not show any phototactic movement in the all wavelengths investigated (n=3, Fig. 5B). At 2.0 W/m^2, adults were positively phototaxis with all the wavelengths tested (n=3, Fig. 5C) with 50-82% of the copepods in the illuminated side. At 3.5 W/m^2, negative phototaxis was observed with all the wavelengths tested (n=3, Fig. 5D).

3.3. Vertical phototactic movement relative to light wavelength and intensity

The percentage of adult individuals that were suspended in the water column further from the light source or referred to as "planktonic" is shown in Fig. 6 (n=5). Planktonic behavior varied with light wavelengths but not with light intensities. Blue light significantly induced highest planktonic behavior (47.4%) in both irradiance tested (2.0 and 3.5 W/m^2), and it was lowest (16.2%) with red light.

4. Discussion

The target species harpacticoid copepod *T. japonicus* is distributed in intertidal rocky shore where it is exposed to harsh light stresses (Davison and Pearson, 1996). To define their survival and dispersal mechanisms in rocky shore, the present study focused on their phototactic behavior which is a critical factor to induce large scale movement of pelagic copepods (diel vertical migration) (Ringelberg, 1995). The target species has been maintained under laboratory conditions for over 25 years, where temperature, food and light are regulated. In our previous studies using marine rotifers *Brachionus plicatilis* s. s., the light absorbance of eyespot was varied with feeding conditions, and the pattern of phototaxis was also changed related to these variation of eyespot (Kim et al., 2014b). The study employed the baker's yeast as food which halved the population growth of rotifers (0.39±0.08) compared to that with *Nannochloropsis oculata*

(0.62±0.02) (Kim et al., 2014b). Our study employed *T. tetrathele* as the food for *T. japonicus* which is known as the optimal food for their population growth, and thus there is a possibility that the cultured copepods have the same pattern of phototaxis as wild individuals.

The light sensor eyespot of *T. japonicus* showed differences in the area (Table 1) and absorbance levels related to developmental stages (Fig. 3), while no obvious differences were detected in the pattern of light absorbance between the two developmental stages. The shorter light wavelengths (400 to 570 nm) were efficiently absorbed compared to the longer light wavelengths (570 to 750 nm) (Fig. 3). This broad spectral range of photosensitivity is not surprising because copepods possess a sophisticated eyespot that can even detect the shortest wavelength such as UV (Martin et al., 2000; Land and Nilsson, 2006; Manor et al., 2009). Copepod *Acartia pacifica* is known to migrate horizontally when exposed to UV (Zengling et al., 2010), in which they could make use of marine macrophytes as protective device against harmful lights. Even though we did not investigate in detail the structure of *T. japonicus* eye, it is assumed that their eye structure and function are similar to that of harpacticoid copepod *T. californicus* described by Martin et al. (2000). The euryhaline rotifer *Brachionus plicatilis* species complex also show active phototactic reactions at a certain light wavelength range which is efficiently absorbed by light sensor eyespot (Kim et al., 2014a, b). Based on the eyespot characteristics in *T. japonicus*, it would be expected that there will be no difference in phototactic behavior related to developmental stages. In spite of our expectation, the employed species showed the different patterns of horizontal phototaxis between nauplius and adult.

The phototactic movement was observed on two different dimensions such as horizontal and vertical. For the horizontal dimension, two different developmental groups were used to observe phototaxis related to light wavelength and intensity. The observation was performed under different salinity conditions, that is, at 34 for nauplius and at 22 for adult to induce best performance in movements (Damgaard and Davenport, 1994). Salinity effects on light refraction and absorption of visible light is negligible (Pegau et al., 1997). Under the all tested light conditions, the adults showed a pattern of negative phototaxis, except at 2.0 W/m^2 where they showed positive phototaxis (Fig. 5). The response of *T. japonicus* on various light wavelengths is similar to that shown by other copepoda species. Broad spectral range (453 to 620 nm) of sensitivity has been reported in calanoid copepods including *A. tonsa* (Stearns and Forward Jr., 1984), *A. pacifica* (Zengling et al., 2010) and *Calanus finmarchicus* (Miljeteig et al., 2014). On the other hand, other migrating copepods including *Centropages typicus*, *Calanopia americana* and *Anomalocera ornata* have narrow range of wavelength responses (Cohen and Forward Jr., 2002). In spite of the pattern of adults, the nauplii showed negative phototaxis under the all tested light conditions (Fig. 4). Even though the pattern of eyespot absorbance was similar, while the absorbance level was different between two developmental stages (Fig. 3). These differences might work as the regulator of different phototaxis related to light intensity. Based on the phenomena observed, we can relate it to the following survival mechanisms: *in situ* the adults and nauplii show negative phototaxis by hiding in dark region of rocky shore to avoid predators. On the other hand, female and male adults might be gather on the water surface where the light intensity is close to 2.0 W/m^2 (Al-Asadi et al., 2007) for fertilization or spawning so as to extend their territory using the surface tide.

The vertical movement of adult *T. japonicus* was investigated at 2.0 and 3.5 W/m^2 which induced the strongest positive and negative phototaxis of adult individuals, respectively. Light wavelength has significant influence on the planktonic behavior of *T. japonicus*, and the blue light makes the adults migrate to surface with 55% of maximum planktonic rate regardless of light intensity (Fig. 6). The target species, *T. japonicus* is a highly fecund copepod, which can produce as high as 1000 nauplii in its lifetime (Fukusho, 1980; Hagiwara et al., 1995). Fukusho (1980) successfully harvested 2-3kg (wet weight) of copepods daily in 200 m^3 tank culture. Considering this large biomass, the potential of *T. japonicus* as a live feed for larviculture is high. However, *T. japonicus* usually remains near the bottom of larval culture tanks which make them unsuitable food source for fish larvae that usually hunt up in the water column. Results of the present study showed that the planktonic behavior of *T. japonicus* can be induced by blue light at 2.0 and 3.5 W/m^2. This is worthwhile to remove the last obstacle to use *T. japonicus* in larviculture facilities as live feed. However, setting up light on the inside or the bottom of fish larval rearing tank could have an impact on the cultured larvae. These factors should be explored further in order to practically apply the information derived from this research.

Acknowledgement

This study was supported by the Ministry of Education, Culture, Sports, Science and Technology Japan, Grant-in-Aid for Scientific Research (B) 2012-2014, No. 24380108 to A.H. The authors deeply appreciate the constructive comments and suggestions from Robert Nesta Kagali and the anonymous reviewers to improve the manuscript.

References

Al-Asadi, M.S., Randerson, P., Benson-Evans, K., 2007. Ecological studies of three west Algerian river: the river Tafna and its tributary the river Remshy. Marina Mesopotanica 22, 37-57.

Cohen, J.H., Forward Jr. R.B., 2002. Spectral sensitivity of vertically migrating marine copepods. Biol. Bull. 203, 307-314.

Cronin, T.W., Shashar, N., Caldwell, R.L., Marshall, J., Cheroske, A.G., Chiou, T.H., 2003. Polarization vision and its role in biological signaling. Integr. Comp. Biol. 43, 549-558.

Cutts, C.J., 2003. Culture of harpacticoid copepods: Potential as live feed for rearing marine fish. Adv. Mar. Biol. 44**,** 295-316.

Dahms, H.-U., Harder, T., Qian, P.-Y., 2004. Effect of meiofauna on macrofauna recruitment: settlement inhibition of the polychaete *Hydroides elegans* by the harpacticoid copepod *Tisbe japonica*. J. Exp. Mar. Biol. Ecol. 311, 47–61.

Damgaard, R.M., Davenport, J., 1994. Salinity tolerance, salinity preference and temperature tolerance in the high-shore harpacticoid copepod *Tigriopus brevicornis*. Marine Biology 118, 443-449.

Davenport, J., Barnett, P.R.O., Mcallen R.J., 1997. Environmental tolerances of three species of the harpacticoid copepod genus *Tigriopus*. J. Mar. Biol. Ass. U.K. 7, 3-16.

Davison, I.R., Pearson, G.A., 1996. Stress tolerance in intertidal seaweeds. Journal of phycology 32, 197-211.

Dethier, M.N., 1980. Tidepools as refuges: predation and the limits of the harpacticoid copepod *Tigriopus japonicus* (Baker). J. Exp. Mar. Biol. Eco. 42, 99-112.

Elofsson, R., 2006. The frontal eye of crustaceans. Arthropod Structural Development 35, 275-291.

Fukusho, K., 1980. Mass production of a copepod, *Tigriopus japonicus* in combination culture with a rotifer *Brachionus plicatilis*, fed omega -yeast as a food source. Nippon Suisan Gakk. 46, 625-629.

Glazier, D.S., Deptola, T.J., 2011. The amphipod *Gammarus minus* has larger eyes in freshwater springs with numerous fish predators. Invertebr. Biol. 130, 60-76.

Hagiwara, A., Kim, H.-J., Matsumoto, H., Ohta, Y., Morita, T., Hatanaka, A., Ishizuka, T., Sakakura, Y., 2016. Production and use of two marine zooplanktons, *Tigriopus japonicus* and *Diaphanosoma celebensis*, as live food for red sea bream *Pagrus major* larvae. Fish. Sci. 82, 799-809.

Hagiwara, A., Lee, C.S., Shiraishi, D.J., 1995. Some reproductive characteristics of the broods of the harpacticoid copepod *Tigriopus japonicus* cultured at different salinities. Fisheries Sci., 61, 618-622.

Hicks, G.R.F., Coull, B.C., 1983. The ecology of marine meiobenthic copepods. Oceanogr. Mar. Biol. 21, 67-175.

Ito, T., 1970. The biology of a harpacticoid copepod, *Tigriopus japonicus* Mori. Journal of Faculty of Science Hokkaido University Series VI, Zoology 17, 474-499.

Jung, S.-O., Lee, Y.-M., Park, T.-J., Park, H.G., Hagiwara, A., Leung, K.M.Y., Dahms, H.-U., Lee, W., Lee, J.-S., 2006. The complete mitochondrial genome of the intertidal copepod *Tigriopus* sp. (Copepoda, Harpactidae) from Korea and phylogenetic considerations. J. Exp. Mar. Biol. Ecol. 333, 251-262.

Kim, H.-J., Sawada, C., Hagiwara, A., 2014a. Behavior and reproduction of the rotifer *Brachionus plicatilis* species complex under different light wavelengths and intensities. Int. Rev. Hydrobiol. 99, 151-156.

Kim, H.-J., Sawada, C., Rhee, J.-S., Lee, J.-S., Suga, K., Hagiwara, A., 2014b. Nutritional effects on the visual system of the rotifer Brachionus plicatilis sensu strcito (Rotifera: Monogononta). JEMBE 460, 177-183.

Land, M.F., Nilsson D.-E., 2006. General purpose and special purpose visual systems. In: Invertebrate Vision. Cambridge University Press, Cambridge, UK, pp. 167-210.

Manor, S., Polak, O., Saidel, W.M., Goulet, T.L., Shashar, N., 2009. Light intensity mediated polarotaxis in *Pontella karachiensis* (Pontellidae, Copepoda). Vision Res. 49, 2371-2378.

Martin, G.G., Speekmann, C., Beidler, S., 2000. Photobehavior of the harpacticoid copepod *Tigriopus californicus* and the fine structure of its nauplius eye. Invertebr. Biol. 119, 110-124.

Martynova, D.M., Gordeeva, A.V., 2010. Light-dependent behavior of abundant zooplankton species in the White Sea. J. Plankton Res. 32, 441-456.

Miliou, H., 1992. Effects of light (photoperiod, spectral composition) on the population dynamics of *Tisbe holothuriae* Humes (Copepoda, Harpacticoida). Hydrobiologia 232, 201-209.

Miljeteig, C., Olsen, A.J., Batnes, A.S., Altin, D., Nordtug, T., Alver, M.O., Speed, J.D.M., Jenssen, B.M., 2014. Sex and life stage dependent phototactic response of the marine copepod *Calanus finmarchicus* (Copepoda: Calanoida). J. Exp. Mar. Biol. Ecol. 451, 16-24.

Pegau, W.S., Gray, D., Zaneveld, V., 1997. Absorption and attenuation of visible and near-infrared light in water: dependence on temperature and salinity. Applied Optics 36, 6035-6046.

Raisuddin, S., Kwok, K.W.H., Leung, K.M.Y., Schlenk, D., Lee J.-S., 2007. The copepod *Tigriopus*: A promising marine model organism for ecotoxicology and environmental genomics. Aquat. Toxicol. 83, 161-173.

Ribeiro, A.C.B., Souza-Santos L.P., 2011. Mass culture and offspring production of marine harpacticoid copepod *Tisbe biminiensis.* Aquaculture 321, 280-288.

Ringelberg, J., 1995. Changes in light intensity and diel vertical migration: acomparison of marine and freshwater environments. J. Mar. Biol. Ass. U. K. 75, 15-25.

Stearns, D.E., Forward Jr. R.B., 1984. Photosensitivity of the calanoid copepod *Acartia tonsa*. Mar. Biol. 82, 85-89.

Stottrup, J.G., 2000. The elusive copepods their productivity and suitability in marine aquaculture. Aquacult. Res. 31, 703-711.

Zengling, M.A., Wei L.I., Kunshan, G.A.O., 2010. Horizontal migration of *Acartia pacifica* Steuer (copepoda) in response to UV-radiation. J. Photochem. Photobio. 101, 233-237.

Table 1

Eyespot area of *Tigriopus japonicus* related to the developmental stages i.e., nauplius and adult (comprising of female and male)

		Area (μm^2)
Nauplius		247.6 ± 25.3^{b}
Adult	Female	1806.6 ± 293.4^{a}
	Male	1918.3 ± 472.2^{a}

Values are the mean±SD. Lower case alphabetical letters represent significant differences (a>b, Tukey-Kramer *post hoc* test, $p<0.05$, $n=5$).

Figure 1. The eyespot of marine harpacticoid *Tigriopus japonicas* on different developmental stages: nauplius (A), male (B) and female (C). Open arrows indicate the eyespot locations.

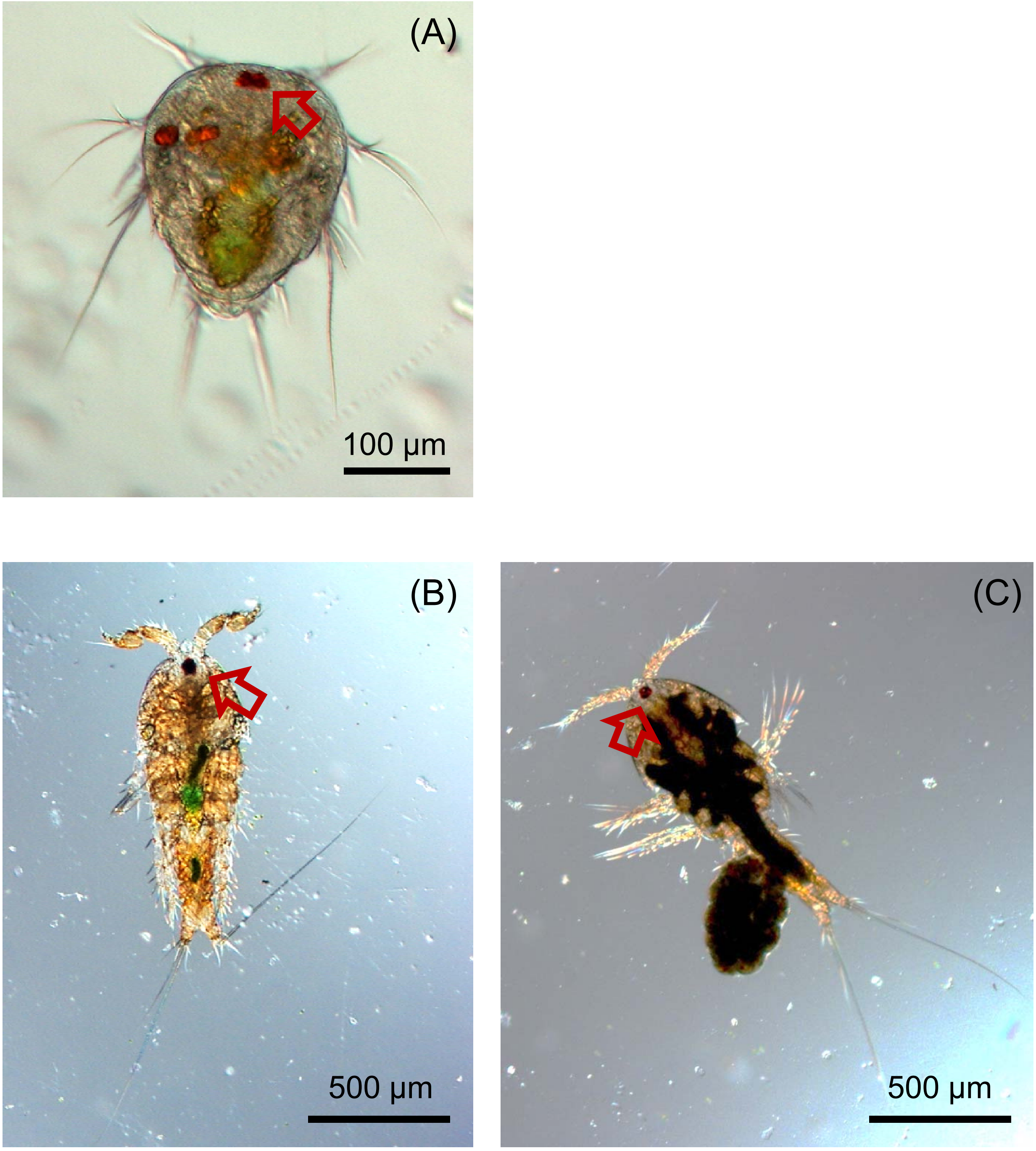

Figure 2. Experimental container used for horizontal (A) and vertical (B) observation of phototaxis in *Tigriopus japonicus*. Closed arrows indicate the position of the partitions.

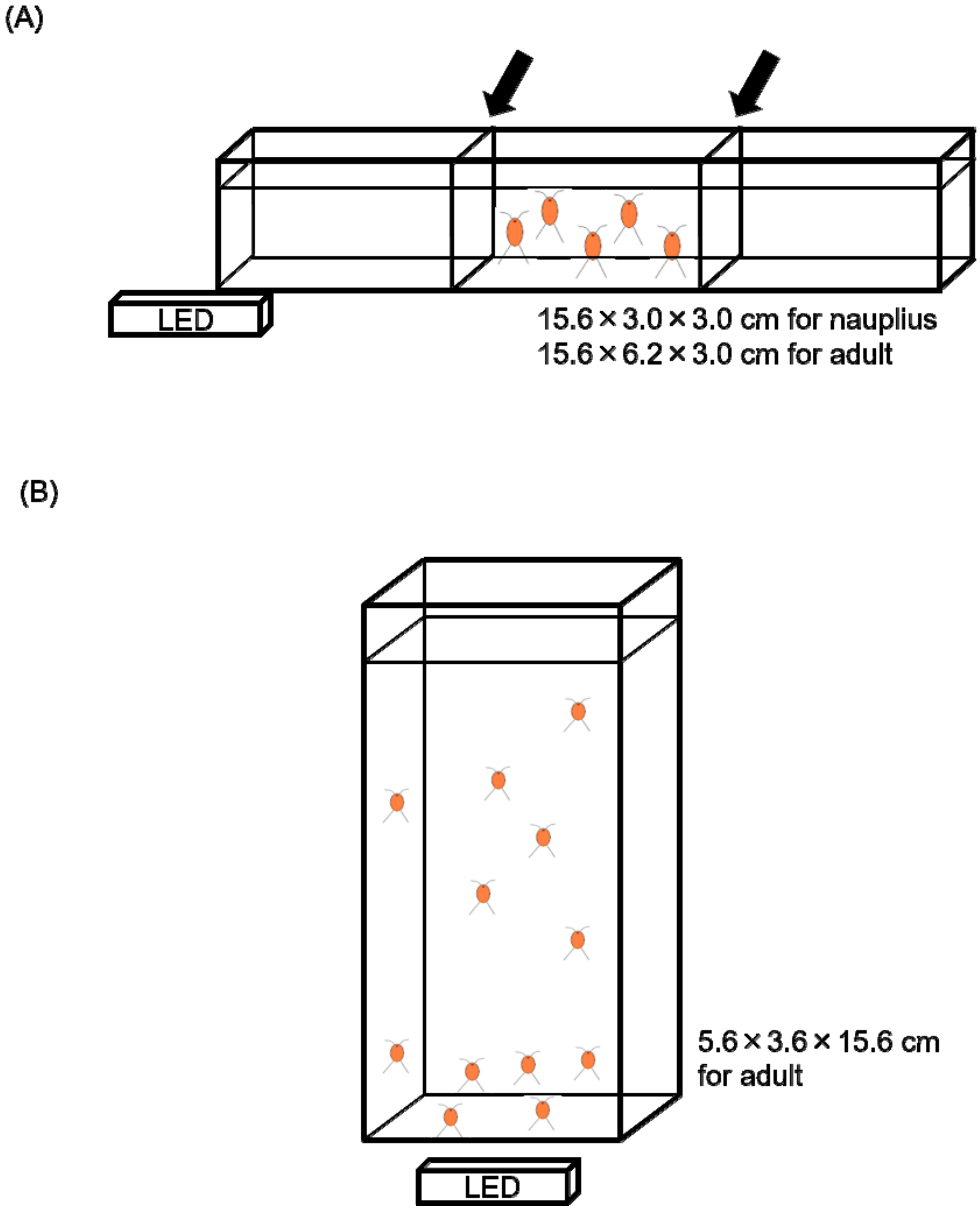

Figure 3. Light absorbance spectrum of the eyespot of *Tigriopus japonicus.* Three different lines denote the mean absorbances of males (solid), females (broken) and nauplii (broken line with dots), respectively (n = 10).

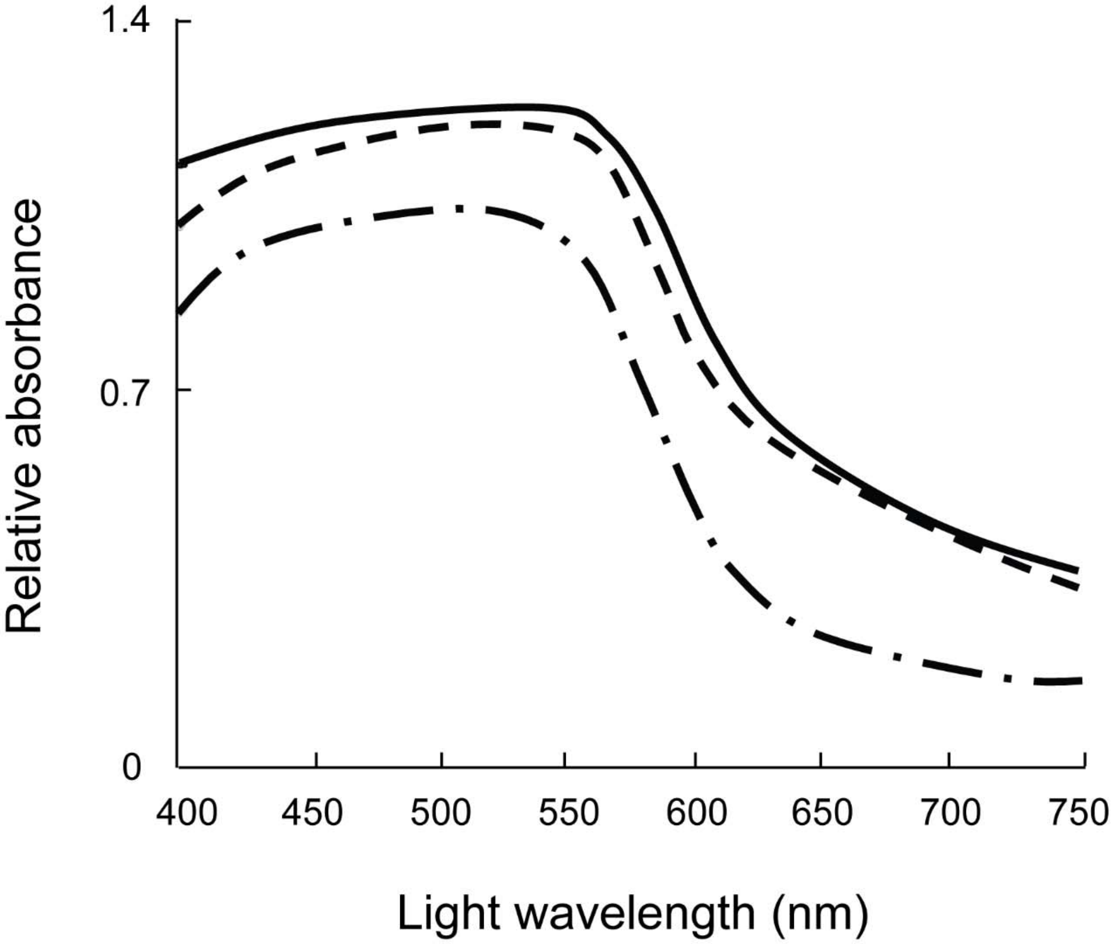

Figure 4. Distribution of nauplii under different wavelengths and intensities (A: Control in total darkness, B: 0.5 W/m^2, C: 2.0 W/m^2, D: 3.5 W/m^2, E: 5.0 W/m^2, F: 15.0 W/m^2) in *Tigriopus japonicus*. Lower case alphabetical letters represent significant differences (a>b>c, Tukey-Kramer *post hoc* test, *P*<0.05, *n*=4).

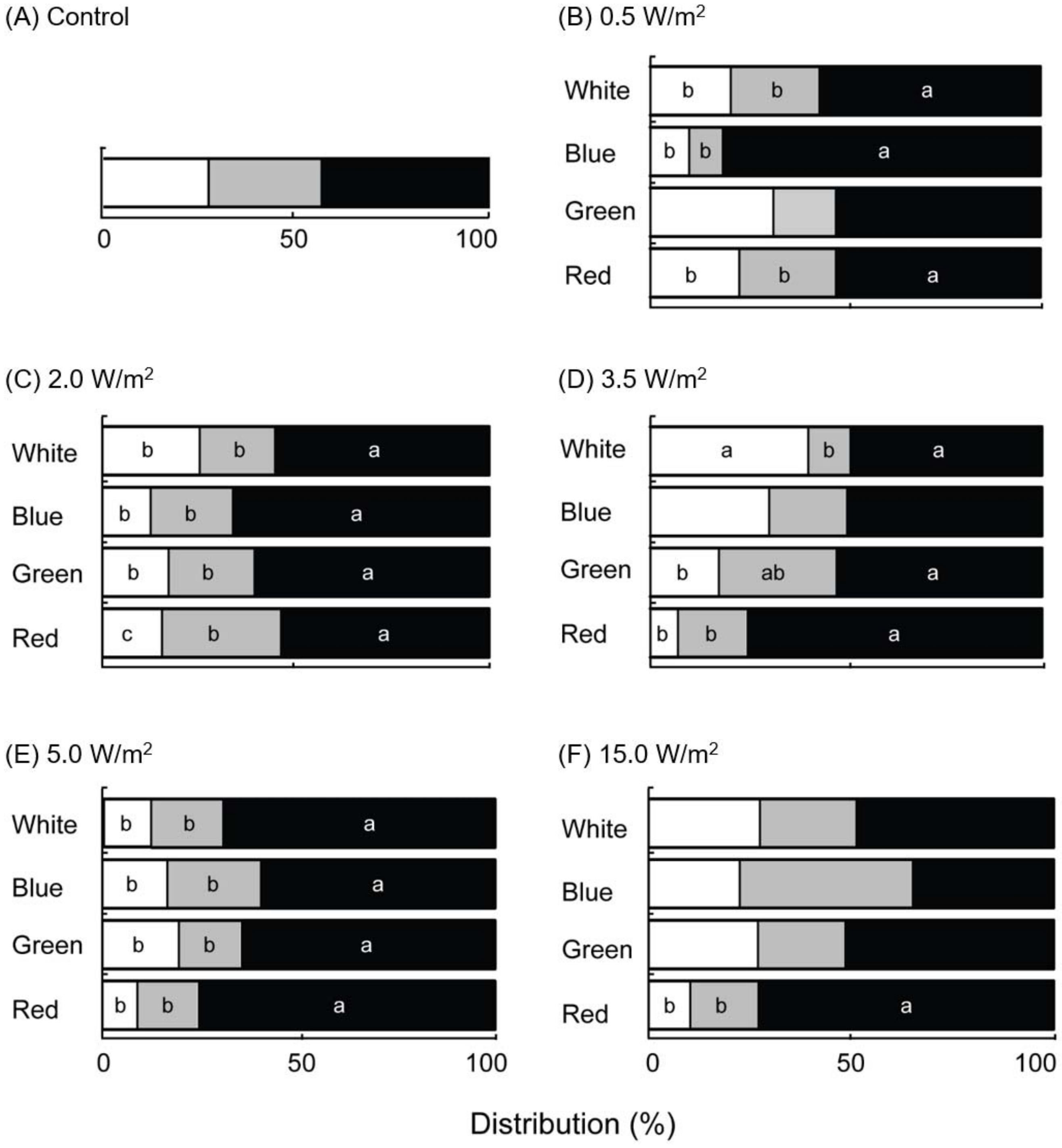

Figure 5. Distribution of adults comprising of males and females randomly selected under different wavelengths (white, blue, green, and red) and intensities (A: Control in total darkness, B: 0.5 W/m^2, C: 2.0 W/m^2, D: 3.5 W/m^2). The color gradation represent the compartments in the experimental vessel (lighting side with white, middle area with gray, dark side with black). Lower case alphabetical letters represent significant differences (a>b>c, Tukey-Kramer *post hoc* test, P<0.05, n = 3).

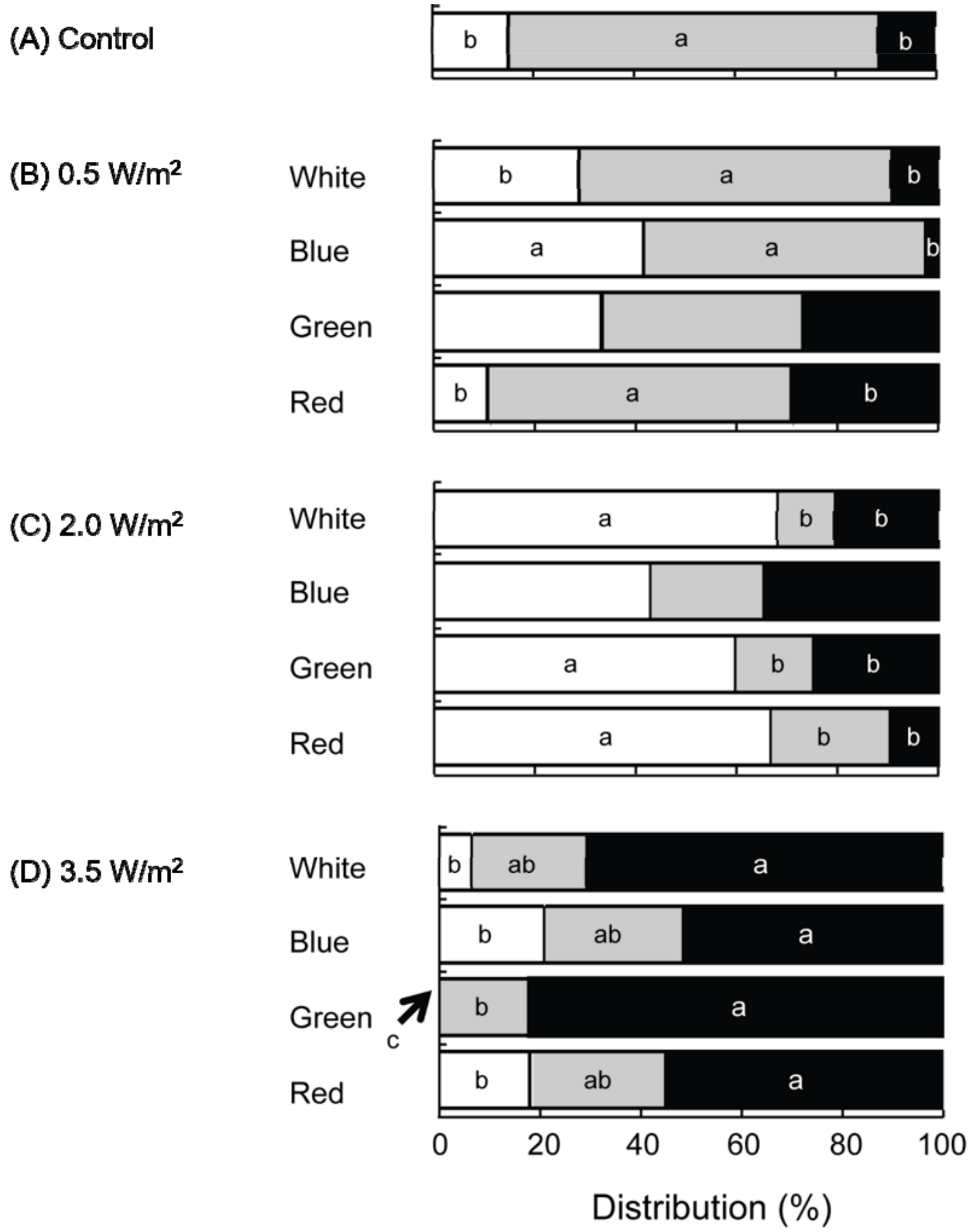

Figure 6. Percent planktonic adults under different light wavelengths (control in total darkness, white, blue, green, and red) and intensities (A: 2.0 W/m^2, B: 3.5 W/m^2). Columns and bars indicate the mean (%) of planktonic individuals and standard deviation, respectively. Lower case alphabetical letters represent significant differences (a>b>c>d, Tukey-Kramer *post hoc* test, $P<0.05$, $n=5$).