Behavior and reproduction of the rotifer *Brachionus plicatilis* species complex under different light wavelengths and intensities

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We investigated the influence of light on phototactic behavior and reproduction in two species of rotifer from the *Brachionus plicatilis* species complex (*Brachionus plicatilis* sensu stricto (s. s.) and *Brachionus manjavacas*). This was done to understand how light effects these species so that we might use this knowledge to establish a more efficient aquaculture protocol. We used four different light wavelengths (white, with peaks at 460 and 570 nm; blue at 470 nm; green at 525 nm; and red at 660 nm) and four intensities (i.e., 0.5 to 30.0 W/m^2). Using micro-spectrophotometry we determined that eyespots of these two *Brachionus* species absorbed blue and green light 5.5 times more than red light. *Brachionus plicatilis* s. s. showed positive phototaxis under white, blue, and green light at lower light intensities, but no phototaxis under red light at all intensities (0.5, 6.2, 15.0 and 30.0 W/m^2). Similar patterns of phototaxis were observed in *B. manjavacas* and did not differ among mictic, amictic females and male rotifers. Population growth rate of *B. plicatilis* s. s. under dark condition was 1.1-1.2 times higher than that under white light condition. No significant differences were observed in population growth rate at 3.8 and 6.2 W/m^2 at all light wavelengths. On the other hand, population growth rates at 0.5 and 1.6 W/m^2 were the lowest under blue light. According to these results both wavelength and intensity of light affect the population growth of rotifers, which in turn may be influenced by the rotifers' wavelength-dependent phototaxis.

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1 Introduction

Many rotifers show a variety of phototactic responses, including diel vertical distributions [1-5] and avoidance of the shore [6]. 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 [7]). The rotifer photo-sensor (eyespot) consists of two pigments, an accessory pigment provides an orientation response and a sensory pigment elicits other responses [8-10]. Through the joint action of these two pigments, rotifers can determine the direction, as well as light wavelength and intensity [9]. Previous studies of rotifer phototaxis employed the freshwater species *Brachionus calyciflorus*. That work reported different patterns of phototaxis that varied with light wavelength and intensity [8, 10].

The monogonont rotifer *Brachionus plicatilis* species complex has an eyespot whose structure is similar to *B. calyciflorus* with only two differences: in relay neurons and endoplasmic reticulum [9]. As Krebs [11] points out organisms are adapted to express different phenotypes related to the environmental conditions. We hypothesized that light sensing system of monogonont rotifers are affected by ambient lighting condition (e.g., light wavelength and intensity). To study this hypothesis we investigated the following four questions. (1) Does the micro-spectrophotometry of the eyespot in two different species from the *B. plicatilis* species complex (*B. plicatilis* sensu stricto (s. s.) and *B. manjavacas*) differ? (2) Does the phototaxic response of these rotifers vary by wavelength and/or light intensity? (3) The monogonont rotifer *B. manjavacas* exhibits cyclical parthenogenesis: Does phototaxis in amictic and mictic females and males differ? As part of that study we compared the photometric data of brackish-water species, *B. plicatilis* s. s. and *B. manjavacas* to evaluate it in relation to published information on the freshwater species *B. calyciflorus*. (4) Does wavelength and intensity of light effect asexual reproduction of *B. plicatilis*? Our goal in this research was to facilitate the use of phototactic characteristics to enhance our

understanding of rotifer light adaptation, thus improving the efficiency of raising rotifers for aquaculture.

2 Materials and Methods

2.1 Light absorbance of rotifer eyespot

Two species from the rotifer *B. plicatilis* species complex, *B. plicatilis* s. s. Makishima strain and *B. manjavacas* Australian strain [12], were employed to investigate phototactic responses. Culture medium (22 ppt of salinity) was prepared by dilution of natural seawater with Milli-Q water (Millipore 0.22 μm) followed by GF/C filtration and autoclaving (121°C, 15 min). Rotifer stocks (100 ml) were cultured with *Nannochloropsis oculata* (7×10^6 cells/ml) at 25°C in total darkness. From the stock cultures, three rotifer individuals were randomly selected and used as specimens to measure the relative absorbance of the eyespot (pigmented spot). Each specimen was prepared using an individual rotifer by transferring it onto a glass slide and then trapping it under a cover glass without anesthesia. The reference absorbance (lorica near pigmented spot) and pigmented spot (lorica + eyespot) were immediately measured by a microscope spectrophotometer system (Spectrophotometry 308 PV™, Craic Technologies™ + Optical microscope BX 61, Olympus), and were calculated by following equation:

$$\text{Absorbance} = \log (I_0/I),$$

where I_0 is the intensity of radiant energy striking the sample (i.e., emitted from the light source of microscope) and I is the intensity of energy emerging from sample. To calculate a net absorbance of pigmented spot, the reference absorbance subtracted from measured pigmented spot absorbance. The resulting data of the two species were compared graphically.

2.2 Phototaxis

We randomly selected 20 female individuals of *B. plicatilis* s. s. from the stock culture and immediately inoculated them into the middle part of an experimental vessel that was divided

into three parts by two sliding partitions (Fig. 1a). To limit vertical movements of rotifers, 20 ml of culture medium was put into the experimental vessel, resulting in less than 4 mm of water depth. We subjected these rotifers to dark adaptation for 5 min and then they were illuminated from the side of the experimental vessel for 15 min by different light emitting diodes (LEDs: i.e., white, with peaks at 460 and 570 nm; blue at 470 nm; green at 525 nm; and red at 660 nm; CCS Inc., Japan) one by one without partitions (Fig. 1b). The light intensity was adjusted to various levels (0.5, 6.2, 15.0 and 30.0 W/m^2) using a fiber optic spectrophotometer (USB 4000, Ocean Optics Inc., USA). After irradiation, two sliding partitions were put back into the experimental vessel and the number of rotifers in each compartment was counted under a stereomicroscope (Olympus, SZX-ILLD2-100) to investigate the pattern of phototaxis (Fig. 1c). In each trial, the proportion of distributed rotifers in the three compartments among total individuals was calculated by the mean values of triplicate observations.

Specimens of *B. manjavacas* were classified into four types by reproductive stages: non-egg carrying females, female-producing amictic females (amictic), male-producing mictic females (mictic), and males. Each type (30 rotifers each; total 120 inds.) was inoculated into the middle compartment of the experimental vessel and then subjected them to the same experimental procedure as *B. plicatilis* s. s (Fig. 1a). However, in this case a pair of LEDs consisting of two different light wavelength LEDs synchro-illuminated either side of experimental vessel at 1.4 W/m^2 (Fig. 1b-1, Table 1). After 15-min of illumination, the partition was replaced in the middle of experimental vessel (Fig. 1c-1) and the number of distributed individuals was counted. The proportion of rotifers in either side was calculated by the same method as for *B. plicatilis* s. s.

2.3 Population growth

In our experiments only the Makishima strain of *B. plicatilis* s. s. reproduces asexually. We inoculated specimens of this strain into 20 ml of diluted natural seawater (22 ppt) at a density of 1 ind./ml. The rotifers were cultured at 25.0±0.5°C on a daily feeding of *N. oculata* (7.0×10^6 cells/ml) for 10 days in triplicate samples. The food was centrifuged at 3968×*g* for 10 min, and re-suspended in rotifer culture medium. Four different wavelength LEDs (white, blue, green and red) were used for the light source, and the batch cultures were illuminated at 0.5, 1.6, 3.8 and 6.2 W/m^2 and the control was kept in complete darkness. The number of

female rotifers was counted as a daily observation and the mean values of triplicate samples were used for estimating population growth by the following equation:

$$\text{Population growth rate } (r): \ln (N_t/N_0) / t,$$

where t is the culture days, and N_0 and N_t are the number of female rotifers on day 0 and t, respectively.

2.4 Statistical analysis

Differences in the distribution associated with light wavelengths and intensities were evaluated with arcsine-transformed data for the analysis of variance (ANOVA) followed by Tukey-Kramer multi-comparison test (*B. plicatilis*) and for the *t*-test associated with light wavelengths (*B. manjavacas*). Tukey-Kramer test also was performed to confirm the effect of light wavelength and intensity on the population growth of rotifer *B. plicatilis* s. s. after ANOVA. All statistical analyses were performed using Statview version 5.0 software (SAS Institute, Inc., USA).

3 Results

3.1 Light absorbance of rotifer eyespot

The eyespot of two rotifer species (*B. plicatilis* s. s. and *B. manjavacas*) showed the same pattern of absorbance associated with light wavelength (Fig. 2). The eyespot absorbed 5.5 times more at the range of 450 to 540 nm (including blue to green) than 660 nm (red).

3.2 Phototaxis

In the experiments at lower intensity (0.5 and 6.2 W/m^2), *B. plicatilis* s. s. showed positive phototaxis to light at 470 and 525 nm, but no phototaxis was observed at 660 nm (Tukey-Kramer test, $p < 0.05$**,** Fig. 3). Only 20-30% of rotifers accumulated on the side of 660 nm light while 74-90% of rotifers accumulated at other light wavelengths. However, rotifers lost

positive phototaxis with increasing light intensity (15.0 and 30.0 W/m^2), even under wavelengths in the white, blue, and green range. For rotifers under a light intensity 15.0 W/m^2, 19-56% individuals accumulated on the illuminated side, while 28-45% accumulated on the illuminated side at an intensity of 30.0 W/m^2. The same patterns of phototaxis were observed in *B. manjavacas* regardless of the type of rotifers (Table 1). When synchro-illumination was applied on either side of experimental vessel at either 470 or 525 nm vs. 660 nm significantly more *B. manjavacas* accumulated in the compartment of the shorter wavelength light: 470 nm, 89.4% (*t*-test, $p < 0.001$); 525 nm, 71.9% (*t*-test, $p < 0.001$). When rotifers were synchro-illuminated by light of 470 and 525 nm, 79.4% of rotifers accumulated in the compartment illuminated at 470 nm (*t*-test, $p = 0.0014$).

3.3 Population growth

Brachionus plicatilis s. s. under complete darkness showed the highest population growth rate ($r = 0.64 \pm 0.03$ to 0.67 ± 0.01) compared to all illuminated treatments (Tukey-Kramer test, $p < 0.05$, Fig. 4), except the rotifers under lowest intensity (0.5 W/m^2) light. The rotifers showed no significant differences in population growth rate among the treatments illuminated with different wavelength lights ($r = 0.53 \pm 0.02$ to 0.55 ± 0.04 at 3.8 W/m^2 and 0.56 ± 0.01 to 0.60 ± 0.03 at 6.2 W/m^2). Under 0.5 and 1.6 W/m^2 of light intensity condition, the 470-nm light induced lowest population growth rates ($r = 0.56 \pm 0.03$ and 0.57 ± 0.00, respectively) than other wavelengths (Tukey-Kramer test, $p < 0.05$). In the lowest intensity treatments (0.5 W/m^2), higher population growth rate (the same level of population growth rate as darkness treatment) was shown at 525 and 660 nm ($r = 0.66 \pm 0.02$ and 0.67 ± 0.01, respectively).

4 Discussion

The eyespots of the brachionid rotifers examined here absorbed light at 450-550 nm more efficiently than at 660 nm with little difference in the absorption patterns of the two species (Fig. 2). This absorbance pattern correlates well to the strong positive phototaxis at 470 and 525 nm, which became weak at 660 nm (Fig. 3). Previous studies of rotifer eyespots mainly employed the freshwater rotifer *B. calyciflorus* [8, 10]. Using methods comparable to ours, those studies reported eyespot absorption patterns in the range of 400 to 540 nm. Although

both of the brackish rotifer species we studied and freshwater *B. calyciflorus* have a very similar eyespot structure [9], their eyespot absorbance varies. This may explains the differences in their patterns of phototaxis. On the other hand, we could not find any differences in the patterns of these parameters between our two test species. We also could not find any differences in light sensitivity among the four types of females or between males and females. This is probably due to similar absorbance of eyespots among female types and male.

Littoral rotifers show reverse diel vertical migration compared to other zooplankton and phytoplankton [13, 14]. They migrate up in the morning with the highest densities in the surface at midday (about 480-960 W/m^2 [13, 15]) and down at night. In this study, all light treatments induced positive phototaxis of rotifers compared to darkness. The rotifers showed strong positive phototaxis under 470 and 525 nm of lower intensity light (at 0.5 and 6.2 W/m^2), and positive phototaxis that became weak or absent with the increase light intensity (at 15.0 and 30.0 W/m^2), even under 470 and 525 nm of light. The results of our study differ to the reverse migration seen in rotifers in nature. Thus, the migration pattern is possibly affected not by light intensity directly but by other factors, especially competition with cladocerans that are the main predator of rotifers in the wild [16].

Both wavelength and intensity of light influenced population growth of rotifers. Rotifers cultured under the lowest light intensity (0.5 W/m^2) exhibited different patterns of population growth with respect to light wavelengths, showing higher values at 525 and 660 nm. However, population growth at the higher intensities (3.8 and 6.2 W/m^2) was lower compared to those cultures in complete darkness. Besides negatively affected the population growth, these higher light intensities also influenced phototaxic behavior. We posit that photokinesis reduced population growth by increasing energy use by elevating swimming speed and reducing turning frequency. Similar behaviors have been observed in *Asplanchna brightwellii* [7] and *B. calyciflorus* [17, 18]. In this study, the highest population growth occurred in cultures raised in total darkness; this may be the result of lower photokinetic movements. Even if the amount of supplied energy (food amount) was same among the treatments during culture, the rotifers under the light may spend more energy for movement compared to those in complete darkness, resulting in reduced energy available for reproduction. The photokinesis of *Brachionus* species rotifers is also affected by light wavelength and intensity, and the linear speed increases from red to blue light wavelengths at weak intensities [18]. Thus, the causes mentioned above can be applied to the patterns of population growth in

relation to light wavelengths and intensity. Additionally, other possibilities include the local decrease of food density even though no food limitation was applied prior to the experiment, as well as local oxygen concentration or increase of ammonia in the experimental condition. Consequently, reproduction in our species is simultaneously affected by light wavelength and intensity, and those patterns can possibly be affected by phototaxis, as well as other phototactic responses such as photokinesis. We recommend additional research on the influence of light conditions on rotifer growth thus allowing further improvements in the production of rotifers for aquaculture.

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6 References

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Tables

Table 1. Phototaxis of *Brachionus manjavacas* at specific wavelengths of light. The numbers in the table indicate the proportion (mean ± SD%) of distributed rotifers on the illuminated side (n = 3). Symbols = ?♀ (non-egg carrying female), F♀ (amictic female), M♀ (unfertilized mictic female), ♂ (male). All pairs are significantly different (*t*-test, $p < 0.05$).

Rotifer Types	Light wavelength (nm)					
	470	525	470	660	525	660
Total	79.4±9.0	20.6±9.0	89.4±8.7	10.6±8.7	71.9±5.1	28.1±5.1
?♀	75.7±16.0	24.3±16.0	88.0±12.3	12.0±12.3	73.0±5.2	27.0±5.2
F♀	80.3±8.5	19.3±9.1	90.0±7.0	10.0±7.0	65.7±5.1	34.3±5.1
M♀	76.0±13.1	24.0±13.1	87.0±12.1	13.0±12.1	72.3±7.5	28.0±7.8
♂	85.7±4.0	14.3±4.0	94.3±3.1	5.7±3.1	78.7±9.6	21.3±9.6

Figures

Figure 1. Experimental design for phototaxis analysis. (a), dark adaptation, rotifers were inoculated into the middle part of experimental vessel (for 5 min) (b), illumination using a LED bulb in the *B. plicatilis* (b-1), synchro-illumination using two LED bulbs in the *B. manjavacas* for 15 min after the removal of partitions (c), counting of distributed *B. plicatilis* and (c-1), *B. manjavacas* individuals after replacing partitions. The colours of LEDs (black and white) indicate light off and on, respectively.

Figure 2. Results of absorbance of the eyespots of *Brachionus plicatilis* s. s. and *Brachionus manjavacas* by microscope spectrophotometer system. The graph was drawn through the mean value of three individuals in each species.

Figure 3. Distribution of *Brachionus plicatilis* s. s. as a function of wavelength and intensity. The white parts of the horizontal histogram represent an illuminated side and the color gradation to dark means the declining illumination, moreover, these areas indicate the proportion of rotifers distributed in each compartment (Fig.1). The abbreviations (W, B, G and R) present white, blue, green and red light wavelengths. Different letters indicate statistically significant differences (a > b > c, Tukey-Kramer test, $p < 0.05$, n = 3).

Figure 4. Population growth rate of *Brachionus plicatilis* s. s. under different light wavelength and intensity. The abbreviations W, B, G, R and D present white, blue, green, red and darkness, respectively. Error bars and different letters indicate standard deviations and significant differences (a>b>c>d, Tukey-Kramer test, $p<0.05$, $n=3$), respectively.

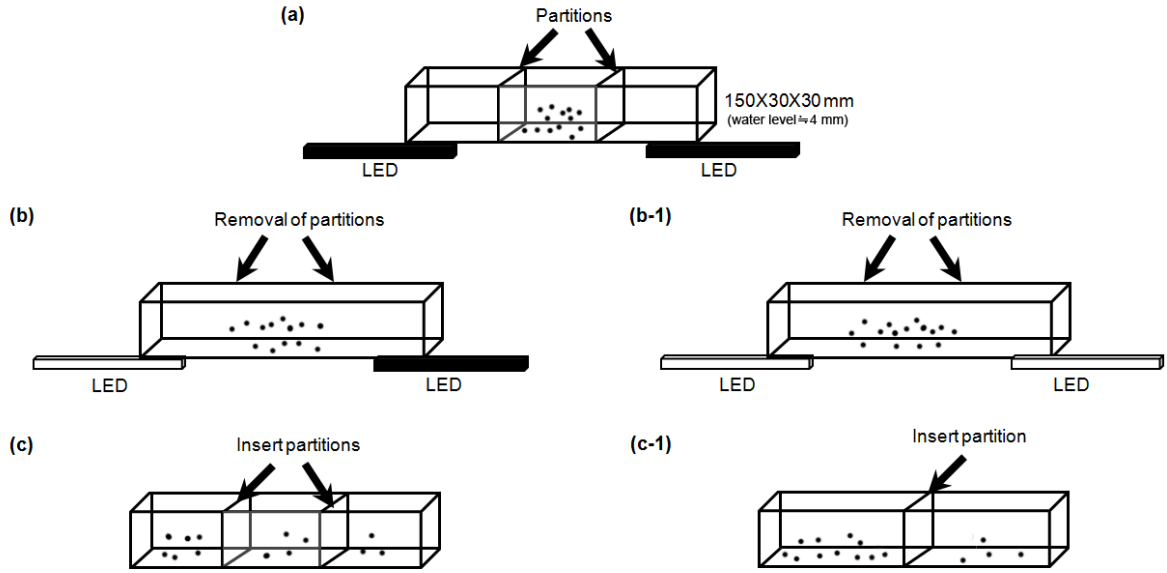
(a)
Partitions
150X30X30 mm
(water level≒4 mm)
LED
LED
(b)
Removal of partitions
LED
LED
(b-1)
Removal of partitions
LED
LED
(c)
Insert partitions
(c-1)
Insert partition

Fig. 1

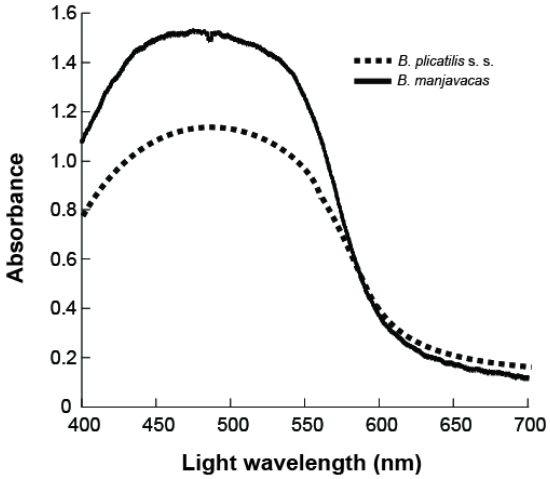
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0
Absorbance
400
450
500
550
600
650
700
Light wavelength (nm)
B. plicatilis s. s.
B. manjavacas

Fig. 2

.

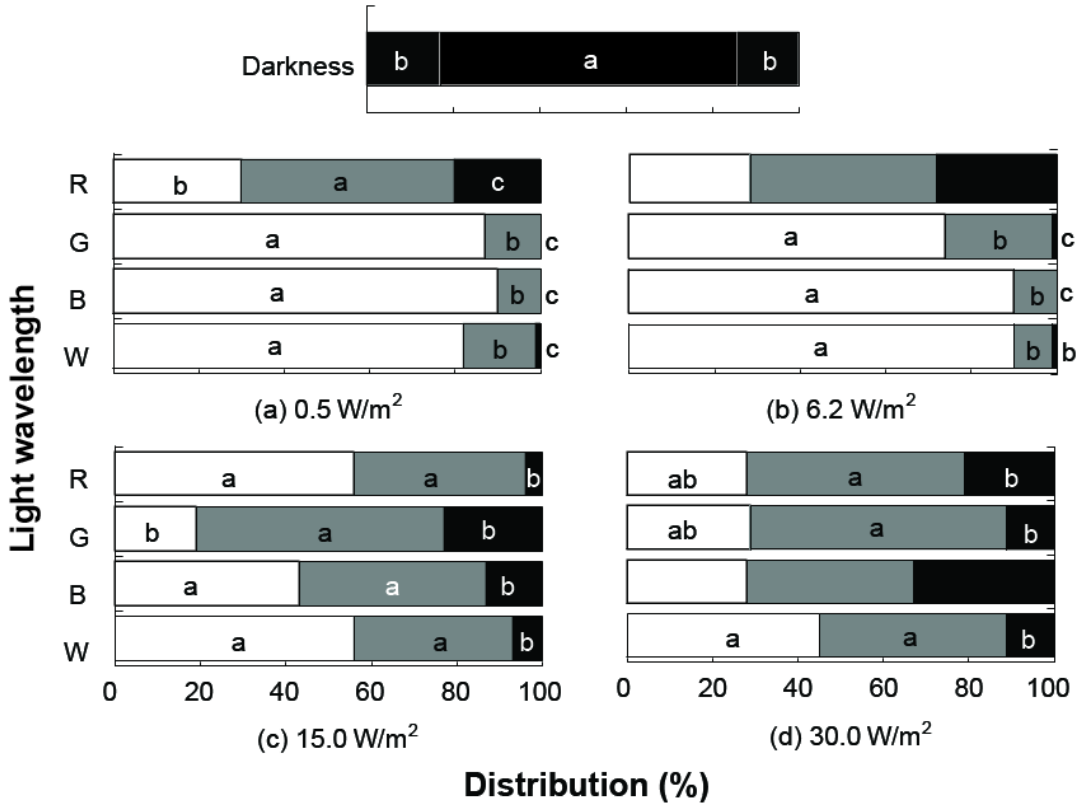
Darkness
b
a
b
R
G
B
W
b
a
c
a
b
c
a
b
c
a
b
c
(a) 0.5 W/m2
a
b
c
a
b
c
a
b
b
(b) 6.2 W/m2
a
a
b
b
a
b
a
a
b
a
a
b
ab
a
b
ab
a
b
a
a
b
0
20
40
60
80
100
(c) 15.0 W/m2
(d) 30.0 W/m2
Light wavelength
Distribution (%)

Fig. 3

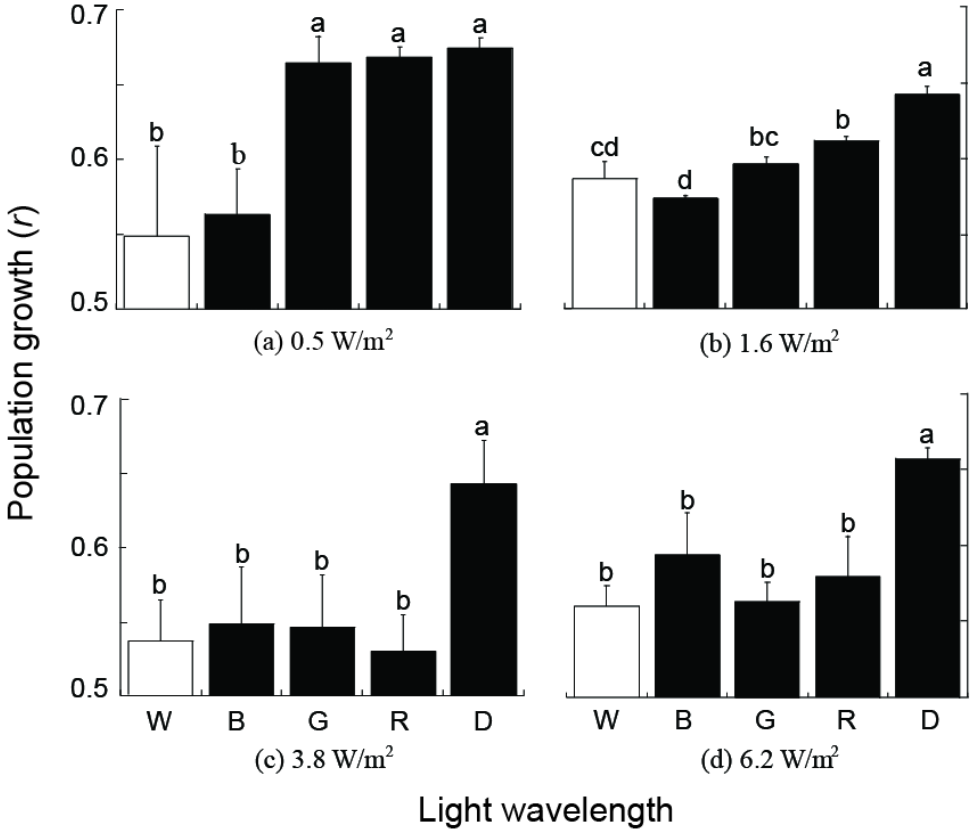
Population growth (r)
0.7
0.6
0.5
a
a
a
b
b
cd
d
bc
b
a
(a) 0.5 W/m2
(b) 1.6 W/m2
b
b
b
b
a
b
b
b
b
a
W
B
G
R
D
(c) 3.8 W/m2
(d) 6.2 W/m2
Light wavelength

Fig. 4