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Photoresponse in the Ciliated Protozoan *Colpoda cucullus*

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Abstract. We found that vegetative cells of *Colpoda cucullus* Nag-1 accumulated in shaded areas of a container when grown in the laboratory and then formed resting cysts. The photodispersal (negative photoaccumulation) of *C. cucullus* was mediated, at least in part, by a difference in forward swimming velocity between the illuminated region and the shaded area of the Petri dish (motion slowed or stopped in the shaded area). When *C. cucullus* was stimulated by continuous light irradiation, the forward swimming velocity increased and reached a steady state within 10 s. When the light intensity decreased, the forward swimming velocity gradually decreased, and eventually returned to its original level for approximately 1 min. The action spectrum of the photokinetic response (steady-state swimming acceleration driven by continuous light stimulation) implies the involvement of blue light receptors.

Key words: *Colpoda*, resting cyst, photodispersal, photokinetic response, action spectrum

INTRODUCTION

In ciliated protozoa, photodispersal (negative photoaccumulation) is mediated by a phototactic orientation response away from the light source (Kuhlmann 1998, Marangoni *et al*. 2000, Cadetti 2000) and photoresponses that occur irrespective of light direction such as step-up photophobic response (directional changes that occur in response to a relative increase in light intensity) and photokinetic responses (an increase in steady-state swimming velocity or steady-state frequency of directional changes that is induced by continuous exposure to light) (Wood 1976; Matsuoka 1983a, b; Kraml and Marwan 1983; Iwatsuki and Naitoh 1983; Iwatsuki 1992; Kuhlmann 1998; Hinrichsen and Peters 2013). We found that the terrestrial ciliate *Colpoda cucullus* Nag-1 accumulated in the shaded area of a container when cultured in the laboratory and formed resting cysts, and that this behavior is mediated by a type of photokinetic response (an increase in steady-state swimming velocity induced by continuous exposure to light). Here, we describe the presumed photoreceptors mediating the photokinetic response of this ciliate based on the action spectrum determination. The ecological advantage of the photobehavior of ciliated protozoa may allow them

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to approach their food source, avoid harmful light, or contact a partner for sexual reproduction (Kuhlmann 1998). Below, we further discuss the ecological advantage of photodispersal in *C. cucullus*.

MATERIALS AND METHODS

Colpoda cucullus Nag-1 (Funadani *et al*. 2016) was cultured in a 0.05% (w/v) infusion of dried wheat leaves. The vegetative cells of *C. cucullus* Nag-1 cultured for 1–2 days were washed 2–3 times with 1 mM Tris-HCl (pH 7.2) after sedimentation (1,500 × *g* for 2 min). Cells were then resuspended in a solution consisting of 1 mM Tris-HCl (pH 7.2) and 0.1 mM $CaCl_2$ prior to measurement. A tungsten lamp was used for the visible range (403 to 642 nm), and a mercury lamp was used for UV-A range (334 nm). Except for the measurements obtained in the stepwise gradient of light (Fig. 2), temperature-controlled water (23°C) supplied by a thermo-regulator (Coolnics, Yamato, Japan) was circulated beneath chambers to maintain a constant temperature for the cell suspension (Figs 1, 3, 4). To determine the temperature of the *C. cucullus* suspension, a small thermistor (1 mm in diameter) (Takara Thermistor Instruments Co., Ltd.) was employed. The light intensity of the visible and UV lights was determined with a UV light meter (TM-208, Tenmars electronics Co., Ltd., Taiwan) or handmade photometer equipped with a silicon photodiode (S1226-5BK, Hamamatsu Photonics, Japan). In Fig. 3, the background light intensity was measured using the Solar Power Meter (TM-207, Tenmars electronics Co., Ltd., Taiwan). The dissecting microscope or light microscope was equipped with a CCD camera (Ristra 40P, Microscope Network, Japan) and DVD recorder (Hitachi DV-DH250S, Japan). Quasi-monochromatic light was obtained using interference filters in the visible range or a band-pass filter in the UV-A range. Columns (circles) and attached bars indicate the mean of five (Figs 1d, 2d, 3c) or six (Fig. 2c) identical

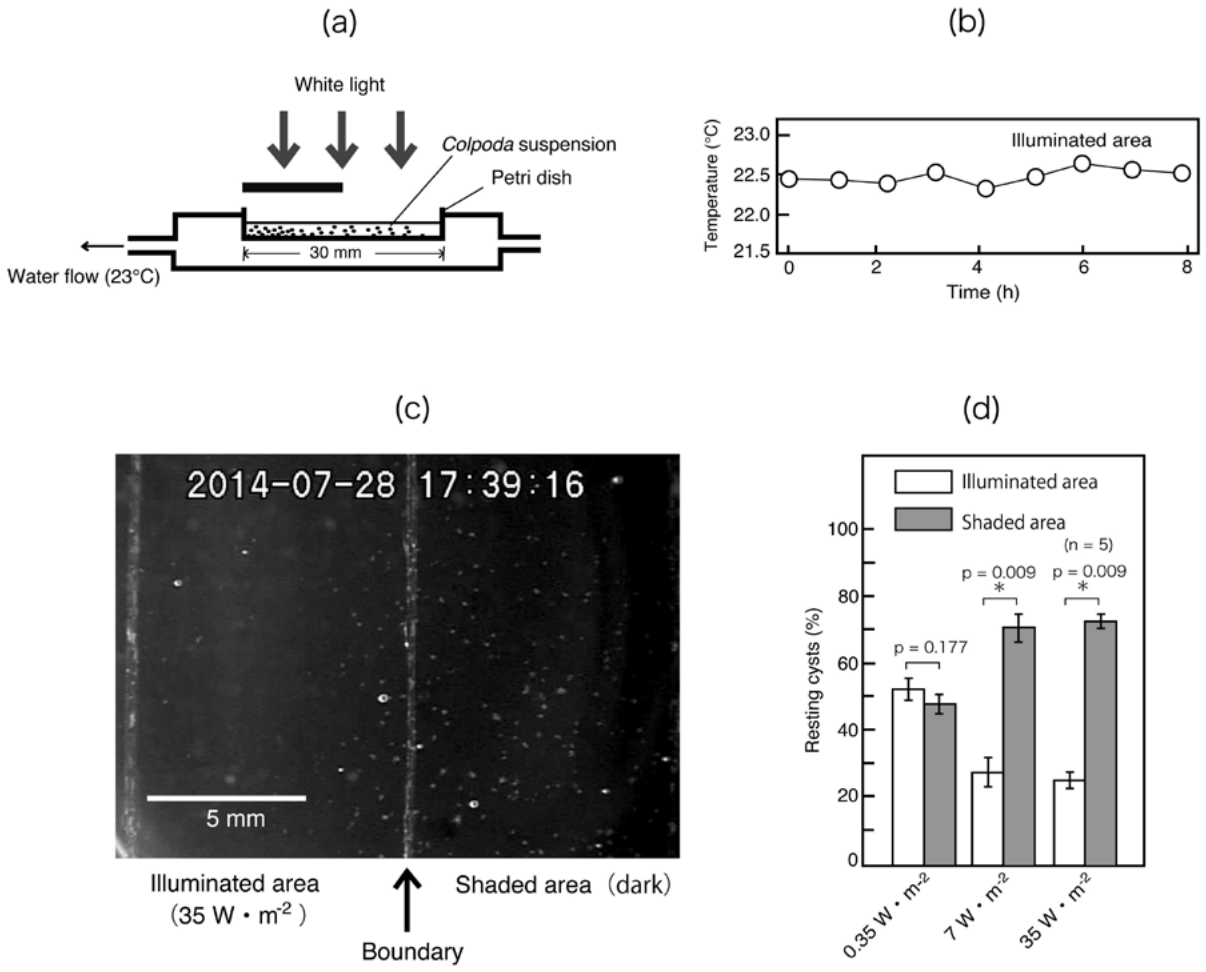


Fig. 1. Resting cyst formation of *C. cucullus* Nag-1 in illuminated and shaded areas. (a) Side-view of a chamber (Petri dish) utilized for encystment induction, half of which was illuminated. (b) Temperature of the cell suspension (at 5 mm depth) in the illuminated area after the onset of light irradiation. (c) A video-recorded photograph (dark-field image) showing that the resting cysts formed in the shaded region of the chamber. (d) The rate of the resting cysts attached to the bottom of the chamber in the illuminated (0.35 W·m^{-2}, 7 W·m^{-2} and 35 W·m^{-2}) and shaded areas, which is expressed as a percentage of the total number of tested cells (100 cells).

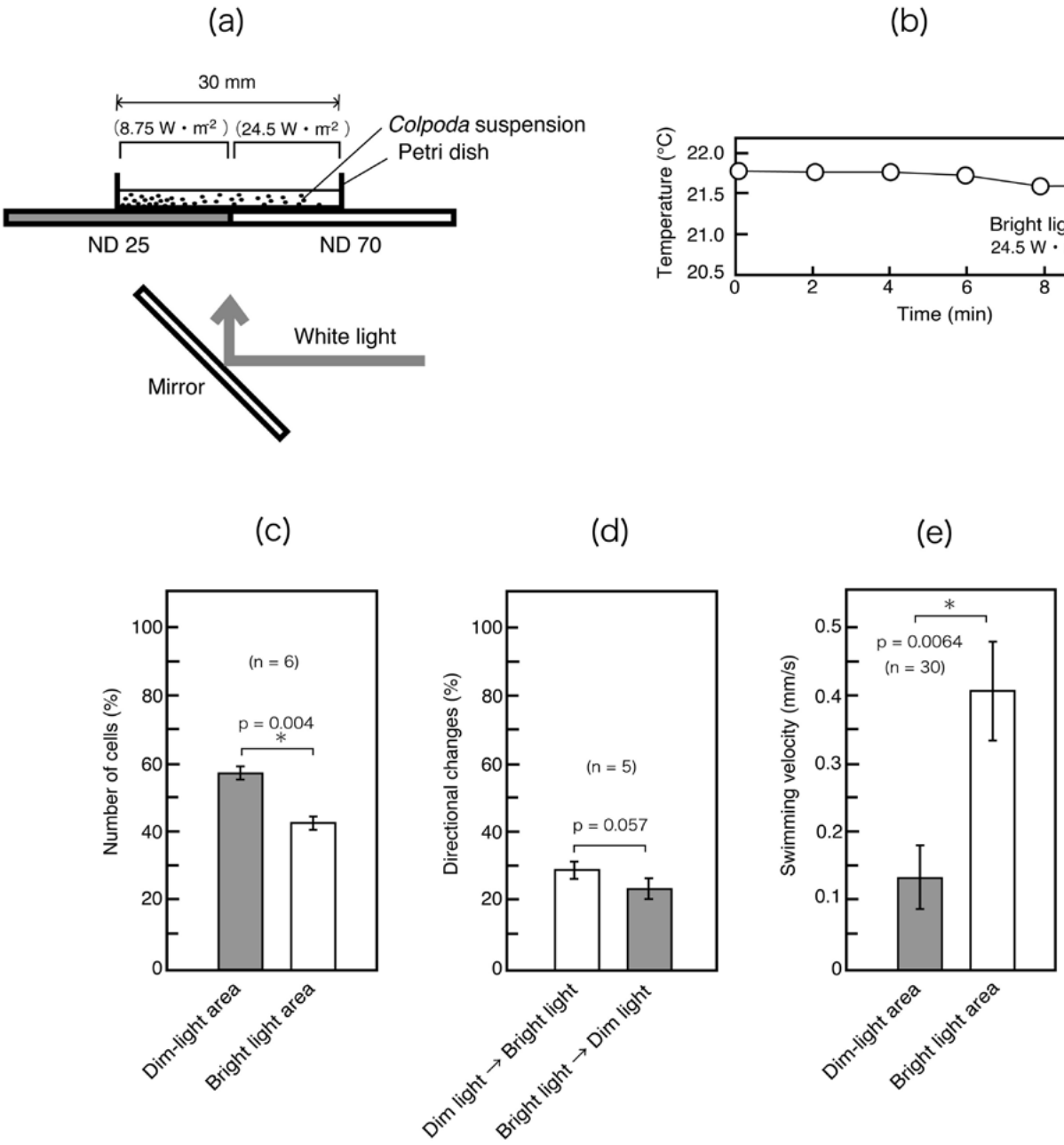
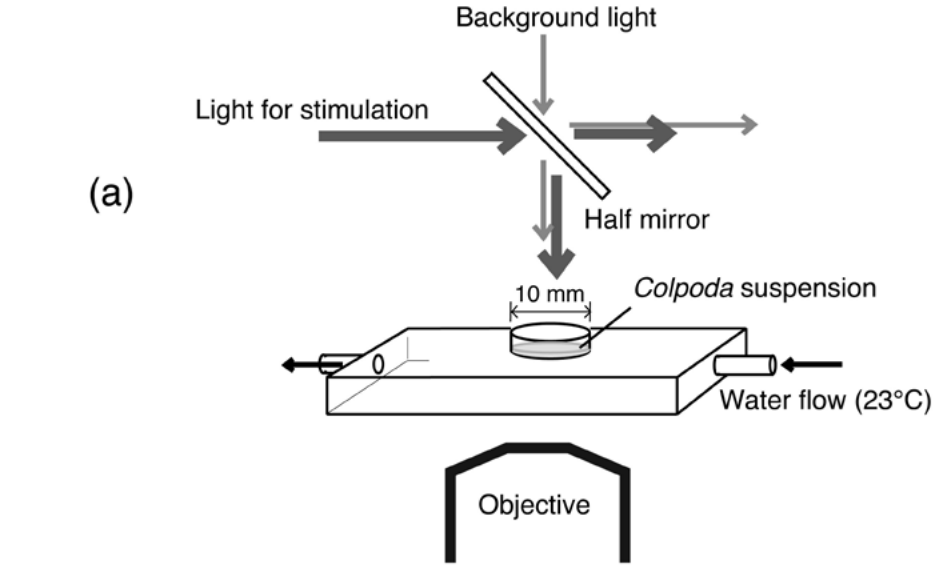
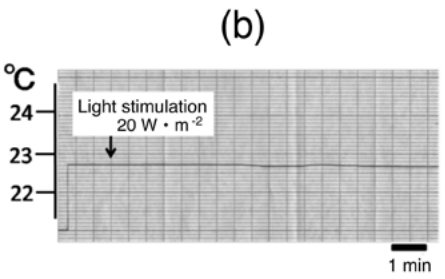
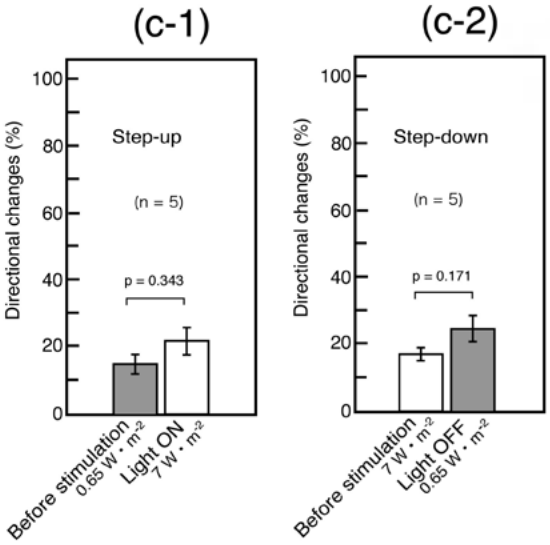
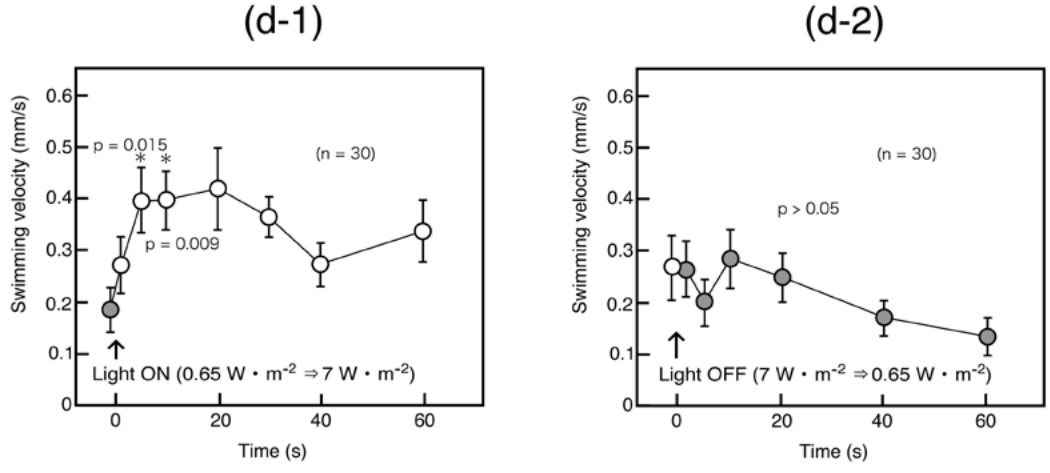


Fig. 2. Behavioral responses of *C. cucullus* Nag-1 in the stepwise light gradient. (a) Side-view of a Petri dish for the observation of photoresponse of the cells. The white light was applied from the bottom of the chamber (Petri dish) through two neutral density filters with different transmission rates (70% transmission; ND 70) and 25% transmission (ND 25), producing a stepwise light gradient (24.5 $W \cdot m^{-2}$ [bright area]; 8.75 $W \cdot m^{-2}$ [dim-light area]). The depth of the cell suspension was maintained at 7 mm. (b) The temperature of the cell suspension in the bright area of the chamber. (c) Photodispersal (negative photoaccumulation) of the cells in the dim-light area. The number of the cells in the dim-light area or bright light area was counted after the cells were placed for 3 min in such stepwise light gradient, and the rate of cell number is expressed as a percentage of the total number of counted cells (100 cells). (d) Directional alteration (photophobic response) of the forward swimming motion of the cells at the boundary between the dim-light and bright light area. The rate of cells which turned more than 90 degrees for 2 s after moving from dim-light to bright light (and vice versa) is expressed as a percentage of the total number of counted cells (100 cells). The measurement was performed after the cells were placed for 3 min under the stepwise light gradient. (e) Forward swimming velocity (mm/s) of the cells in the dim-light and bright light areas. The swimming velocity was measured after the cells were placed for 3 min in the stepwise light gradient.


(a)
Background light
Light for stimulation
Half mirror
10 mm
Colpoda suspension
Water flow (23°C)
Objective


(b)
°C
24
23
22
Light stimulation
20 W • m-2
1 min


(c-1)
(c-2)
Directional changes (%)
Step-up
(n = 5)
p = 0.343
Step-down
(n = 5)
p = 0.171
Before stimulation
0.65 W • m-2
Light ON
7 W • m-2
Before stimulation
7 W • m-2
Light OFF
0.65 W • m-2


(d-1)
(d-2)
Swimming velocity (mm/s)
p = 0.015
p = 0.009
(n = 30)
Light ON (0.65 W • m-2 ⇒ 7 W • m-2)
p > 0.05
Light OFF (7 W • m-2 ⇒ 0.65 W • m-2)
Time (s)

measurements (100 cells per measurement) and standard errors, or correspond to the mean of 30 cells (Figs 2e, 3d) or 60 cells (Fig. 4) and standard errors.

RESULTS AND DISCUSSION

Vegetative cells of *C. cucullus,* suspended in the encystment-inducing medium consisting of 1 mM Tris-HCl (pH 7.2) and 0.1 mM $CaCl_2$, were transferred to a Petri dish to form a water layer 5 mm thick (Fig. 1a). One half of the chamber was illuminated at various intensities of white light (0.35 $W \cdot m^{-2}$, 7 $W \cdot m^{-2}$, and 35 $W \cdot m^{-2}$), while the other half was shaded (Fig. 1a). After 8 h of cell suspension incubation under the different light conditions, the majority of the cells were attached to the bottom of the chamber and encysted. We found that a larger number of resting cysts formed in the shaded area than in the areas irradiated at 7 $W \cdot m^{-2}$ or 35 $W \cdot m^{-2}$ (Fig. 1c, d). There was a significant difference between the percentage of resting cysts found in the illuminated area and that in the shaded area (Fig. 1d, $p < 0.05$, Mann-Whitney test).

The formation of resting cysts in the shaded area may be the result of the photomotile response of vegetative cells since *C. cucullus* cyst formation is not affected by light irradiation (unpublished data). Under the stepwise light gradient (24.5 $W \cdot m^{-2}$ [bright area]; 8.75 $W \cdot m^{-2}$ [dim-light area]) shown in Fig. 2a, the temperature of the cell suspension did not increase in the bright area (Fig. 2b), although no temperature-controlled water circulated beneath the chamber. When the cells were placed in such stepwise light gradient, the cells tended to accumulate in the dim-light area (Fig. 2c). In this case, there was a significant difference between the number of cells in the bright area and that in the dim-light area ($p < 0.05$, Mann-Whitney test). Fig. 2d shows the rate (%) of the cells which altered forward swimming direction when they moved from the dim-light to the bright area (open column) and vice versa (shaded column). In this case, there was no significant difference between these experimental groups ($p > 0.05$, Mann-Whitney test). The forward swimming velocity significantly increased in the bright area ($p < 0.05$, Mann-Whitney test) (Fig. 2e).

Using the apparatus shown in Fig. 3a, step-up (Fig. 3c-1) and step-down (Fig. 3c-2) photophobic responses (changes in forward swimming direction), as well as the light-dependent swimming acceleration response (Fig. 3d) were analyzed. Both the step-up (from 0.65 $W \cdot m^{-2}$

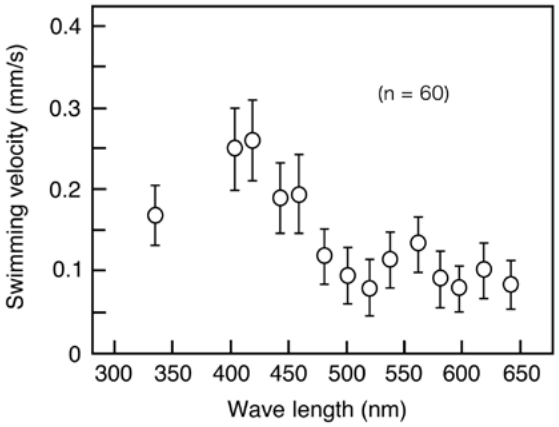


Fig. 4. Action spectrum (open circles) for the photokinetic response (fluence rate-dependent steady-state swimming acceleration) of *C. cucullus* Nag-1. The cells were adapted for 1 min in the background dim white light (0.65 $W \cdot m^{-2}$) and a given fluence rate (5×10^{17} quanta $\cdot m^{-2} \cdot s^{-1}$) of quasi-monochromatic light was applied for step-up stimulation by using the apparatus shown in Fig. 3a. Only a 334 nm light beam was directly applied to the cell suspension obliquely from the top. Swimming velocity (open circles) was measured at 20 s after light stimulation was continuously applied.

Fig. 3. Photoresponse of *C. cucullus* Nag-1 to an increase or decrease in white light intensity. (a) Side-view of a chamber to observe the photoresponse of the cells. The cell suspension was kept 1-mm thick. (b) The temperature of the cell suspension after stimulation light was applied (arrow). (c) Directional changes (photophobic response) of the cells to a sudden increase (c-1) or decrease (c-2) in white light intensity. Prior to "ON" and "OFF" stimulation, the cells were adapted for 1 min in the background dim-light (0.65 $W \cdot m^{-2}$) and bright light (7 $W \cdot m^{-2}$), respectively. The cells responded by turning more than 90 degrees in 2 s after light stimulation was expressed as the percentage of the total number of tested cells (100 cells). (d) Alteration in steady-state forward swimming velocity of the cells induced by continuous light stimulation (photokinetic response). Prior to "ON" and "OFF" stimulation, the cells were adapted for 1 min in dim-light (0.65 $W \cdot m^{-2}$) and bright light (7 $W \cdot m^{-2}$), respectively. (d-1) Time course of forward swimming velocity after the onset of step-up light stimulation. The shaded circles indicate the swimming velocity at 1 s before light intensity was increased. Asterisks indicate significant differences in the swimming velocity before light stimulation (shaded circle) ($p < 0.05$, Mann-Whitney test). (d-2) Time course of forward swimming velocity after the onset of step-down light stimulation. The open circle is swimming velocity at 1 s before light intensity is decreased.

to 7 $W \cdot m^{-2}$) (Fig. 3c-1) and step-down light stimulation (from 7 $W \cdot m^{-2}$ to 0.65 $W \cdot m^{-2}$) (Fig. 3c-2) did not induce changes in forward swimming direction. There was no significant difference between the rates of spontaneous responses (Fig. 3c, "Before stimulation") and those induced by the step-up or down stimulation (Fig. 3c, "Light ON" or "Light OFF") ($p > 0.05$, Mann-Whitney test). On the other hand, the forward swimming velocity increased in response to continuous light irradiation (light intensity raised from 0.65 $W \cdot m^{-2}$ to 7 $W \cdot m^{-2}$) and reached a steady-state level in 5 s (Fig. 3d-1). When the light intensity decreased from 7 $W \cdot m^{-2}$ to 0.65 $W \cdot m^{-2}$, the means of the forward swimming velocity gradually decreased, although there was no significant difference from the velocity before the step-down stimulation (open circle) ($p > 0.05$, Mann-Whitney test) (Fig. 3d-2). The steady-state increase in forward swimming velocity caused by continuous light irradiation is a type of photokinetic response that may be responsible for photodispersal of *C. cucullus* vegetative cells.

Fig. 4 shows the action spectrum of the photokinetic response (the increase in steady-state swimming velocity) of *C. cucullus* vegetative cells. The action spectrum had a peak in the blue light region, implying the involvement of blue light receptors (Briggs and Christie 2002, Iseki *et al*. 2002, Yu *et al*. 2010).

Ciliate encystment induction and motile responses are influenced by changes in temperature (Corliss and Esser 1974, Nakaoka and Oosawa 1977, Matsuoka *et al*. 1990). In the present study, the temperature of the cell suspension did not increase due to light irradiation (Figs 1b, 2b, 3b), indicating that the formation of *C. cucullus* resting cysts in the shaded area, the accumulation of vegetative cells in the shaded area, and the acceleration of swimming movement driven by light irradiation is related to light reception and not influenced by thermoreception. The fluctuation in cell suspension temperature (Figs 1b, 2b) may be due to temperature control capability of the thermo-regulator.

It has been suggested that in some ciliates such as *Fabrea salina*, positive photoaccumulation may serve nutrient needs, since the green algae that constitute a food source for *F. salina* accumulate in illuminated areas (Colombetti *et al*. 1992). In case of the pink-pigmented ciliate *Blepharisma*, the cells are killed by photosensitization of the endogenous quinone pigment known as blepharismin (Giese 1973, Checcucci *et al*. 1997, Maeda *et al*. 1997, Spitzner *et al*. 1998), which has antimicrobial activity (Noda Terazima *et al*. 1999) when extruded. Therefore, ciliates may need to escape illuminated areas for survival (Matsuoka *et al*. 2010). Additionally, blepharismin is believed to function as a photoreceptor, mediating photodispersal (Scevoli *et al*. 1987, Matsuoka *et al*. 1992, Checcucci *et al*. 1993). What is the ecological significance of photodispersal in *C. cucullus*? The terrestrial ciliate *C. cucullus* needs to form resting cysts resistant to desiccation before transient puddles desiccate. If the puddle dries quickly due to exposure to sun light, *Colpoda* may fail to complete the formation of resting cysts. It is also possible that the accumulation of *Colpoda* in the shaded regions play a role for the avoidance of vegetative cells and resting cysts from UV radiation which may be lethal for even the resting cysts, as pointed out by Häder *et al.* (2011) in aquatic organisms.

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