Effect of Light Wavelength on the Sexual and Asexual Reproduction of the Monogonont Rotifer *Brachionus manjavacas*

Hee-Jin KIM, Koushirou SUGA and Atsushi HAGIWARA*[1]

Abstract: The monogonont rotifer *Brachionus manjavacas* (Australian strain) showed a steadily increasing population growth and larger number of resting egg production under continuous white light compared to under total darkness when they were batch cultured. By comparing different wavelength of light, such as white (control), 470 (blue), 525 (green) and 660 (red) nm, rotifers showed no significant differences in specific population growth rate, but sexual reproduction showed different patterns associated with light wavelengths. Although there were no significant differences with regard to mixis induction, the resting egg formation actively occurred at 525 nm. We further observed the movement of female rotifers to find the influential factor of different sexual reproduction. There was no significant difference in the mean swimming speed of 10 female rotifers, but the proportion of settling individuals varied with light wavelength. Under 525 nm light, no individuals continuously settled for one minute, while under other light wavelengths the percent of settling females for a minute ranged between 6.1±5.4 and 23.8±6.5%. The higher ratio of swimming females at 525 nm should enhance the male/female encounters, which resulted in higher resting egg formation.

Graduate School of Fisheries Science and Environmental Studies, Nagasaki University, Bunkyo 1-14, Nagasaki 852-8521, Japan.
* Corresponding author: E-mail, hagiwara@nagasaki-u.ac.jp (A. Hagiwara).

Key words: *Brachionus manjavacas*; Light wavelength; Reproduction; Behavior

Aquatic organisms living near the surface such as planktonic metazoan including zooplankton and marine invertebrate larvae are exposed to solar light, and they show the phototactic responses such as the diel and ontogenetic vertical distribution (Forward 1988; Ringelberg 1999; Jékely et al. 2008). They have eyespots that cannot form images but can sense the direction of light (Jékely et al. 2008). The common planktonic invertebrate, the monogonont rotifer Brachionus, has a cerebral eye (red eye spot) consisting of two types of pigment-bearing cells: epithelial cells consisting of cup-shaped pigmented cells (accessory pigments), and sensory neurons to process a specialized membranous structure associated with the photoreceptor pigment (sensory pigment) (Clément 1980; Clément et al. 1983; Cornillac et al. 1983). Rotifers can detect the direction of light with the functional cerebral eye. They show sensitivity to direction, quantity, quality, duration and wavelength of light (Clément et al. 1983). We focused on the light wavelength-dependent phototaxis (Clément 1980; Clément et al. 1983; Cornillac et al. 1983) effect on rotifer reproduction in the present study.

Euryhaline monogonont rotifers have a cyclically parthenogenetic life cycle with both asexual (amictic) and sexual (mictic) reproduction and it is affected by various internal and external factors (Ricci 2001; Serra et al. 2004; Hagiwara et al. 2007; Gilbert 2010). Asexual reproduction predominates in the rotifer's 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. Among the environmental factors, light plays an important role in the behavior of numerous plankton species with phototaxis (Forward 1988; Buskey et al. 1989; Storz and Paul 1998). In the case of rotifers, the light affects reproduction as well as behavior. In the light, *Asplanchna brightwelli* swims at higher speeds and with fewer turns than darkness as a phototactic response (Mimouni et al. 1993). *B. rubens* shows sensitivity to light cycles in male production (Laderman and Gutman 1974; Gilbert 2004) and *Notommata* sp. and *Trichocera* sp. are affected by a long photoperiod in mictic female production (Gilbert 2004). The previous studies have been made on freshwater rotifers and little attention has been given to euryhaline rotifers. Euryhaline rotifer *Brachionus* species, they are widely used as the live food in marine larviculture, and the optimization of these rotifer culture condition has a primary importance. In this study, we tried to optimize the rotifer culture condition by the regulation of illuminating light wavelength.

We predicted the effect of continuous lighting on the reproduction of the euryhaline rotifer *Brachionus manjavacas* as a function of rotifer eyespot. We firstly investigated the effect of presence of light on the reproduction of rotifers using continuous white light composed of broad range of wavelength including blue, green and red regions. Secondly, the effect of specific wavelength on the behavior and reproduction of the rotifer *B. manjavacas* was studied. If the rotifers show different reproductive patterns against light wavelengths, different behavior such as swimming and settlement movements associated with four light wavelengths may affect those patterns. Thus, we monitored the movements of female rotifers associated with different light wavelengths.

Materials and Methods

Lighting effects on rotifer reproduction

We employed Australian strain of the monogonont rotifer *Brachionus manjavacas* (Fontaneto et al. 2007), which belongs to *B. plicatilis* species complex for this study. This strain shows both active sexual and asexual reproduction (Araujo and Hagiwara 2005; Kim and Hagiwara 2011). The resting eggs of rotifers were produced at 11 psu, 25.0±0.5°C, and the eggs were hatched under 1.4 W/m^2 of fluorescence light with photoperiod at 24L:0D. New born neonates from resting eggs were inoculated into 30 *ml* mayonnaise bottles containing 20 *ml* of 11 psu culture medium at 1 ind/*ml*. The culture medium was prepared by the dilution of natural seawater with mili-Q water (Millipore 0.22 μm) followed by GF/C filtration (Whatman) and sterilization (121^{o}C, 20 minutes). The rotifers were cultured at 25.0±0.5^{o}C with the daily feeding of *Tetraselmis tetrathele* (0.24x10^6 cells/*ml*) and no aeration for 8 days in triplicates. The food of rotifer, *T. tetrathele* cultured in Erd-Schreiber medium was centrifuged at 2,000 x*g* for 10 minutes, and resuspended in the rotifer culture medium. *T. tetrathele* is effective for enhancing both sexual and asexual reproduction of *B. plicatilis* species complex (Korstad et al. 1989; Hagiwara and Hino 1990). Light-emitting diodes (LEDs, Keystone Technology Inc., Japan) were used for the light source, and the rotifers were equally illuminated from side of a bottle and the light intensity was equal in triplicate cultures. Control rotifers were cultured in total darkness, and treatments were under continuous white-LED light (with peaks at 460 and 570 nm, also see Fig. 1) at 1.4 W/m^2 measured by fiber optic spectrometer (USB 4000, Ocean optics Inc., USA). The mean number of rotifers was estimated by daily count of female and male rotifers without fixation by pipetting out 1 *ml* samples from triplicate cultures. Female rotifers were classified into 4 types on the basis of carrying egg types: non-egg carrying female,

female-producing amictic female (F♀), male-producing mictic female (M♀), and resting egg-producing mictic female (R♀). The category non-egg carrying female includes immature females before laying eggs, post-reproductive females, and non-spawning adult females (Hagiwara et al. 1988). The mean values of triplicates were used for estimating population growth (*r*), mixis (%) and fertilization (%). We calculated these reproduction parameters by the following three equations (Hagiwara and Hino 1988):

Population growth rate (*r*): $\ln (N_t/N_0) / t$

Mixis (%): [(M♀+R♀)/(F♀+M♀+R♀)]x100

Fertilization (%): [(R♀)/(M♀+R♀)]x100

Where t is the culture days, and N_0 and N_t are the number of all the types of female rotifers on Day 0 and t, respectively. The number of produced resting eggs was also counted daily.

Fig. 1

Light wavelength effects on rotifer reproduction

To test the effect of light wavelength on the reproduction of rotifers, we cultured the rotifers for 8 days under four different wavelength LED lights (Keystone Technology, Inc., Japan): white (control), 470 (blue), 525 (green) and 660 (red) nm as shown in Fig 1. Other culture conditions and observations were set up the same as in the experiment of lighting effects.

Light wavelength effects on rotifer movement

The swimming behavior such as swimming speed and the number of swimming and settling rotifers under control and three different wavelength lights (white, 470, 525 and 660 nm) were monitored on Day 8. We pipetted out 300 *μl* of medium from 20 *ml* cultures containing about 30 rotifers into a well of 48-well microplate with three replicates. Movements of rotifers were recorded for 1 minute under a stereomicroscope at x12.5 (SteREO Discovery V8, Carl Zeiss, Inc., USA). We analyzed swimming speed of 10 female rotifers and the proportion of settling individuals using Dipp Motion Pro version 2.01 (DITECT Co. Ltd., Japan).

Statistical analysis

We used *t*-test to evaluate the effect of presence of light on the pattern of reproduction and the movements of rotifers. Analysis of variance (ANOVA) was performed to examine the effect of light wavelength followed by Tukey-Kramer multi-comparison test. All statistical analyses were performed using Statview version 5.0 software (SAS Institute, Inc., USA).

Results

Lighting effects on rotifer reproduction

Initial population growth until Day 4 was higher in total darkness (r=1.21±0.01/day) than with continuous light (r=0.98±0.02/day; n=3, *t*-test, P<0.05). Rotifers under continuous light showed a steady increase of population growth until the end of culture, while no population growth was observed in total darkness since Day 5 (Fig. 2a). In the sexual reproduction, percent mixis (4.8±0.8% in total darkness and 4.3±1.2% under continuous light) and fertilization (17.7±5.3% in total darkness and 21.5±1.5% under continuous light) showed no significant differences between under lighting and darkness. The rotifers cultured under continuous light formed a larger number of resting eggs (0.9±0.2 eggs/*ml*) than those in total darkness (0.5±0.3 eggs/*ml*) for 8 days (Fig. 2b).

Fig. 2

Light wavelength effects on rotifer reproduction

The population of total female rotifers (consisting of four female types) continuously increased until the end of culture (see Fig. 3). There was no significant difference in the population growth rate (*r*) during the first five days among three different light wavelengths and control (white), which ranged from 0.98±0.02 to 1.01±0.02/day (Table 1). On the other hand, the initial population growth until Day 3 was higher at 470 nm (Tukey-Kramer test, $P<0.05$).

Fig. 3

Male-producing females (unfertilized mictic females) initially appeared on Day 2. The density of male-producing females showed no difference among all cultures, while the density of resting egg-producing females at 525 nm was higher than others (Fig. 3). Sexual reproductive parameters showed different patterns associated with light wavelength (Table 1). Although there was no significant difference in mixis rate (3.3±0.5% - 4.9±1.4%), the fertilization rate was the lowest under white light (21.5±1.5%). On the other hand, the rotifers produced more males at 660 nm (126.6±13.4 males/*ml*; Fig 4a), while a higher number of resting egg was produced at 525 nm (3.2±1.6 resting eggs/*ml*, Fig 4b). Moreover, the initiation of resting egg production was the earliest at 525 nm on Day 4, but the rotifers at other light wavelengths started to produce resting eggs on Day 5 (Fig. 4b). The production of resting eggs at 525 nm was also maintained at a high level (0.9±0.5 resting eggs/*ml*) until the last day of culture.

Table 1

Fig. 4

Light wavelength effects on rotifer movement

The mean swimming speed of 10 female rotifers in each culture was not significantly different among all cultures (Table 2). However the proportion of settling rotifers was different associated with light wavelengths. No individuals continuously attached on the side and bottom of a well of multiwall plate at 525 nm light (0%) for a minute, while rotifers under other wavelengths showed 6.1±5.4 (control) – 23.8±6.5% (660 nm) of settlement.

Table 2

Discussion

The population growth of euryhaline rotifer *Brachionus manjavacas* (Australian strain) is affected by continuous irradiation of light. The population growth under continuous white light increased until the last day of culture in contrast to that in total darkness ceased on Day 4 (Fig. 2a). The rotifers under both treatments were daily fed on the same amount of food ($0.24x10^6$ *T. tetrathele* cells/*ml*) during culture period. This feeding amount did not support increasing population, and the rotifers in total darkness maintained the density at the end of culture (Snell 1986; Kirk 1997). On the other hand, the rotifers cultured under white light showed continuous population growth under food limitation condition. The initial population growth (until Day 4) in complete darkness is higher than that under light. Freshwater rotifer *Asplancha brighwelli* exhibits photokinesis and they show active orthokinesis reaction (moving faster and more dispersion) under light (Mimouni et al. 1993). The same phenomenon was also reported with *B. calyciflorus* (Viaud 1940; Clément 1977). It is speculated that the tested rotifers spent more energy to move under light than darkness during the period of light adaptation (until Day 4), even though feeding amount was same.

Therefore, the rotifers under light might experience the energy shortage for reproduction resulting in lower population growth until Day 4. In the sexual reproduction, the higher number of produced resting eggs was shown under light. There were no significant differences in sexual reproductive parameters and in the number of produced resting eggs per each single sexual female (Table 3). Thus, the higher number of sexual female rotifers should affect the number of produced resting eggs. Table 3

Light quality and quantity such as intensity and day length are important factors regulating the growth of phytoplankton (Aidar et al. 1994; Meseck et al. 2005). *Tetraselmis chui* cultured under longer day length (for 24 hours) and higher light intensities (220 μEinst/m^2/s) showed higher biomass (Meseck et al. 2005), although phytoplankton loses energy by respiration in total darkness (Nybakken 2001). Moreover, *Tetraselmis gracilis* is more stimulated to synthesize pigments and protein when incubated under white light (Aidar et al. 1994). The nutrient value of *T. tetrathele* is expected to be higher under continuous white light as the same mechanisms of the reported *Tetraselmis* species. The rotifers under continuous light should obtain more chances of intake higher quality food and showed active population growth until the last day of culture (Fig. 2a).

The white LED light which is composed of three dominant light wavelengths, blue (470 nm), green (525 nm) and red (660 nm, Fig. 1, Thornton 1971) enhanced the reproduction of rotifers in this study. The rotifer *B. manjavacas* shows light wavelength-dependent phototaxis and we hypothesized that the irradiation of different wavelengths should lead to different reproductive patterns caused by their phototaxis. As the results of this study, the asexual reproduction showed the same pattern of population growth among light treatments. On the other hand, the sexual reproduction was different by four different light wavelengths. The sexual reproduction initiated on

Day 2 and significantly affected the logistic population growth after Day 5 (Fig. 3). A large number of fertilized mictic females appeared at 525 nm on Day 5 with the earliest production of resting eggs on Day 4 (Fig. 4). On the other hand, a number of sexual females at 660 nm remain unfertilized (Fig. 3) and the males actively appeared on Day 5 (Fig. 4). We postulated that the key factor of active resting egg production is the mechanism of fertilization, and observed female behaviors under four different light wavelengths. There were no significant differences in swimming speed against four different light wavelengths, while no individuals continuously settled on a wall of microplate at 525 nm (Table 2). Rotifer males continue to swim (Hagiwara et al. 1988), so that they may have more chances to encounter with swimming females than settling ones and these movements could lead to active fertilization. To support this view, it is required to examine the behavior of male rotifer under different light wavelengths. Because, male rotifers also have an eyespot and are possible to show different movement patterns associated with light wavelengths. In this study, *T. tetrathele* was employed as a food source for the tested rotifers. The flagellated phytoplankton *Tetraselmis* species show strong phototactic response (Melkonian and Robenek 1979; Foster and Smyth 1980) and these effects on the rotifer reproduction cannot be ignored. Therefore, the further study with other phytoplankton having no phototaxis such as *Chlorella* is needed to make use of the light regulating culture system of rotifer in aquaculture.

Acknowledgements

This research was supported by a Ministry of Education, Science, Sports and Culture Grant-in-Aid for Scientific Research (B) (2009-2011, No. 21380125; 2012-2014, No.

24380108) to A. H., Rotary Yoneyama Memorial Foundation and the Nagasaki University Special Researcher Scholarship to H.-J. K. The authors would like to thank Prof. David L. G. Noakes and Eli Bjoernoe Sjulstad for improving the manuscript.

References

Aidar, E., S. M. F. Gianessella-Galvao, T. C. S. Sigaud, C. S. Asano, T. H. Liang, K. R. V. Rezende, M. K. Oishi, F. J. Aranha, G. M. Milani and M. A. L. Sandes (1994) Effects of light quality on growth, biochemical composition and photosynthetic production in *Cyclotella caspia* Grunow and *Tetraselmis gracilis* (Kylin) Butcher. *J. Exp. Mar. Biol. Ecol.*, **180**, 175-187.

Araujo, A. and A. Hagiwara (2005) Screening methods for improving rotifer culture quality. *Hydrobiologia*, **181**, 553-558.

Buskey, E. J., K. S. Baker, R. C. Smith and E. Swift (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.

Clément, P. (1977) Phototaxis in rotifers (action spectra). *Arch. Hydrobiol. Beih.*, **8**, 47-49.

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

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

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

Fontaneto, D., I. Gioradani, G. Melone and M. Serra (2007) Disentangling the morphological stasis in two rotifer species of the *Brachionus plicatilis* species complex. *Hydrobiologia*, **583**, 297-307.

Forward, R. (1988) Diel, B. vertical migration: zooplankton photobiology and behavior. *Oceanogr. Mar. Biol. Annu. Rev.*, **26**, 361-393.

Foster, K. W. and R. D. Smyth (1980) Light antennas in phototactic algae. *Microbiol. Rev.*, **44**, 527-630.

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. and A. Hino (1988) Effect of incubation and preservation on resting egg hatching and mixis in the derived clones of the rotifer *Brachionus plicatilis*. *Hydrobiologia*, **186/187**, 415-421.

Hagiwara, A. and A. Hino (1990) Feeding history and hatching of resting eggs in the marine rotifer *Brachionus plicatilis*. *Nippon Suisan Gakkaishi*, **56**, 1965-1971.

Hagiwara, A., A. Hino and R. Hirano (1988) Effects of temperature and chlorinity on resting egg formation in the rotifer *Brachionus plicatilis*. *Nippon Suisan Gakkaishi*, **54**, 569–575.

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

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

Kim, H.-J. and A. Hagiwara (2011) Effect of female aging on the morphology and hatchability of resting eggs in the rotifer *Brachionus plicatilis* Müller. *Hydrobiologia*, **662**, 107-111.

Kirk, K. L. (1997) Life-history responses to variable environments: Starvation and reproduction in planktonic rotifers. *Ecology*, **78**, 434-441.

Korstad, J., Y. Olsen and O. Vadstein (1989) Life history characteristics of *Brachionus plicatilis* (rotifera) fed different algae. *Hydrobiologia*, **186/187**, 43-50.

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

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

Meseck, S. L., J. H. Alix and G. H. Wikfors (2005) Photoperiod and light intensity effects on growth and utilization of nutrients by the aquaculture feed microalga, *Tetraselmis chui* (PLY 429). *Aquaculture*, **246**, 393-404.

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

Nybakken, J. W. (2001) *Marine Biology: An Ecological Approach. 5th ed.* Bengamin Cummings, an imprint of Addison Wesley Longman, Inc., San Francisco, 516 pp.

Ricci, C. (2001) Dormancy patterns in rotifers. *Hydrobiologia*, **446/447**, 1-11.

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

Serra, M., T. W. Snell and C. E. King (2004) The timing of sex in cyclically parthenogenetic rotifers. In “*Evolution. From Molecules to Ecosystem*” (ed. by A. Moya and E. Font), Oxford University Press, Oxford, pp. 135-146.

Snell, T. W. (1986) Effect of temperature, salinity and food level on sexual and asexual reproduction in *Brachionus plicatilis* (Rotifera). *Mar. Biol.*, **92**, 157-162.

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

Thornton, W. A. (1971) Luminosity and color-rendering capability of white light. *J. Opt. Soc. Am.*, **61**, 1155-1163.

Viaud, G. (1940) Rechèrches expérimentales sur le phototropisme des rotiférs, *I*. *Bull. Biol. Fr. Belg.*, **74**, 249-308.

ワムシの生殖に与える光波長の影響

金 禧珍・菅 向志郎・萩原篤志

シオミズツボワムシ複合種 *Brachionus manjavacas*（いわゆる L 型オーストラリア株、以下ワムシと略）を *Tetraselmis* 給餌によってバッチ培養したところ、暗黒下での培養よりも白色光の連続照射下で活発な個体群増殖と耐久卵形成を示した。次に、青（470 nm)、緑（525 nm)、赤（660 nm)および白色光（対照）の連続光照射下（約 1.4 W/m^2）で培養し、個体群増殖率（r）、両性生殖誘導率、受精率、耐久卵数を求めた。異なる波長光の照射によってワムシの個体群増殖率と両性生殖誘導率に変化はなかったが、耐久卵形成は光波長 525 nm のとき最も活発に起こった。雌ワムシの遊泳速度は照射光波長にかかわらず一定だったが、付着行動を示す雌ワムシは光波長 660 nm のとき最も多くなった。一方、光波長 525 nm では付着個体が出現せず、全個体が遊泳した。光波長 525 nm のとき、雌ワムシの活発な遊泳行動によって、雄ワムシとの接触頻度が増大し、受精と耐久卵形成が促進された可能性がある。

Tables

Table 1. Reproduction parameters of the Australian rotifer *Brachionus manjavacas* under different light wavelengths

Light wavelength	Population growth (/day)	Mixis (%)	Fertilization (%)
White	0.98±0.02	4.3±1.2	21.5± 1.5[b]
470 nm	1.01±0.02	3.3±0.5	31.5± 0.2[a]
525 nm	0.98±0.03	4.9±1.4	38.4±11.2[a]
660 nm	1.00±0.00	4.1±0.3	34.4± 9.5[a]

Values are mean ± standard deviations of three replicates. Superscript letters indicate the significant differences (a>b, Tukey-Kramer test, $P<0.05$).

Table 2. Movements of the Australian rotifer *Brachionus manjavacas* (swimming speed of 10 female rotifers and the number of attached individuals in triplicates) at different light wavelengths

	White	470 nm	525 nm	660 nm
Swimming speed (mm/sec)	0.46±0.24	0.77±0.21	0.49±0.25	0.52±0.36
Settling individuals (%)	6.1 ± 5.4^{b}	7.6 ± 0.8^{b}	0^{b}	23.8 ± 6.5^{a}

Values are mean ± standard deviations. Superscript letters indicate the significant differences (a>b, Tukey-Kramer test, *P*<0.05).

Table 3. The number of produced resting eggs per a sexual female from the initial day of resting egg production to the end of culture

	Darkness	Light
Day 5	0.11±0.19	0.06±0.06
Day 6	1.39±0.79	0.52±0.19
Day 7	2.67±2.31	1.48±0.67
Day 8	0	1.54±1.28

Values are mean ± standard deviations.

Figure captions

Fig. 1. Luminescence spectrum of four LEDs: white, blue, green, and red from Keystone Technology Inc (Japan). Lines indicate light wavelengths such as black, blue, green and red lines indicate the white (with peaks at 460 and 570 nm), blue (470 nm), green (525 nm), and red (660 nm) LED's spectrum, respectively.

Fig. 2. Lighting effect on the reproductive pattern of the Australian rotifer *Brachionus manjavacas*; (A) variation of total female density. Closed and open circles indicate the density of total female rotifers in a total darkness and light, respectively. (B) Total number of produced resting eggs for 8 days. Black and white columns indicate the number of produced resting eggs in a total darkness and light, and vertical bars represent standard deviations. Asterisk on (B) shows statistically significant difference (*t*-test, $P<0.05$).

Fig. 3. Density variations of total females and these female types of rotifers for 8 days associated with different light wavelengths. Closed circles, open diamonds, closed squares, and open triangles indicate the population growth of each female rotifers under white, 470, 525 and 660 nm light, respectively.

Fig. 4. Male (A) and resting egg production (B) in response to different light wavelengths by the Australian rotifer *Brachionus manjavacas*. (A): Closed circles, open diamonds, closed squares, and open triangles indicate the production of male rotifers under white, 470, 525 and 660 nm light, respectively. (B): Marks on bars indicate resting egg production on Day 4 , Day 5 , Day 6 , Day 7

and Day 8 . The arrow and alphabets on (B) indicate the production of resting eggs on Day 4 and significant differences (a>b, Tukey-Kramer test, $P<0.05$), respectively.

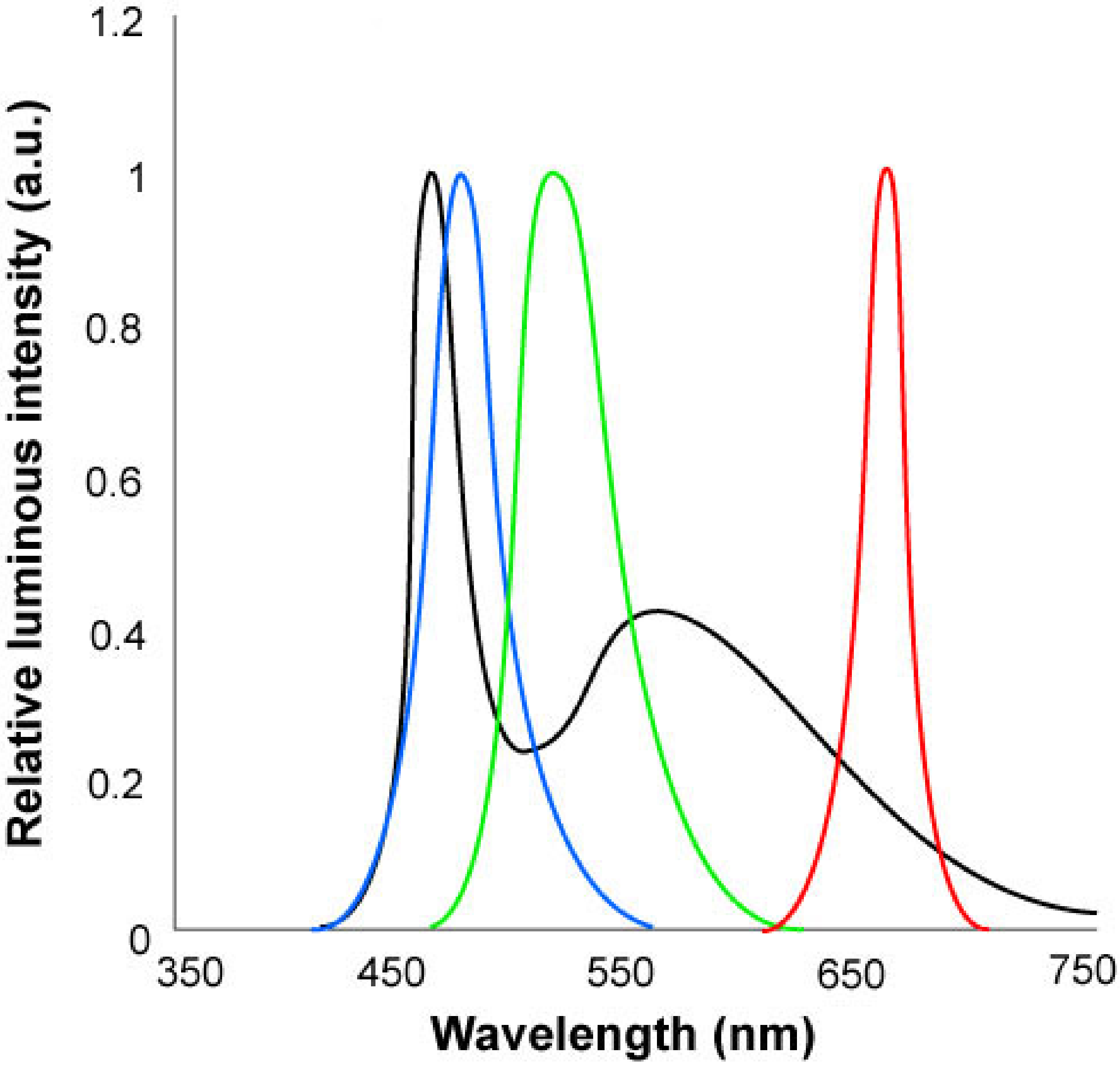

Fig. 1

Kim et al. (50%)

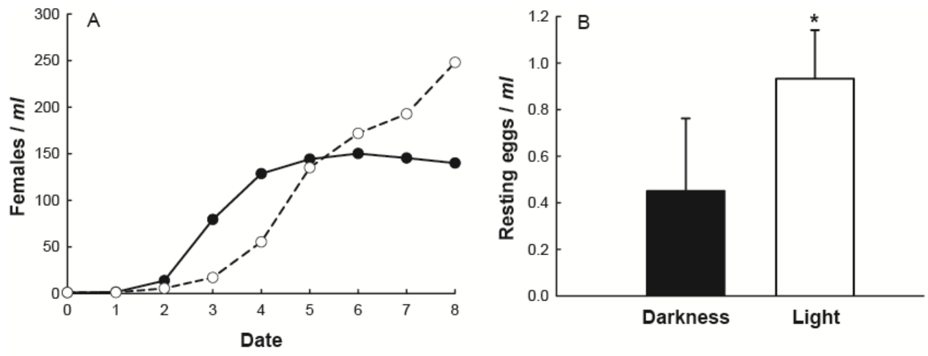

Fig. 2

Kim et al. (100%)

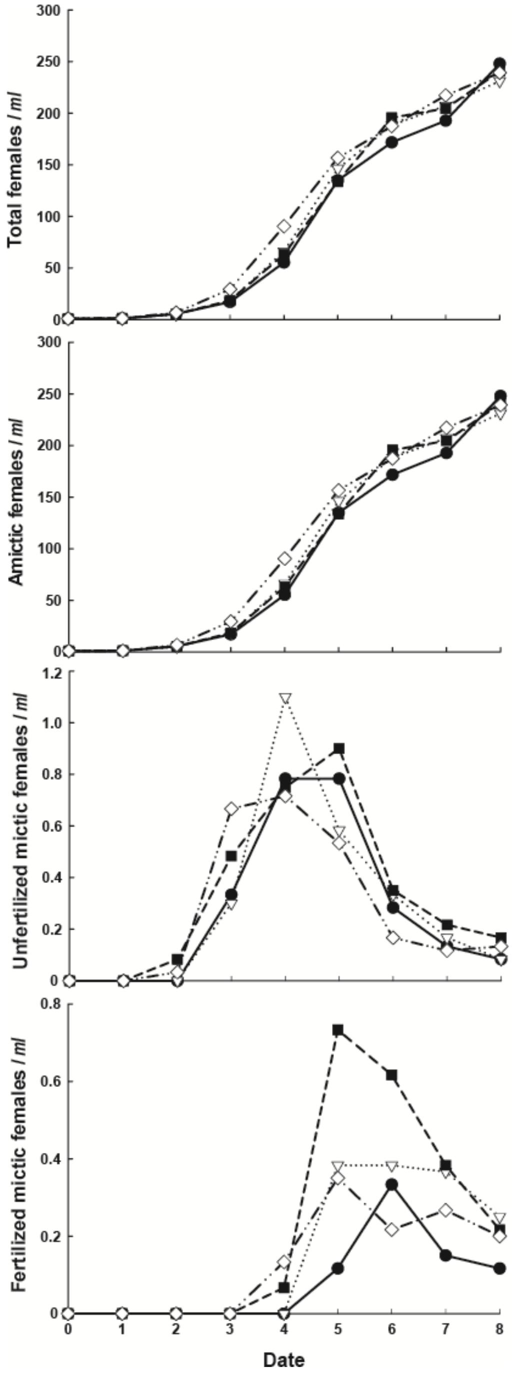

Fig. 3

Kim et al. (100%)

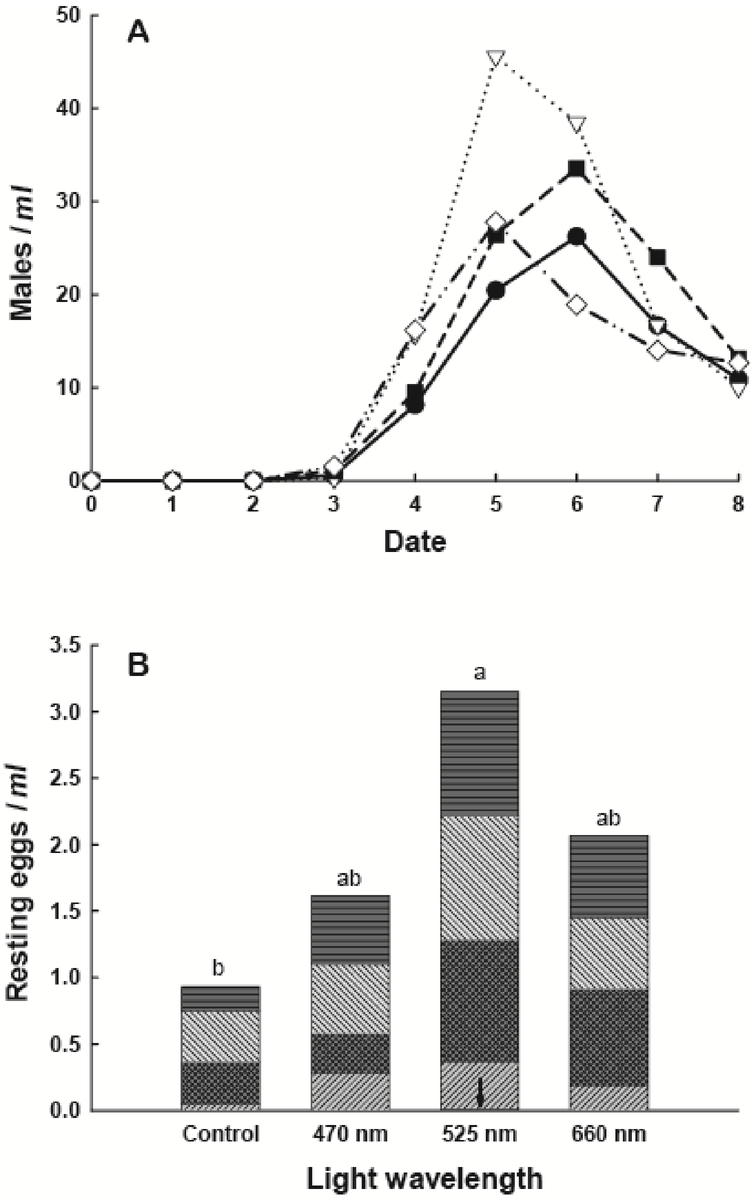

Fig. 4

Kim et al. (70%)