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# Light intensity and wavelength influence development, reproduction and locomotor activity in the predatory flower bug *Orius sauteri* (Poppius) (Hemiptera: Anthocoridae)

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**Abstract** Light wavelength and intensity are physical factors that can affect arthropod development and reproduction. The present study examined the development, reproduction and locomotor activity of the predatory flower bug, *Orius sauteri* (Poppius) (Hemiptera: Anthocoridae), under five light intensities (1000, 2000, 3000, 4000 and 5000 lux) and five wavelengths [red (678.5 nm), green (620.0 nm), yellow (581.7 nm), blue (478.1 nm) and white (all wavelengths)] at constant temperature (25 °C) and RH (70%). The duration of nymphal development was extended at lower light intensities, primarily due to effects on the first three instars. Under white, yellow and green light, *O. sauteri* completed development in 18.0 d, but blue light extended development by 3.2 d and red light extended it by 7.4 d. Although lower light intensities extended the preoviposition period and reduced fecundity, they improved egg fertility. Both red and blue light negatively affected preoviposition period, fecundity and egg fertility. Whereas adult female mean walking speed over a five min period was reduced at lower light intensities, longer wavelengths (yellow and red) increased it, ostensibly reflecting an avoidance response. The respiration quotient of adult *O. sauteri* females was also elevated under red light conditions. These findings are informative for optimizing *O. sauteri* mass-rearing procedures and maximizing its efficacy as a biological control agent in greenhouse cultures.

**Keywords** fecundity, fertility, locomotion, mating, oviposition, respiration quotient

## **Introduction**

Various aspects of the photic environment, including photoperiod, intensity, wavelength and even polarization, may influence the development, metabolism and reproduction of arthropods. In particular, light intensity and wavelength are known to influence biological and physiological processes in insects (Bishop 1969; Manuwoto and Scriber 1985; Sakai et al. 2002). Predators and parasitoids have received substantial attention in this regard because of the need to understand photic influences on biological control outcomes (e.g., Omkar et al. 2005; Malaquias et al. 2010) and the suitability of lighting regimes for mass rearing natural enemies for augmentative biological control applications (Chambers 1977). For example, the aphid parasitoid *Aphidius ervi* Haliday (Hymenoptera: Aphidiinae) orients preferentially to green light as opposed to blue-green or yellow-green, ostensibly because of the association of aphid hosts with green foliage (Goff and Nault 1984). The predatory stonefly *Eusthenia costalis* Banks (Plecoptera: Eustheniidae) alters its foraging behaviour in response to small differences in wavelength between red and infrared light (Turnbull & Barmuta 2006). Adults of *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae) prefer to aggregate on white or highly reflective objects during flights to overwintering sites (Wang et al. 2011). Both photoperiod and light spectrum affect development and reproductive capacity in the aphidophagous coccinellid *Coelophora saucia* (Mulsant) (Coleoptera: Coccinellidae) (Omkar and Pathak 2006). Changes in light intensity have been linked to the induction and termination of diapause in various predatory Hemiptera and Coleoptera (Danks 1987). Diminishing light intensity eventually limits the ability of the bee *Megalopta genalis* Meade-Waldo (Hymenoptera: Halictidae) to find the nest entrance and thus determines the duration of crepuscular foraging behavior (Kelber et al. 2006). The whitefly parasitoids *Encarsia formosa* Gahan and *Eretmocerus eremicus* Rose and Zolnerowich (Hymenoptera: Aphelinidae) parasitized approximately twice as many hosts under conditions of high light intensity and long daylength as under low light intensity and short daylength (Zilahi-balogh et al. 2006).

Flower bugs of the genus *Orius* (Wolff) (Hemiptera: Anthocoridae) are efficient predators of small insect pests, especially thrips, in many agroecosystems (Jacobson 1993; Pfannenstiel and Yeorgan 1998; Lattin 1999). Previous work suggests that photic conditions affect many aspects of *Orius* spp. biology and behavior, including development, reproduction and orientation (Askari and Stern 1972; Smith and Stadelbacher 1978; Abenes and Khan 1990). For example, short daylength induces reproductive diapause in *Orius strigicollis* (Poppius) (Hemiptera: Anthocoridae), whereas long daylength tends to increase developmental duration, albeit with an interaction with temperature (Musolin et al. 2004). Similar results have been reported for *O. insidiosus* (Say) (Ruberson et al. 1991). Similarly, photoperiod was shown to influence survival, fecundity and longevity in *O. thyestes* Herring (Carvalho et al. 2006) and *O. niger* Wolff (Bahşi and Tunç 2008). Fewer studies have examined the effect of light intensity on the locomotor activity of *Orius* spp. or their distribution in habitats (Shields 1980; Gossner 2009), and studies of spectral effects (color) have not yet been conducted.

The present study examined the effects of different light intensities and wavelengths on development, reproduction and locomotor activity of the predatory flower bug *Orius sauteri* (Poppius) (Hemiptera: Anthocoridae). This beneficial species is mass-reared for augmentative release in vegetable greenhouses in Asia for suppression of arthropod pests such as thrips and aphids (Nakata 1995; Nagai and Yano 2000). Short daylength induces reproductive diapause in *O. sauteri* (Ito and Nakata 1998a; b) and photic conditions are known to influence other aspects of its development and reproduction in mass-reared cultures (Saulich and Musolin 2009). The primary objective of this study was generate better understanding of photic effects on aspects of *O. sauteri* developmental and reproductive biology and behavior that may affect either the efficacy of mass-rearing procedures or the insect's performance as a biological control agent following release in augmentation programs.

## **Materials and Methods**

Insects

A total of 390 *O. sauteri* adults (213 females and 177 males) were collected from alfalfa (*Medicago sativa*) fields around Wang Jia-Yuan Biodiversity Research Station (40°14' N, 116°13' E) in Changping County, Beijing, China during the season of April to July, 2011. Species identity was confirmed by Dr. Cai Wanzhi, taxonomist of Hemiptera at Chinese Agricultural University, Beijing, China and these bugs were used to establish a colony in the Natural Enemy Research Laboratory at the Beijing Academy of Agriculture and Forestry Sciences, Haidian District, Beijing. Bugs were reared in aluminum frame cages (45.0 × 50.0 × 60.0 cm) covered with fabric net (100 mesh), beginning with 30 bugs/cage, in a climate-controlled growth chamber (MH-351, Sanyo, Japan) set to 25 ± 1°C, 70 ± 2 % RH, 16:8 L:D photoperiod, 3000 lux fluorescent light. The cowpea aphid, *Aphis craccivora* Koch (Hemiptera: Aphididae), reared on broad bean, *Vicia faba* L., was provided as food and kidney bean plants, *Phaseolus vulgaris* L., as an oviposition substrate (Murai et al. 2001). The stock colony was managed as a continuous culture. New plants were introduced every four days and heavily damaged plants removed, their stems clipped and left in cages until bugs eclosed.

#### Luminosity and wavelength treatments

Experiments were conducted in climate-controlled growth chambers under the same physical conditions as above, unless otherwise specified. For the luminosity treatments, white fluorescent lights were set to one of five light intensities (1000, 2000, 3000, 4000 or 5000 lux). For wavelength treatments, colored LED lamps (LENSER-Illuminate™, Shenzhen, China) were used in place of the stock fluorescent lamps and luminosity was set at 3000 lux in all treatments. The following wavelength treatments were established: 678.5 nm (red), 620.0 nm (yellow), 581.7 nm (green), 478.1 nm (blue) and fluorescent white (control). Actual wavelengths were measured to an accuracy of ± 10-15 nm using an LED analyzer (Feasa Enterprises limited, Limerick, Ireland). A thermal probe was used to verify that temperatures experienced by the insects in various containers remained at 25 ± 1 °C across all lighting treatments.

#### Development assay

Bugs for experiments were obtained by placing kidney bean stems bearing freshly laid eggs of *O. sauteri* ( $24 \pm 2$  h old post-moulting) in clean plastic Petri dishes (10.0 cm diameter), three per dish. As nymphs hatched, they were transferred to smaller Petri dishes (4.5 cm diameter, 1 bug per dish) within one h of eclosion. Each dish was provisioned with ca. 40 3rd instar aphids (replaced every 24 h without removing containers from growth chambers) and covered with perforated Parafilm<sup>®</sup>. Nymphs in experiments were examined every four h, using an infrared camera to make night-time observations, the developmental status of each was recorded, and the proportion surviving to the adult stage was tallied. A total of 25 replicates were conducted simultaneously at each light intensity and wavelength treatment.

#### Reproduction assay

Virgin *O. sauteri* adults ( $24 \pm 2$  h old) were paired in plastic Petri dishes (10.0 cm diam) with abundant food ( $> 60$  aphids, replaced daily) and assigned to one of the five light intensity or wavelength treatments. Pairs were observed continuously from onset of copulation (maintenance of genital contact for  $\geq 5$  min) until male and female separated and then every four h thereafter. Males were then removed and each female provided five *P. vulgaris* stems as oviposition substrates. The duration of copula and preoviposition period were determined by direct observation. Bean stems were replaced daily and held under the same physical conditions until eclosion of neonate bugs; fecundity and egg fertility was followed for five days by examining all oviposition punctures and dividing the number of bugs eclosing by the total number of hatched and unhatched eggs. The test was repeated five times with five pairs in each light intensity for a total of 25 replicates per treatment.

#### Respiration assay

We reasoned that changes in the respiratory quotient should be evident if light conditions affect *O. sauteri* metabolic rate. A respirometry system (Sable Systems International,

Las Vegas, NV, USA) was used to measure the respiratory quotient of individual newly molted adult female bugs ( $24 \pm 2$  h old) under the various conditions of luminosity and wavelength described above. The apparatus consisted of a CO<sub>2</sub> infrared analyzer (Testo 535, Testo, Lenzkirch, Germany), an O<sub>2</sub> gas sensor (InPro 6000G, Mettler-Toledo, Greifensee, Switzerland), an air pump, a flow meter, a CO<sub>2</sub> purification tube, a drying tube, a glass sample chamber (2.0 cm ID × 10.0 cm), a data collector (Vernier LabPro, Beaverton, OR, USA) and a temperature sensor (TMP-112, Texas Instruments, Dallas, TX, USA). The apparatus was assembled within an aluminum box (65.0 x 40.0 x 40.0 cm) with light sources mounted on the inside and temperature was maintained at  $25 \pm 1^\circ\text{C}$ . Air flow was adjusted to 0.1 L/min in the apparatus and initial O<sub>2</sub> and CO<sub>2</sub> concentrations were recorded. Once all readings were stable, insects (n = 10 adult females per treatment) were placed singly in the sample chamber and measurements made on each for a period of 30 min. The respiratory quotient (RQ) was calculated according to manufacturer's specifications as:

$$\text{RQ} = (\% \text{CO}_2 \text{ expired} - \% \text{CO}_2 \text{ inspired}) / (\% \text{O}_2 \text{ inspired} - \% \text{O}_2 \text{ expired})$$

#### Locomotion assay

A tracksphere locomotion compensator with behavior recorder (Syntech LC-100, Hilversum, Netherlands) was used to monitor differences in locomotor activity under various light intensities and wavelengths. After adjustment of focus, the device was covered with tin foil shade to exclude ambient light and four white LED lamps were placed in the shade. Light intensity was regulated by an auto regulator (light intensity measurer, TPM-Z1, Leaderlab<sup>®</sup>, Hangzhou, China). Newly emerged *O. sauteri* females were placed singly on top of the monitoring sphere. The sphere adjusted its speed to maintain the insect on the top and the bug's movement was recorded continuously for five min. The behavior recorder sums all movements of the sphere to yield an estimate of linear distance that is then divided by the time of the observation to yield a walking speed. A total of 10 females were observed under each light intensity treatment, and 35 under each wavelength treatment.

## Statistical analysis

Data from all experiments were analyzed by one-way ANOVA (SPSS 1998) and means separated by LSD ( $\alpha = 0.05$ ).

## Results

The total duration of *O. sauteri* development decreased with increasing light intensity, largely due to faster development in the first, second and third nymphal instars, the fourth and fifth instars remaining largely unaffected (Table 1). Nymphal development was fastest under white, green and yellow light, slower under blue light, and very slow under red light, with significant effects of wavelength on the durations of all nymphal instars (Table 2).

There were significant effects of light intensity on all reproductive parameters. Duration of copula and preoviposition period were both decreased by increasing light intensity, but fecundity was maximal at intermediate light intensity and egg fertility was maximal at low intensity (Table 3). White, green and yellow light yielded the longest durations of copula, shortest preoviposition periods, and the highest levels of fecundity and egg fertility (Table 4). Light intensity did not significantly affect the respiration quotient ( $F_{4,45} = 1.86$ ,  $P = 0.133$ ) but wavelength did ( $F_{4,45} = 2.97$ ,  $P = 0.029$ ). Bugs under red light exhibited a significantly higher RQ value than did those under other wavelength treatments, none of which differed significantly (Fig. 1).

The walking speed of *O. sauteri* females averaged over five one-minute intervals increased almost two-fold as light intensity was increased from 1000 to 5000 lux (Table 5). Vagility was highest under red light conditions, intermediate under yellow, and lowest under white, green and blue (Table 6).

## Discussion

Although *O. sauteri* was able to develop and reproduce successfully under the full range of photic conditions tested, both light intensity and wavelength exerted significant influences on its biology and behavior. Early instar nymphs of *O. sauteri* were particularly sensitive to light intensity and their development was delayed below 4000 lux, suggesting a diurnal species adapted to development in brightly lit habitats. The delayed development observed under red and blue light is perhaps suggestive of adaptation to development under light in the yellow-green range, consistent with what would be expected in a natural plant canopy. The equivalent suitability of white light to that of yellow and green suggests that it is not so much the perception of wavelengths longer or shorter than yellow-green that delays development, but rather the absence of wavelengths within this optimal range. Nevertheless, previous studies have demonstrated negative effects of short wavelength light on mating behaviour in *Drosophila melanogaster* (Jacobs 1960; Sakai et al. 2002) and on feeding behaviour in both Diptera (Lunau and Wacht 1997), and Lepidoptera (White et al. 1994), whereas red light has been shown to impede development in coccinellids (Omkar et al. 2005).

Since developmental rate was almost 20% faster at the highest light intensity compared to the lowest, cultures of *O. sauteri* should employ light intensity higher than 4000 lux if the objective is to speed the production of large numbers. Likewise, adult locomotor activity increased with light intensity (Table 5), which might translate into a beneficial effect on foraging behaviour under supplementary lighting in a greenhouse environment. White light was generally most suitable for reproduction, although not significantly superior to yellow or green (Table 4). However, bugs reproducing under red light took longer to lay eggs and had reduced fecundity and fertility. Thus, the greater walking speed of adult females under red light (Table 6) likely reflects an avoidance response to this wavelength of light, an inference that would be consistent with the higher observed RQ (Fig. 1). This might result in lower rates of prey consumption and thus account for the negative effect on reproduction. Both red and blue light were shown to cause erratic reproductive activity in two species of Coccinellidae (Omkar et al. 2005). Notably, Castrejon and Rojas (2010) found differences between larvae and mated female adults of *Estigmene acrea* (Drury) (Lepidoptera: Arctiidae) in selective orientation to different wavelengths of light, the former preferring green, the color of food plants, and

the latter wavelengths in the range of blue to UV, ostensibly useful as cues for orienting to oviposition sites.

Light intensity had inconsistent effects on different parameters of *O. sauteri* reproduction (Table 3). Notably, Nissenen et al. (2008) found that gravid and virgin female carrot psyllids, *Triozza apicalis* Forster (Hemiptera: Triozidae), responded differently to subtle differences in light intensity. Higher luminosities shortened the duration of copula for *O. sauteri*, accelerated the onset of oviposition, and yielded higher fecundities, but significantly reduced fertility, possibly due in part to the effect on mating time. Thus, the high light intensities that favor rapid bug development and reproduction are associated with reductions in egg fertility. One possible application of this finding in commercial production of *O. sauteri* might be the regular harvest of egg-bearing plant parts so that these can be held under lower light conditions until emergence of nymphs. Alternatively, the cage environment could be modified so that oviposition sites are shaded and experience lower light intensity than the rest of the enclosure. Further experiments could test whether females respond to differences in light intensity when selecting oviposition sites.

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**Table 3.** Mean reproductive parameters ( $\pm$  SE) for *O. sauteri* adult pairs (n = 5 replicates of 5 pairs per treatment) when reared at  $25 \pm 1$  °C under five different intensities of white fluorescent light. Values bearing the same letter were not significantly different within columns (LSD test,  $\alpha = 0.05$ )

Light intensity (lux)	Parameter			
	Duration of copula (min)	Preoviposition period (d)	5-day fecundity (no. eggs)	Fertility (% eggs hatching)
1000	11.6 $\pm$ 0.4a	11.2 $\pm$ 0.4a	30.2 $\pm$ 1.4b	92.7 $\pm$ 2.0a
2000	10.4 $\pm$ 0.3a	11.1 $\pm$ 0.6ab	34.1 $\pm$ 1.8b	85.3 $\pm$ 3.2b
3000	7.6 $\pm$ 0.4b	9.8 $\pm$ 0.3b	40.3 $\pm$ 2.1a	73.8 $\pm$ 2.1c
4000	6.1 $\pm$ 0.4c	8.4 $\pm$ 0.4c	39.1 $\pm$ 1.9a	61.7 $\pm$ 4.0d
5000	5.9 $\pm$ 0.3c	7.7 $\pm$ 0.8c	39.3 $\pm$ 1.1a	54.2 $\pm$ 2.5e
<i>F</i>	66.848	18.210	13.725	91.977
df	4,120	4,120	4,120	4,120
<i>P</i>	< 0.001	< 0.001	< 0.001	< 0.001

**Table 4.** Mean reproductive parameters ( $\pm$  SE) for *O. sauteri* adult pairs (n = 5 replicates of 5 bugs per treatment) when reared at  $25 \pm 1$  °C under five different wavelengths of light at 3000 lux. Values bearing the same letter were not significantly different within columns (LSD test,  $\alpha = 0.05$ )

Wavelength (nm)	Variable			
	Duration of copula (min)	Preoviposition period (d)	5-day fecundity (no. eggs)	Fertility (% eggs hatching)
Red (678.5)	6.2 $\pm$ 0.3b	14.9 $\pm$ 1.1a	37.2 $\pm$ 2.3b	59.1 $\pm$ 2.6b
Yellow (620.0)	7.8 $\pm$ 0.4ab	10.6 $\pm$ 0.6bc	42.5 $\pm$ 1.3a	72.7 $\pm$ 2.5a
Green (581.7)	8.2 $\pm$ 0.5a	9.9 $\pm$ 0.5c	43.1 $\pm$ 1.4a	74.1 $\pm$ 2.4a
Blue (478.1)	6.9 $\pm$ 0.2b	12.4 $\pm$ 0.6b	33.3 $\pm$ 1.7b	63.6 $\pm$ 3.6b
White	7.4 $\pm$ 0.2ab	9.5 $\pm$ 0.3c	41.8 $\pm$ 1.8a	74.5 $\pm$ 2.8a
<i>F</i>	12.687	25.893	13.536	14.718
df	4,120	4,120	4,120	4,120
<i>P</i>	< 0.001	< 0.001	< 0.001	< 0.001

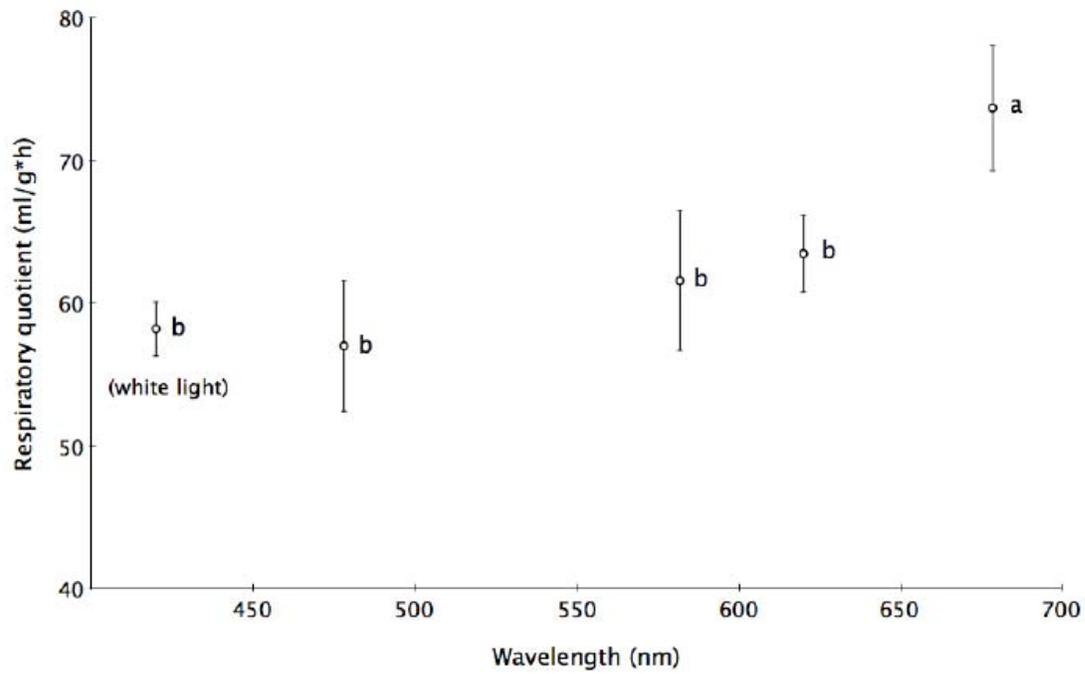
**Table 5.** Mean walking speed (mm/sec  $\pm$  SE) of *O. sauteri* female adults (n = 10 per treatment) in one-minute intervals under five different intensities of white fluorescent light. Values bearing the same letter were not significantly different within columns (LSD test,  $\alpha = 0.05$ )

Light intensity (lux)	Time interval					Average
	First	Second	Third	Fourth	Fifth	
1000	8.8 $\pm$ 0.7b	8.6 $\pm$ 0.9b	7.1 $\pm$ 0.9b	5.7 $\pm$ 0.6d	5.4 $\pm$ 1.1d	7.1 $\pm$ 0.4d
2000	9.0 $\pm$ 1.4b	8.9 $\pm$ 0.8b	7.7 $\pm$ 0.5b	7.4 $\pm$ 0.3c	7.1 $\pm$ 0.2c	8.0 $\pm$ 0.4cd
3000	9.2 $\pm$ 0.7b	8.9 $\pm$ 0.6b	8.2 $\pm$ 0.5b	8.0 $\pm$ 0.6c	7.8 $\pm$ 0.9c	8.4 $\pm$ 0.3c
4000	12.6 $\pm$ 1.3a	11.2 $\pm$ 0.9ab	10.5 $\pm$ 0.7ab	10.2 $\pm$ 0.5b	9.9 $\pm$ 0.7b	10.9 $\pm$ 0.4b
5000	13.1 $\pm$ 0.9a	13.1 $\pm$ 1.0a	12.2 $\pm$ 1.4a	12.3 $\pm$ 0.7a	12.4 $\pm$ 1.2a	12.6 $\pm$ 0.5a
<i>F</i>	4.12	5.10	6.03	20.52	9.71	34.03
<i>df</i>	4,45	4,45	4,45	4,45	4,45	4,45
<i>P</i>	0.006	0.002	0.001	< 0.001	< 0.001	< 0.001

**Table 6.** Mean walking speed (mm/sec  $\pm$  SE) of *O. sauteri* female adults (n = 35 per treatment) during one-minute intervals under five different wavelengths of light. Values bearing the same letter were not significantly different within columns (LSD test,  $\alpha = 0.05$ )

Wavelength (nm)	Time interval					Average
	First	Second	Third	Fourth	Fifth	
Red (678.5)	13.2 $\pm$ 0.7a	11.1 $\pm$ 0.5a	10.1 $\pm$ 0.7a	9.4 $\pm$ 0.2a	7.8 $\pm$ 0.3a	10.3 $\pm$ 0.4a
Yellow (620.0)	11.7 $\pm$ 0.7a	9.7 $\pm$ 0.3b	9.2 $\pm$ 0.4a	9.1 $\pm$ 0.8a	7.4 $\pm$ 0.8a	9.4 $\pm$ 0.8b
Green (581.7)	9.6 $\pm$ 0.7b	9.3 $\pm$ 0.5bc	8.2 $\pm$ 0.3b	7.4 $\pm$ 0.5b	6.7 $\pm$ 0.4a	8.2 $\pm$ 0.6c
Blue (478.1)	8.3 $\pm$ 0.7b	8.3 $\pm$ 0.5c	8.2 $\pm$ 0.4b	7.3 $\pm$ 0.7b	6.4 $\pm$ 0.5a	7.7 $\pm$ 0.9c
White	9.2 $\pm$ 0.4b	9.0 $\pm$ 0.6bc	8.1 $\pm$ 0.4b	7.6 $\pm$ 0.2b	7.0 $\pm$ 0.3a	8.2 $\pm$ 0.6c
<i>F</i>	12.69	6.52	7.10	5.75	0.68	15.18
<i>df</i>	4,45	4,45	4,45	4,45	4,45	4,45
<i>P</i>	< 0.001	< 0.001	< 0.001	< 0.001	0.608	< 0.001

Fig. 1



**Fig. 1.** Mean ( $\pm$  SE) respiration quotients of adult female *O. sauteri* measured under different wavelengths of light. Data points bearing the same letters were not significantly different (LSD test,  $\alpha = 0.05$ ).