Phototactic behavior of live food rotifer *Brachionus plicatilis* species complex and its significance in larviculture: a review

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ABSTRACT

We will review a recent progress of photophysiological studies in the rotifer *Brachionus plicatilis* species complex. The rotifers have a light sensor i.e., eyespot inducing phototactic behavior. For the rotifer *B. plicatilis* sp. complex, the eyespot efficiently absorbs the light wavelength ranging from 450 to 550 nm. The function of eyespot is affected by diet species from 30-day batch cultures fed by either microalgae *Nannochloropsis oculata* or baker's yeast *Saccharomyces cerevisiae*. By feeding baker's yeast, rotifer eyespot gradually lost its function: area (5.5 times) and absorbance (2.2 times) decrease compared to those fed by *N. oculata*. Phototactic behavior and reproductive characteristics of the rotifer *B. plicatilis* sp. complex varied with different light wavelengths and

intensities. The rotifers show light wavelength dependent phototaxis associated with the reception of an eyespot. For the phototactic behavior in horizontal level, light intensity is also a significant factor to regulate phototaxis. The rotifers show strong positive phototaxis under blue (peaks at 470 nm), green (525 nm), and white (460 and 570 nm) lights at 0.5 W/m^2. Rotifer reproduction is also affected by light wavelength and intensities. Asexual reproduction of rotifers is accelerated by green and red lights at 0.5 W/m^2. On the other hand, active sexual reproduction is observed with blue light at 1.4 W/m^2. Under a certain light condition inducing active phototactic behavior, the rotifers show continuous swimming movement without attaching to substrates. The different behaviors associated with light conditions affect the reproductive characteristics of rotifers. The regulation of live food distribution is significant for feeding efficiency of fish larvae. The efficient feeding promotes larval growth and survival: hence it is a significant factor for successful larviculture. Fish larvae also show different phototactic behavior related to light wavelengths and intensities. Therefore, the distributions of fish larvae under the applied light conditions should be considered.

Keywords: Rotifera, *Brachionus* spp., Eyespot, Phototaxis, Larviculture

1. Introduction

Aquatic organisms living near the surface like rotifers *Brachionus plicatilis* species complex are overly exposed to sunlight, and exhibit the phototactic responses such as the diel and ontogenetic vertical distribution (Forward 1988; Ringelberg 1999; Burks et al., 2002). Pelagic organisms exhibit peculiar phototactic behavior, and usually differs according to light sensor (George and Fernando, 1970; Richard and Forward, 1988). The light sensor instructs the movement of possessors which can detect direction of light, while not form visible images (Jékely et al. 2008). The phototactic behavior of rotifers is also significantly influenced by the characteristics of light sensor eyespot. Therefore, this review firstly characterizes rotifer eyespot with light wavelength-dependent absorbance.

The light plays an important role in the behavior of numerous plankton species with phototaxis (Forward 1988; Buskey et al. 1989; Storz and Paul 1998). The light wavelength and intensity have significant role in the phototactic behavior of zooplanktons (Richard and Forward, 1988). Locomotor reactions of rotifers to qualitative or quantitative variations in light conditions can be classified into two categories: oriented reactions (phototaxis) that can be positive or negative, and non-oriented reactions (photokinesis) that are subdivided into orthokinesis (modification of linear speed) and klinokinesis (modification of the rate of change of direction, Mimouni et al. 1993). We secondly reviewed recent studies on phototactic behavior of the euryhaline rotifers under different light conditions (various light wavelengths and intensities).

The monogonont rotifers have a cyclically parthenogenetic life cycle with both sexual (mictic) and asexual (amictic) reproduction and it is affected by various internal and external factors (Hagiwara et al. 2007; Gilbert 2010). Asexual reproduction predominates the rotifer life cycle, while sexual reproduction results from stimulation by various environmental factors such as light, temperature and food density. In sexual reproduction, mictic females produce haploid males, or if fertilized, they produce diploid resting eggs (Gilbert 2004, 2010; Hagiwara et al. 2007). The produced resting eggs can be used as *Artemia* cyst in aquaculture. To date, light effects on rotifer reproduction have been defined for the efficient production of resting eggs and rotifer propagation. In this review, the effects of light conditions on the reproduction of euryhaline rotifer *B. plicatilis* sp. complex was thirdly debated related to the movement pattern of rotifers. Lastly, the predator effects and the application methods were discussed for the further experiments.

2. Light sensor of rotifers

The light sensors detect light signals with visual pigments and the detected signals can modulate the phototactic behavior of organisms (Jékely, 2009). The euryhaline rotifer *B. plicatilis* species complex has a red eyespot which has a similar structure to the freshwater rotifer *Brachionus calyciflorus* with only two differences in relay neuron and endoplasmic reticulum (Fig. 1, Clément et al., 1983). As the common planktonic invertebrate, the monogonont rotifer *Brachionus*, has a cerebral eye (red eye spot) consisted of two types of pigment-bearing cells: one epithelial cell cup containing accessory pigment and one or more sensory neurons (sensory pigment) with membranous structure (Clément 1980; Clément et al. 1983; Cornillac et al. 1983). Through the joint action of these two pigments, they can determine the direction, as well as light wavelength and intensity (Clément et al. 1983). The main visual pigment of rotifer eyespot has been suggested as rhodopsin (Wolken, 1971; Clément, 1980). Red visual pigment, rhodopsin is consisted of opsin protein covalently linked to 11-cis-retinal which is a derivate of vitamin A (Palczewski et al., 2000; Zhong et al., 2012). The rotifer eyespot showed the same absorbance as rhodopsin measured by microspectrophotometer system; the eyespot efficiently absorbs the light ranging from 450 to 550nm of wavelength (Fig. 2A, Kim et al., 2014a, b). The level of absorbance is 5.5 times higher for blue (470 nm) and green (525 nm) lights compared to the level for red light (660 nm). The level slightly differs related to the rotifer morphotypes and species, whereas this pattern is same (Kim et al., 2014a). Recently, the existence of rhodopsin has also been confirmed using genomic DNA analysis, and the findings show that rotifers have 12 opsin-relative genes (Table 1, Kim et al., 2014b).

The rotifer eyespot is significantly affected by the nutritional conditions of food (Kim et al., 2014b). Through 30-day rotifer culture with two different diets; *Nannochloropsis oculata* and baker's yeast (*Saccharomyces cerevisiae*), their eyespot area decreased to 14.7 μm^2 with baker's yeast while it was maintained the initial value with *N. oculata* (82.9 μm^2) (Fig. 2). For the light absorbance of rotifer eyespot, the pattern was not dependent on food species, while the absorbance level gradually decreased with baker's yeast during the culture period (Fig. 3). This feature has significant relationship with the structure of rhodopsin compounded with vitamin A precursor (Kim et al, 2014b). The microalgae, *Nannochloropsis* sp. contains vitamin A and its precursor such as 0.25 $\mu g\ g^{-1}$ of vitamin A and 0.29 ± 0.04 $mg\ g^{-1}$ of β-carotene under continuous fluorescent light (Brown et al., 1999). On the other hand, baker's yeast does not contain any nutrients related to vitamin A (Hamre et al., 2008; Satuito and Hirayama, 1986), nor vitamin B_{12} and ω3 highly unsaturated fatty acids which needed for the rotifer population growth (Hirayama and Funamoto, 1983; Satuito and Hirayama, 1991). Thus, these demonstrate that visual function of rotifers and their phototactic behaviors are significantly affected by the nutrient levels of their diet. The phototactic behavior related to the features of eyespot is discussed below.

3. Phototactic behaviors

Rotifers can detect the direction, quantity, duration and wavelength of light with the function of cerebral eye (Clément et al. 1983). In order to investigate the effects of light on the movement of rotifers, other influential factors including temperature, salinity and food were controlled to limit their effects on experimental results in the previous studies. For one example, food presence affects the movements of rotifers, and the low swimming speed and attachment were frequently observed (Charoy and Clément, 1993; Yúfera, 2007). Therefore, phototactic movement of rotifers was observed in a clear medium; without food. In the movement of rotifers, two notable circumstances occurred with light stimulation: increasing swimming speed and reducing turning frequency (Clément, 1977; Mimouni et al., 1993). Recent studies on rotifer phototactic behavior focused on the light wavelength and intensity. For the phototaxis of euryhaline rotifers, the experimental methods are originated from the previous study by Cornillac et al. (1983) with several modifications (Fig. 4). An experimental vessel which manually constructed with reflective black plastic plank (0.3 mm of thickness), contained 20 mL of the stock culture medium (22 ppt) to make a minimum water depth (<4 mm) suppressing vertical movements of rotifers. The vessel were divided into three parts and partitions were placed after irradiation of LED lights (Kim et al., 2014). The phototactic behavior related to light wavelength and intensity was described on Figure 5. The gradation represents light intensity varied with illumination on left side. The rotifers showed a pattern of positive phototaxis with blue light (470 nm) and it should reflect the light absorbance of eyespot. The light wavelength-dependent phototaxis of rotifers is significantly affected by light intensity, and the pattern was significantly related to the absorbance of eyespot (Kim et al., 2014a, b). For the short light wavelengths (450 to 550 nm of wavelength) where eyespot efficiently absorbs, rotifers can recognize weak light intensities (at 0.5 and 6.2 W/m^2). In this light sector induced the positive phototaxis of rotifers, strong light intensities (at 15.0 and 30.0 W/m^2) disturb the phototactic behavior of rotifers and lost its pattern (Fig. 5). Contrastively, rotifers showed positive phototaxis with strong light intensities (at 15.0 and 30.0 W/m^2) of the longer light wavelengths (over 600 nm, Fig. 5) where the absorbance level of eyespot is low. This patterns at around 470 nm (blue) is significantly different from the freshwater rotifer *Brachionus calyciflorus* (Fig. 6, Viaud 1940; Cornillac et al., 1983). It may reflect the ambient conditions of their habitat like salinity affects the phototactic behavior of rotifers. The euryhaline rotifers show continuous swimming movement without attaching to substrates under the light condition inducing strong phototaxis, while high rate of attaching is observed under the light condition with weak phototaxis, although, no differences were observed in swimming speed among the four tested light wavelengths (white, blue, green and red) (Kim et al., 2013).

4. Population growth with light conditions

The light conditions significantly affect the reproductivity of zooplanktons. The reproduction of marine zooplankton *Artemia franciscanan* is affected by photoperiod (Nambu et al., 2004). The mixis induction (male production) of *Brachionus rubens* is affected by the light cycle (Laderman and Gutman, 1974) and *Notommata* sp. and *Trichocera* sp. are affected by a long photoperiod in mictic female production (Gilbert 2004). The light wavelength and intensity significantly influence the movement of rotifers and these phenomena denote those effects on physiological condition of rotifers. The phototactic behavior of supplied phytoplankton has a possibility to affect the movement and population growth of rotifers. The flagellate *Tetraselmis cordiformis* Stein (Chlorophyceae) showed the phototactic behavior (Melkonian and Robenek, 1979) and biased distribution of it in the culture medium. It is possible that phototaxis of phytoplankton affect the population growth of rotifers in relation to the energy allocation procedure. Therefore, the previous studies conducted on rotifer cultures with phytoplankton which has no phototactic movement (without flagella) e.g., *Nannochloropsis oculata* to investigate population growth of rotifers related to various light conditions (Kim et al., 2014a, b). The density of food *N. oculata* supplied every day was regulated so that all food are consumed by rotifers, thus preventing photosynthetic nutritional variations associated with light wavelength and intensity illuminated (Gaytan-Luna et al., 2016).

Rotifer reproduction is also affected by light wavelength and intensity. Asexual reproduction of rotifers is accelerated by green and red lights at 0.5 W/m^2 of weak light for *B. plicatilis* s. s., whereas the lights at stronger than 0.5 W/m^2 negatively affect population growth of rotifers (Fig. 7, Kim et al., 2014a). At 1.4 W/m^2, asexual reproduction has no significant difference among the tested light wavelengths in *B. manjavacas* (Fig. 8A). Different patterns was observed related to the light wavelengths in the sexual reproduction of *B. manjavacas*. At 1.4 W/m^2 of light intensity, the male production is stimulated by red light illumination (660 nm, Fig. 8B), while the resting egg production was actively occurred with blue light (525 nm) for *B. manjavacas* (Fig. 8C, Kim et al., 2013). It should be significantly related to the movement patterns of rotifers for the encounter of female and male individuals under the different light conditions.

5. Predator effects on phototactic behavior

The light sensor of predator fish showed different absorbance patter from prey zooplankton's. The phototaxis of fish larvae is also affected by light intensity (Bulkowski and Meade, 1983). This phenomena should mean that zooplankton and predator showed different phototactic behavior under the same light conditions. Moreover, the infochemical is defined as a kairomone which released from predators, leads to a behavioral and physiological reaction of receiver prey (Lass and Spaak, 2003). It is generally known that the fresh water rotifer *B. calyciflorus* recognizes predators through innate releasing mechanism to avoid carnivorism through the infochemical (Sarma, 2011). *Daphnia magna* exhibited a full induced change in phototactic behavior after a few hours of exposure to fish kairomone (de Meester and Cousyn, 1997). The predator effects on the phototactic behavior of rotifers have not been tested so far, while the information is needed to figure out actual distribution of rotifers in a larval rearing tank.

6. Conclusion

The regulation of live food distribution in a larval rearing tanks is significant for feeding efficiency of fish larvae. The rotifer *B. plicatilis* sp. complex are commonly used as initial live food sources for fish larviculture. For the efficiency of larviculture, it is desirable that rotifers show even distribution in a larval rearing tank. However, their distribution tends to be biased because of their phototactic behavioral responses. In addition, rotifers occasionally attach to the substrates, and thus the frequency of encounter decreases between fish larvae and the live foods. The rotifer eyespot efficiently absorbs light wavelength from 450 to 550 nm and the phototactic behavior of rotifers reflects its features. The phototactic behavior of rotifer is affected by light wavelengths and intensities. The swimming and attachment behaviors are also affected by light conditions. The previous studies posit that photokinesis reduced population growth by increasing the energy use by elevating swimming speed and reducing turning frequency. That is a reason for the low population growth under light condition that induced strong positive phototaxis. The predator of rotifers, fish larvae also show different phototactic behavior related to light wavelengths and intensities. Moreover, the presence of predator influence the distribution of live food. There is a possibility that the distributions of fish larvae and rotifers show different distribution under the applied light conditions. For the efficient larval feeding, the encounter rate between fish and live food is significant to improve fish larval growth and survival. Therefore, (1) characteristics of phototactic behavior of fish larvae and (2) the predator effects on the phototaxis of live food should be considered for the efficient larviculture with light regulations.

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References

Brown, M.R., Mular, M., Miller, I., Farmer, C., Trenerry, C., 1999. The vitamin content of microalgae used in aquaculture. J. Appl. Phycol. 11, 247–255.

Bulkowski, L., Meade, J. W., 1983. Changes in phototaxis during early development of walleye. Trans. Am. Fish. Soc. 112, 445-447.

Buskey, E. J., Baker, K. S., Smith, R. C., Swift, E., 1989. Photosensitivity of the oceanic copepods *Pleuromamma gracilis* and *Pleuromamma xiphias* and its relationship to light penetration and daytime depth distribution. Mar. Ecol. Prog. Ser. 55, 207-216.

Burks, R. L., Lodge, D. M., Jeppesen, E., Lauridsen T. L., 2002. Diel horizontal migration of zooplankton: costs and benefits of inhabiting the littoral. Freshw. Biol. 47, 343-365.

Charoy, C., Clément, P., 1993. Foraging behavior of *Brachionus calyciflorus* (Pallas): variations in the swimming path according to presence or absence of algal food (*Chlorella*). Hydrobiologia 255/256, 95-100.

Clément, P., 1977. Phototaxis in rotifer (action spectra). Arch. Hydrobiol. Beih. Ergebn. Limnol. 8, 67-70.

Clément, P., 1980. Phylogenetic relationships of rotifers, as derived from photoreceptor morphology and other ultrastructural analyses. Hydrobiologia 73, 93-117.

Clément, P., Wurdark, E., Amsellem, J., 1983. Behavior and ultrastructure of sensory organs in rotifers. Hydrobiologia 104, 89-130.

Cornillac, A., Wurdak, E., Clément, P., 1983. Phototaxis in monochromatic light and microspectrophotometry of the cerebral eye of the rotifer *Brachionus calyciflorus*. Hydrobiologia 104, 191-196.

de Meester, L., Cousyn, C., 1997. The change in phototactic behavior of a *Daphnia magna* clone in the presence of fish kairomones: the effect of exposure time. Hydrobiologia 360, 169-175.

Forward, R. B. Jr., 1988. Diel vertical migration: zooplankton photobiology and behavior. Oceanogr Mar Biol: An Annual Review 26, 361-393.

Gaytán-Luna, D. E., Ochoa-Alfaro, A. E., Rocha-Uribe, A., Pérez-Martínez, A. S., Alpuche-Solís, Á. G., Soria-Guerra, R. E. 2016. Effect of green and red light in lipid accumulation and transcriptional profile of genes implicated in lipid biosynthesis in *Chlamydomona reinhardtii*. Biotechnol. Prog. 32, 1404-1411.

George, M. G., Fernando, C. M., 1970. Diurnal migration in three species of rotifers in Sunfish Lake, Ontario. Limmol. Oceanogr. 15, 218-223.

Gilbert, J. J., 2004. Population density, sexual reproduction and diapause in monogonont rotifers: new data for *Brachionus* and a review. J. Limnol. 63, 32-36.

Gilbert, J. J., 2010. Effect of food concentration on the production and viability of resting eggs of the rotifer *Brachionus*: implications for the timing of sexual reproduction. Freshwater Biol. 55, 2437-2446.

Hagiwara, A., Suga, K., Akazawa, A., Kotani T., Sakakura Y., 2007. Development of rotifer strains with useful traits for rearing fish larvae. Aquaculture 268, 44-52.

Hamre, K., Srivastava, A., RØnnestad, I., Mangor-Jensen, A., Stoss, J., 2008. Several micronutrients in the rotifer *Brachionus* sp. may not fulfill the nutritional requirements of marine fish larvae. Aquacult. Nutr. 14, 51–60.

Hirayama, K.,Funamoto, H., 1983. Supplementary effect of several nutrients on nutritive deficiency of baker's yeast for population growth of the rotifer *Brachionus plicatilis*. Bull. Jpn. Soc. Sci. Fish. 49, 505–510.

Jékely, G., 2009. Evolution of phototaxis. Phil. Trans. R. Soc. B 364, 2795-2808.

Jékely, G., Colombelli, J., Hausen, H., Guy, K., Stelzer, E., Nédélec, F., Arendt, d., 2008. Mechanism of phototaxis in marine zooplankton. Nature 456, 395-399.

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., Suga, K., Hagiwara, A., 2013. Effect of light wavelength on the sexual and asexual reproduction of the monogonont rotifer *Brachionus manjavacas*. Aquaculture Sci. 61, 261-268.

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 stricto (Rotifera: Monogononta). J. Exp. Mar. Biol. Ecol. 460, 177-183.

Laderman, A. D., Gutman, H. N., 1974. Induction of sexuality by alteration of photoperiod in the rotifer *Brachionus rubens*. J. Exp. Zool. 152, 5-11.

Lass, S., Spaak, P., 2003. Chemically induced anti-predator defences in plankton: a review. Hydrobiologia 491, 221-239.

Melkonian, M., Robenek, H., 1979. The eyespot of the flagellate *Tetraselmis cordiformis* Stein (Chlorophyceae): structural specialization of the outer chloroplast membrane and its possible significance in phototaxis of green algae. Protoplasma 100, 183-197.

Mimouni, P., Luciani, A., Clément, P., 1993. How females of the rotifer *Asplanchna brightwelli* swim in darkness and light: An automated tracking study. Hydrobiologia 255/256, 101-108.

Nambu, Z., Tanaka, S., Nambu F., 2004. Influence of photoperiod and temperature on reproductive mode in the brine shrimp, *Artemia franciscana*. J. Exp. Zool. 301A, 542-546.

Richard, B., Forward, J. R., 1988. Diel vertical migration: zooplankton photobiology and behavior. Oceanogr. Mar. Biol. Annu. Rev. Barnes, M., Ed., Aberdeen University Press 26, 316-393.

Ringerberge, J., 1999. The photobehavior of *Daphnia* spp. as a model to explain diel vertical migration in zooplankton. Biol. Rev. 74, 397-423.

Sarma, S. S. S., 2011. Morphometric and demographic responses of brachionid prey (*Brachionus calyciflorus* Pallas and *Plationus macracanthus* (Daday)) in the presence of different densities of the predator *Asplanchna brightwellii* (Rotifera: Asplanchnidae). Hydrobiologia 662, 179-187.

Satuito, C.G., Hirayama, K., 1986. Fat-soluble vitamin requirements of the rotifer *Brachionus plicatilis*. In:

Maclean, J.L., Dizon, L.B., Hosillos, L.V. (Eds.), The First Asian Fisheries Forum. Asian Fisheries Society, Manila, Philippines, pp. 619–622.

Satuito, C.G.,Hirayama, K., 1991. Supplementary effect of vitamin C and squid liver oil on the nutritional value of baker's yeast for the population growth of the rotifer *Brachionus plicatilis*. Bull. Fac. Fish. Nagasaki Univ. 69, 7–11.

Storz, U. C., Paul, R. J., 1998. Phototaxis in water fleas (*Daphnia magna*) is differently influenced by visible and UV light. J. Comp. Physiol. A. 183, 709-717.

Palczewski, K., Kumasaka, T., Hori, T., Behnke, C. A., Motoshima, H., Fox, B. A., Le Trong, L., Teller, D. C., Okada, T., Stenkamp, R. E., Yamamoto, M., Miyano, M., 2000. Crystal structure of rhodopsin: AG protein-coupled receptor. Science 289, 739-745.

Viaud, G., 1940. Recherches experimnetales sur le phototropisme des Rotiferes. I. Bull. Biol. Fr, Belg. 74, 249-308.

Wolken, J. J., 1971. Invertebrate photoreceptors: a comparative analysis, Acad. Press., New York, London.

Yúfera, M., 2007. Swimming behavior of *Brachionus plicatilis* in relation to food concentration and feeding rates. Hydrobiologia 593, 13-18.

Zhong, M., Kawagushi, R., Kassai, M., Sun, H., 2012. Retina, retinol, retinal and the natural history of vitamin A as a light sensor. Nutrients 4, 2069-2096.

Tables

Table 1. Putative opsin-relevant genes identified in the genome database of *Brachionus koreanus*. The values of three parameters i.e., E-value, identities, positives were analyzed by in silico BLASTx search in the NCBI database (Kim et al., 2014b).

Gene	Length (bp)	Accession No.	Species (GenBank No.)	E-value	Identities (%)	Positives (%)
Blue-sensitive opsin-like	267	**KF885941**	*Latimeria chalumnae* (XP_006001498)	7E-10	41	58
C-opsin	882	**KF885939**	*Tribolium castaneum* (NP_001138950)	4E-38	33	54
Ciliary opsin	216	**KF885940**	*Platynereis dumerilii* (AAV63834)	2E-07	33	58
Ciliary opsin	624	**KF885942**	*Terebratalia transversa* (ADZ24786)	1E-31	36	57
GQ-rhodopsin	267	**KF885938**	*Daphnia pulex* (EFX63569)	8E-09	36	58
Melanopsin	747	**KF885936**	*Crassostrea gigas* (EKC19391)	7E-35	32	54
Melanopsin	684	**KF885946**	*Lottia gigantean* (ESO95853)	9E-27	32	47
Melanopsin	276	**KF885945**	*Myotis brandtii* (EPQ10710)	2E-11	36	58
Opsin	273	**KF885944**	*Schmidtea polychroa* (AFB74475)	1E-12	40	59
Opsin (encephalopsin, panopsin)	207	**KF885937**	*Danio rerio* (CAX13063)	7E-10	43	64
Peropsin	792	**KF885943**	*Hasarius adansoni* (BAJ22674)	3E-34	31	50
Rhabdomeric opsin	1,101	**KF885935**	*Platynereis dumerilii* (AGL94565)	2E-53	31	53

Figures

Fig. 1. Diagram of the cerebral eye of freshwater rotifer *Brachionus calyciflorus* (A) and euryhaline rotifer *Brachionus plicatilis* sensu sticto (B) with an electron microscope. **Ax**, axon; **PC**, pigment cup; **P**, platelets; **SC1**, **SC2**, sensory neurons; **RN**, relay neuron; **DL**, dendritic lamellae (Clément et al., 1983).

Fig. 2. Variation of rotifer eyespot area with different two diets *Nannochloropsis oculata* (closed circle) and baker's yeast (*Saccharomyces cerevisiae,* closed triangle). Plots and bars indicate the means and standard deviations, respectively. Different alphabetical letters on the plots denote significant differences (a > b, Tukey-Kramer post hoc test, $p < 0.05$, $n = 3$) (Kim et al., 2014b).

Fig. 3. Light absorbance variation of rotifer eyespot through 30-day culture with two different diets *Nannochloropsis oculata* (solid line) and baker's yeast (*Saccharomyces cerevisiae,* dotted line) from the hatchlings (A). Progress was observed with three culture days, day 10 (B), day 20 (C), day 30 (D) (Kim et al., 2014b).

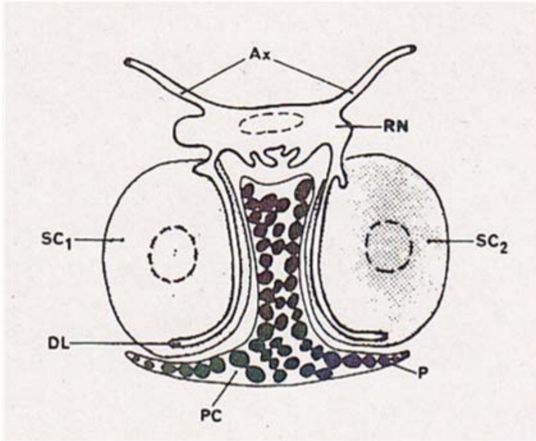
Fig. 4. Methods for the phototaxis of rotifers. (A) Dark adaptation rotifers were inoculated into the middle part of experimental vessel (for 5 min.), (B) Illumination using a LED bulb and (B-1) synchro-illumination using two LED bulbs for 15 min. after the removal of partitions. (C, C-1) Counting of distributed individuals after replacing partitions. The colors of LEDs (black and white) indicate light off and on, respectively (Kim et al., 2014a).

Fig. 5. The patterns of phototactic behavior (phototaxis) of the rotifer *Brachionus plicatilis* s. s. in a horizontal level. The white parts indicate illumination side and the color gradation to dark means the declining illumination in the horizontal histogram. The areas indicate the proportion of rotifers distributed in each compartment. The abbreviations W, B, G, R present white, blue, green, red of light wavelengths. Different alphabetical letters indicate statistically significant differences (a > b > c, Tukey-Kramer test, $p<0.05$, $n=3$) (Kim et al., 2014a).

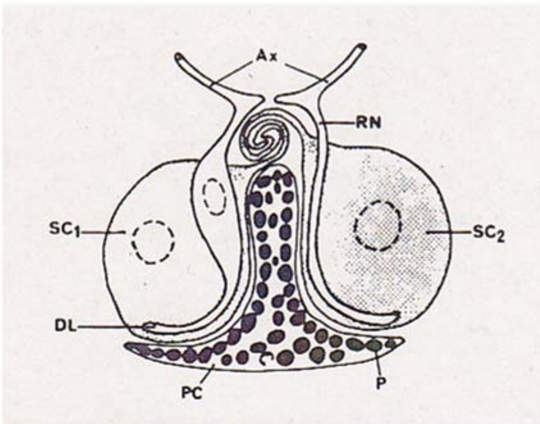
Fig. 6. Phototactic behavior of freshwater rotifer *Brachionus calyciflorus*. Open circles present the percentage of positive phototaxis counted in lighting compartment measured by Cornillac et al. (1983) and closed circles is by Viaud (1940).

Fig. 7. Population growth patterns under different light wavelengths and intensities in the euryhaline rotifer *Brachionus plicatilis* s. s. The abbreviations W, B, G, R present white, blue, green, red of light wavelengths. Bars and error bars indicate means and standard deviations, respectively. Different alphabetical letters indicate significant differences (a > b > c > d, Tukey-Kramer test, $p<0.05$, $n=3$) (Kim et al., 2014a).

Fig. 8. The patterns of sexual and asexual reproduction of rotifer *Brachionus manjavacas* related to different light wavelengths. Closed circles, open diamonds, closed squares, and open triangles indicated population growth of female rotifers (A), male production (B), and resting egg production (C) with white, blue, green, and red light, respectively.



(A)



(B)

Fig. 1.

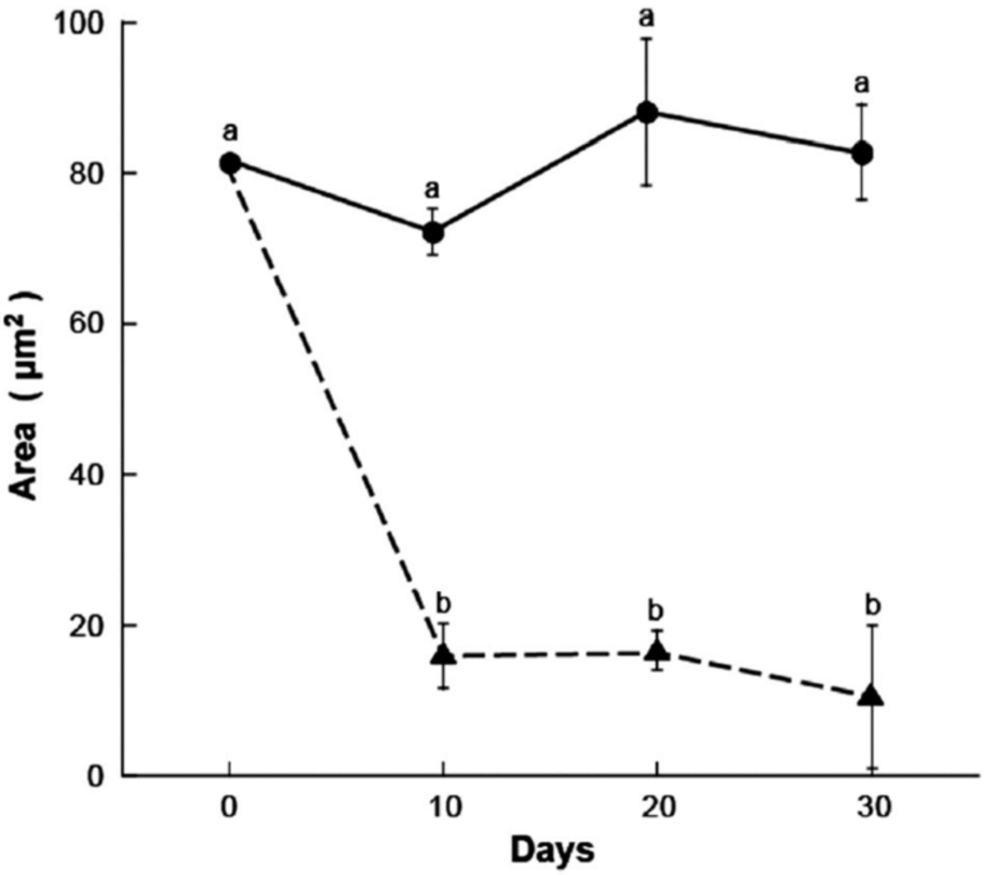


Fig. 2.

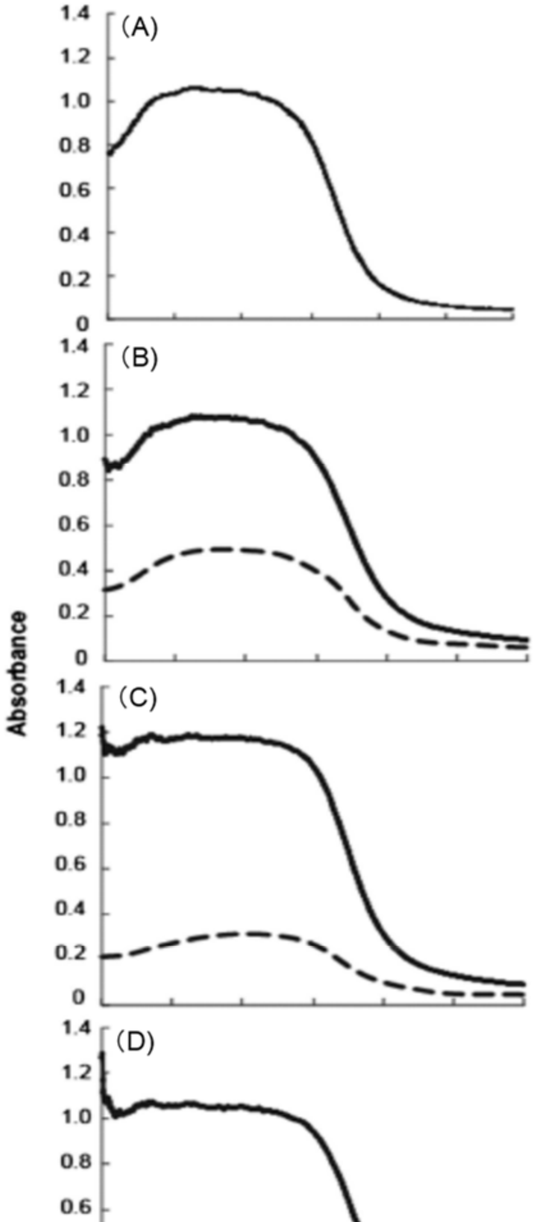

(A)
(B)
(C)
(D)
Absorbance
0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
400
450
500
550
600
650
700
Light wavelength (nm)

Fig. 3.

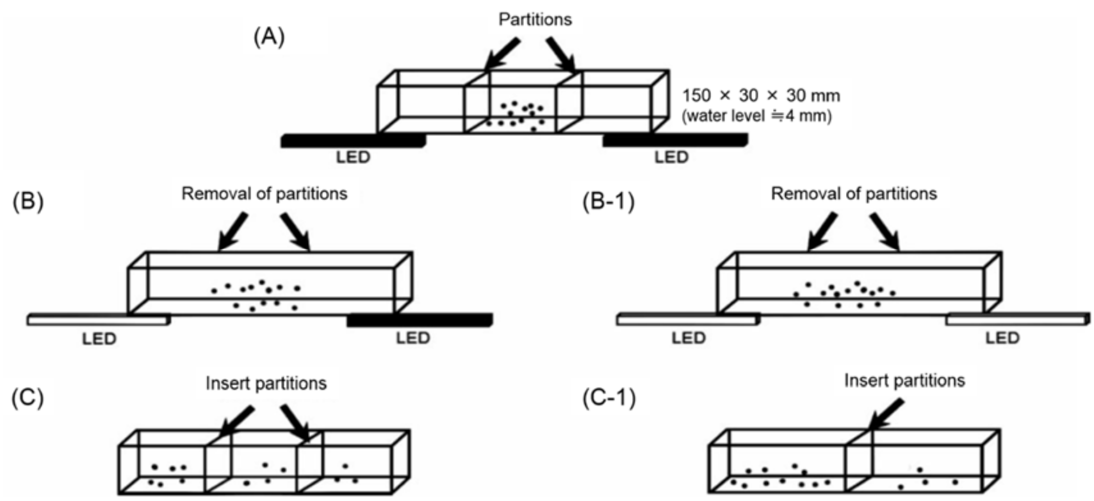

(A)
Partitions
150 × 30 × 30 mm
(water level ≒4 mm)
LED
LED
(B)
Removal of partitions
LED
LED
(B-1)
Removal of partitions
LED
LED
(C)
Insert partitions
(C-1)
Insert partitions

Fig. 4.

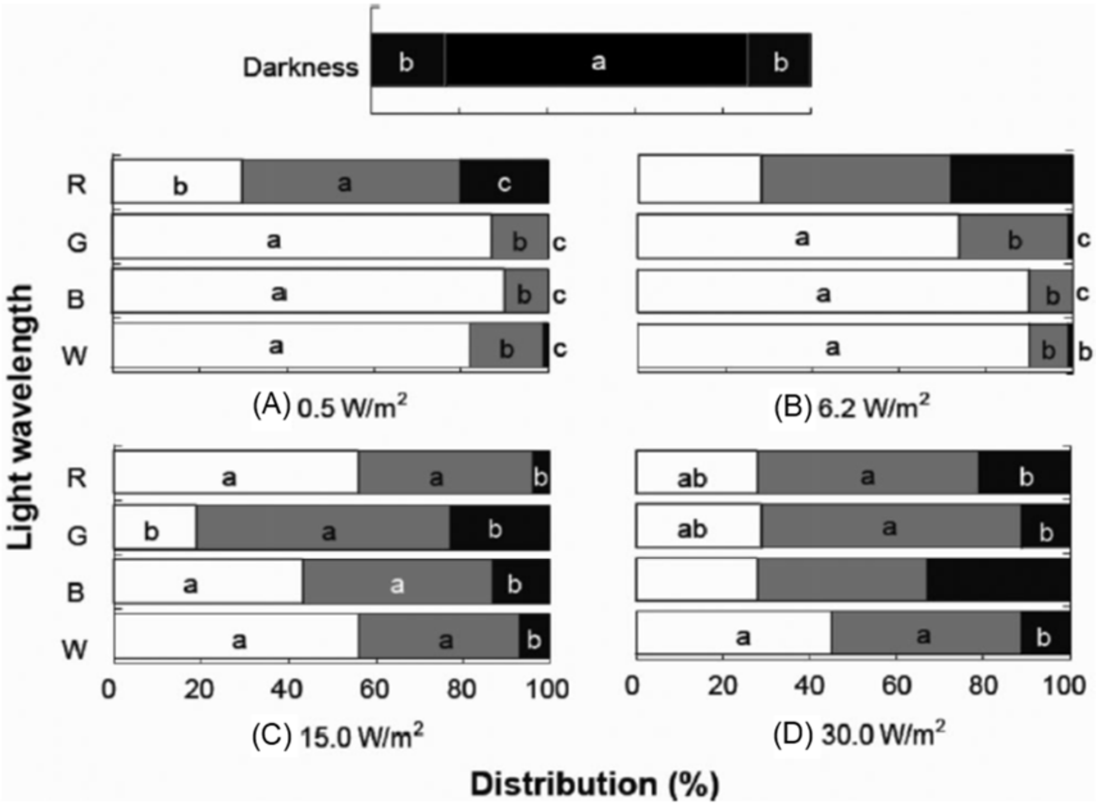

Darkness
R
G
B
W
Light wavelength
(A) 0.5 W/m2
(B) 6.2 W/m2
(C) 15.0 W/m2
(D) 30.0 W/m2
0 20 40 60 80 100
Distribution (%)

Fig. 5.

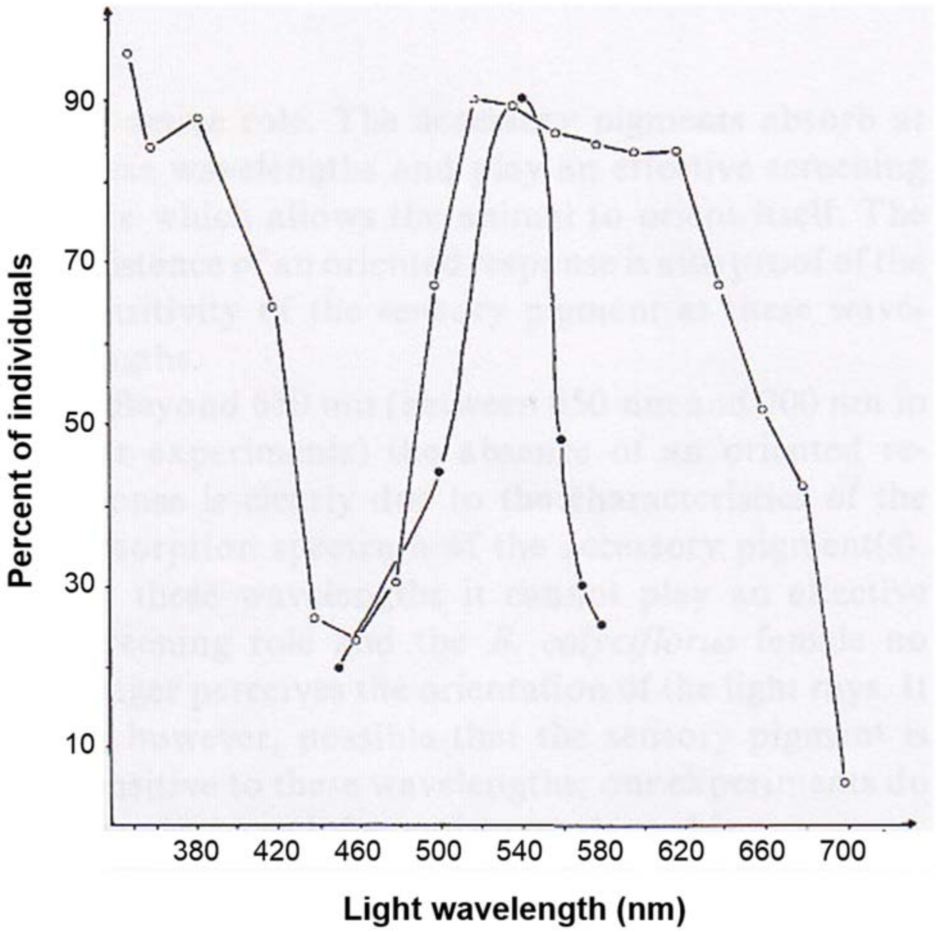

Percent of individuals
90
70
50
30
10
380
420
460
500
540
580
620
660
700
Light wavelength (nm)

Fig. 6.

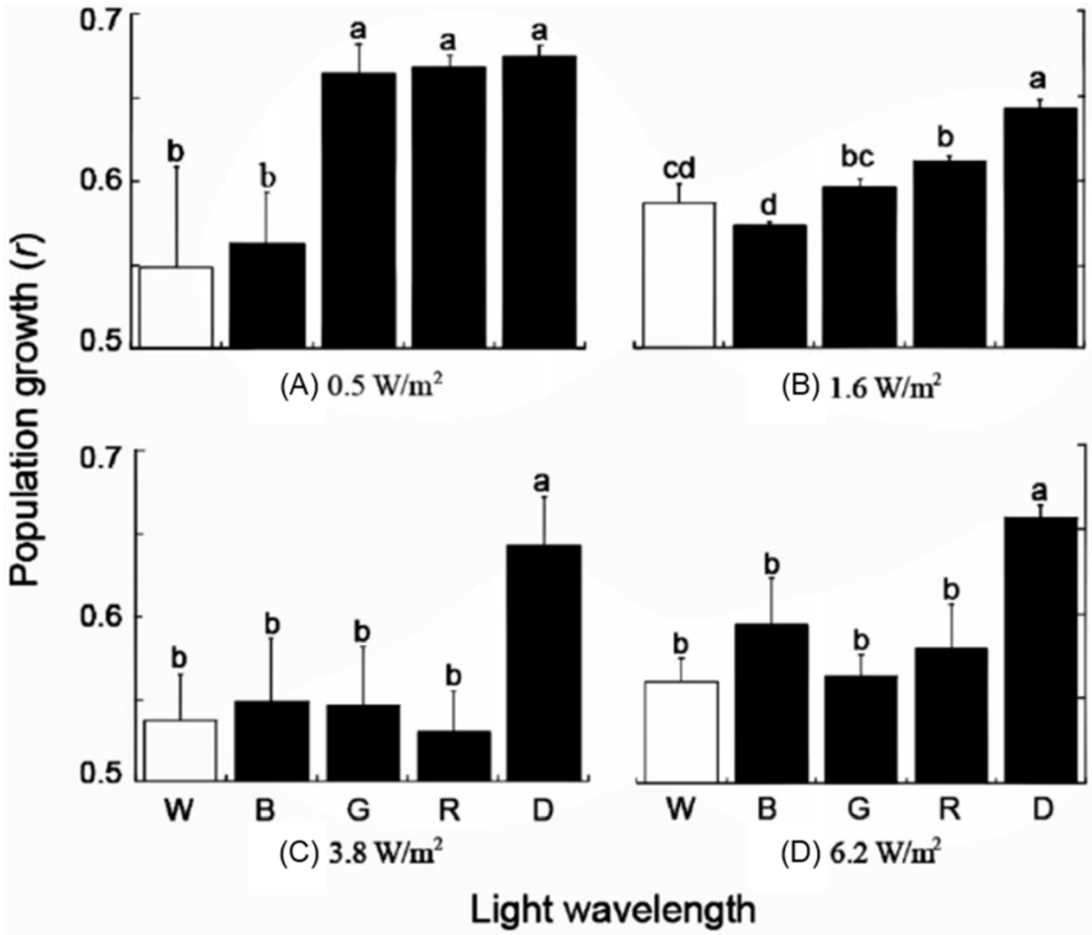
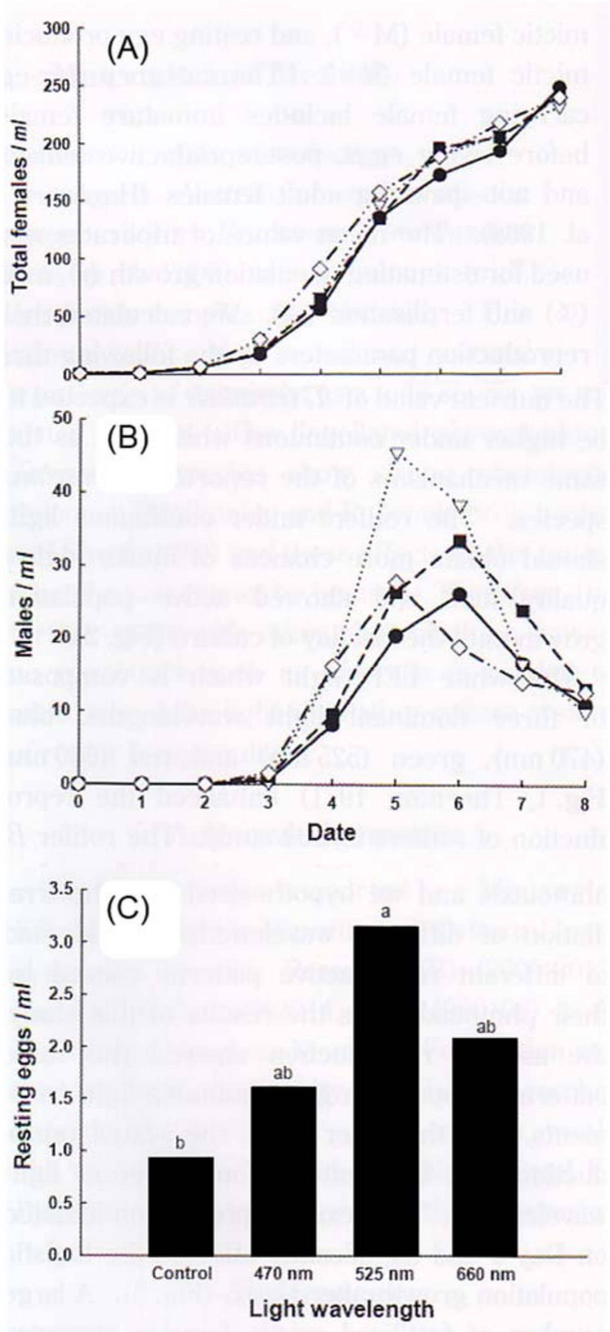

Population growth (r)
0.7
0.6
0.5
b
b
a
a
a
(A) 0.5 W/m²
cd
d
bc
b
a
(B) 1.6 W/m²
b
b
b
b
a
W
B
G
R
D
(C) 3.8 W/m²
b
b
b
b
a
W
B
G
R
D
(D) 6.2 W/m²
Light wavelength

Fig. 7.



(A)
Total females / ml
300
250
200
150
100
50
0
(B)
Males / ml
50
40
30
20
10
0
0
1
2
3
4
5
6
7
8
Date
(C)
Resting eggs / ml
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
b
ab
a
ab
Control
470 nm
525 nm
660 nm
Light wavelength

Fig. 8.