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RESEARCH ARTICLE

Behaviour of the plathelminth *Symsagittifera roscoffensis* under different light conditions and the consequences for the symbiotic algae *Tetraselmis convolutae*

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ABSTRACT

Symsagittifera roscoffensis is a plathelminth living in symbiosis with the green algae *Tetraselmis convolutae*. Host and symbiont are a model system for the study of endosymbiosis, which has so far mainly focused on their biochemical interactions. *Symsagittifera roscoffensis* is well known for its positive phototaxis that is hypothesized to optimize the symbiont's light perception for photosynthesis. In this study, we conducted a detailed analysis of phototaxis using light sources of different wavelength and brightness by videotracking. Furthermore, we compared the behavioural data with the electron transfer rate of the photosystem from cultured symbiotic cells. The symbiotic algae is adapted to low light conditions, showing a positive electron transfer rate at a photosynthetically active radiation of 0.112 µmol photons m^{-2} s^{-1}, and *S. roscoffensis* showed a positive phototactic behaviour for light intensities up to 459.17 µmol photons m^{-2} s^{-1}, which is not optimal regarding the needs of the symbiotic cells and may even harm host and symbiont. Red light cannot be detected by the animals and therefore their eyes seem not to be suitable for measuring the exact photosynthetically active radiation to the benefit of the photosymbionts.

KEY WORDS: Endosymbionts, Endosymbioses, Photoautotroph, Phototaxis, Host

INTRODUCTION

Oxygen-dependent photosynthesis is a significant pathway for many organisms. It is suggested that the development of oxygen only occurred once in evolution in the cyanobacteria or late Archaean (Cavalier-Smith, 2006). Later, the pathway was passed over by endosymbiosis to basal eukaryotes, leading to primary, secondary and tertiary plastids of various algal species as well as the plastids of higher plants that evolved from green algae (Johnson, 2011; Taylor, 1973; Trench, 1993). However, there are also many groups of organisms that developed mechanisms to capture photosynthetic products through the formation of symbiotic associations. These include the phyla Porifera (marine and freshwater sponges), Cnidaria (corals, sea anemones and freshwater hydra), Acoelomorpha (marine turbellaria), Mollusca (e.g. giant clams and nudibranchs) and Chordata (marine ascidians) (Serôdio et al., 2014; Trench, 1979; Venn et al., 2008). One special variety of photosynthesis in the animal kingdom occurs in the sea slug taxon *Sacoglossa*. They feed on algae, sequestering the chloroplasts. Some species of slugs are able to keep these chloroplasts alive within their own cells for several months (Händeler et al., 2009; Kremer, 1976, 1977). A horizontal gene transfer from the host algae to the slugs was suggested, but this suggestion is still controversial and is an area of ongoing research (Bhattacharya et al., 2013; Rumpho et al., 2008; Schwartz et al., 2014; Wägele et al., 2010).

The plathelminth of the order Turbellaria *Symsagittifera roscoffensis* (Graff 1891), formally known as *Convoluta roscoffensis*, is a simply organized unsegmented worm, living in symbiosis with the algae *Tetraselmis convolutae* (Norris et al., 1980; Parke and Manton, 1967). It is 2–4 mm long and contains 20,000–70,000 endosymbiotic cells of the algae within its body cavities (Doonan and Gooday, 1982). These cells are not passed on maternally, but must be acquired by each individual during the first days after hatching (Keeble and Gamble, 1907). This is a critical process, as the symbiosis is obligatory for the animals to survive. Thus, the worm becomes a photoautotrophic organism, consuming nutrients provided by the symbiotic algae (Keeble, 1910; Muscatine et al., 1974). The algae also profit from the symbiosis because they reach a higher rate of carbon fixation, compared with a free-living phytoplankton organism in habitats with tidal changes (Doonan and Gooday, 1982; Gooday, 1970). Doonan and Gooday discussed that the worms may regulate the photosynthesis of their symbionts, but only little is known about the worm's influence. Behavioural and physiological studies are rare but it is known that *S. roscoffensis* shows a positive phototaxis and escapes into the sandy sediment upon vibration (Keeble, 1910). Artificial light experiments with a light gradient suggest *S. roscoffensis* optimize photosynthesis by choosing the optimal light conditions for the algae (Serôdio et al., 2011). In general, the eyes of turbellarians are without lenses, consisting of a shading pigment cup and nerve cells (Yamasu, 1991). Nevertheless, this type of eye is capable of assessing the intensity and locating the direction of a light source (Jékely et al., 2008). In order to show whether *S. roscoffensis*'s phototactic behaviour is directed to optimize light perception of the symbionts, we used light of different wavelengths and intensities to trigger phototaxis.

RESULTS

Light curve measurement

As a first step towards understanding the mutualism between *S. roscoffensis* and *T. convolutae*, optimal light intensity for photosynthesis in the algae was determined. The electron transfer rate (ETR) of photosystem II was recorded as a function of varying intensities of photosynthetically active radiation (PAR) in the range

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2–105 µmol photons m^{-2} s^{-1} using chlorophyll fluorescence (N=6 repeats; Fig. 1). While an almost linear correlation of ETR with irradiation was observed for photon flux densities below 40 µmol photons m^{-2} s^{-1}, onset of saturation became obvious for intensities higher than 50 µmol photons m^{-2} s^{-1}. The obtained data for *T. convolutae* and two closely related algae, *Tetraselmis striata* and *Tetraselmis suecia*, were fitted to an exponential growth curve:

$$\mathrm{ETR} = \mathrm{ETR}_0 + \mathrm{ETR}_{\max} \times (1 - \mathrm{e}^{(-k \times \mathrm{PAR})}), \quad (1)$$

where ETR is a dependent parameter, ETR_0 is the electron transfer rate at zero light, ETR_{max} is the maximal electron transfer rate, PAR is an independent parameter and k is a constant factor (for details, see Table 1). Interestingly, the minimal light intensity for an ETR larger than zero calculated from the exponential growth curve solved for PAR (Eqn 2) revealed that at light intensities above 0.112 µmol photons m^{-2} s^{-1} a positive ETR could be detected for isolated algae, clearly demonstrating a low light adaptation of *T. convolutae*:

$$\mathrm{PAR} = \ln(1 - (\mathrm{ETR} - \mathrm{ETR}_0) / \mathrm{ETR}_{\max}) / -k. \quad (2)$$

Measurement of photosynthetic oxygen formation in isolated algae

Oxygen formation was measured using suspensions of algae isolated from the symbionts to gain information about the suitability of the light sources for photosynthesis (Fig. 2). The oxygen concentration of the medium increased with a rate of 0.19±0.12 µmol O_2 l^{-1} s^{-1} g^{-1} biomass µmol^{-1} photons for blue light and 0.50±0 µmol O_2 l^{-1} s^{-1} g^{-1} biomass µmol^{-1} photons for red light, respectively. A t-test revealed a significantly higher oxygen evolution rate for red light than for blue light (P=0.043, N=5).

Behaviour of *S. roscoffensis* exposed to varying light regimes

To test whether symbiotic *S. roscoffensis* preferred illuminated areas over shaded ones, the animals (N=57) were exposed to three different light colours each at a photon flux density of 5 µmol photons m^{-2} s^{-1}. The experimental setup is depicted in Fig. 3. A significantly positive response to illumination, i.e. a higher allocation time in illuminated versus shaded areas, was observed for green and blue light but not for red light (Fig. 4). In red light, average stay time was 510±257 s versus 385±255 s in the shaded area (P=0.08), while the allocation time for green light was 566±200 s versus 333±201 s in the shaded area (P=0.00005) and for blue light it was 550±182 s versus 348±183 s in the shaded area (P=0.0002). When the allocation times in areas illuminated by different light sources were compared in an ANOVA on ranks, no effect of light colour could be revealed.

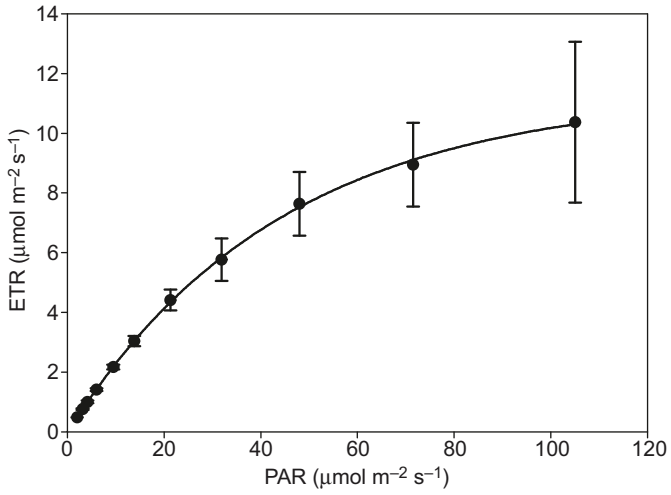

Fig. 1. Electron transfer rate (ETR) of *Tetraselmis convolutae* versus photosynthetically active radiation (PAR). Data are means±s.d., N=6.

Table 1. Photosynthetic parameters obtained for *Tetraselmis convolutae*, *Tetraselmis striata* and *Tetraselmis suecia*

	T. convoluta	*T. striata*	*T. suecia*
ETR_0 (µmol photons m^{-2} s^{-1})	–0.0290±0.2444	0.0745±0.1722	0.1719±0.2713
ETR_{max} (µmol photons m^{-2} s^{-1})	11.38±0.6107	20.3466±1.9507	18.7784±2.2331
k	0.0227±0.0032	0.0104±0.0017	0.0121±0.0026

Data are means±s.e.
ETR_0, electron transfer rate at zero light; ETR_{max}, maximal electron transfer rate; k, constant factor.

When the animals (N=40) had the choice between all three colours and a shaded sector at the same time, the blue area was the most preferred, with a mean stay time of 455±193 s, and values of 162±114 s for green, 130±99 s for red and 131 s±138 s for shaded sectors (Fig. 5). Even if the blue light was less intense, it was still preferred over a brighter red sector (N=30) (Fig. 6). The allocation time in the blue sector of the arena (600±274 s) was significantly longer than that in the red sector (239±274 s; P=0.006).

Behaviour of *S. roscoffensis* within a light gradient

The behaviour of *S. roscoffensis* (N=18) within a light gradient was tested in Petri dishes with 3.2 cm diameter. The arena was divided into two areas of the same size: a highly illuminated part and a part with a gradient of light (the umbra). Allocation times of 18 animals were recorded over 1800 s. The animals tended to prefer the fully illuminated half of the arena, with an average allocation time of 1235±732 s compared with 563±733 s in the umbra (P=0.06) (Fig. 7).

Overall activity of the turbellarians

All data from the previous experiments were analysed to characterize the movement behaviour of *S. roscoffensis*. During 71 h observation time in total, the animals showed measureable movement for 34 h. The overall distance travelled by all animals was 135.3 m, resulting in an average speed of 1.1 mm s^{-1} (3.9 m h^{-1}). With a probability of 99%, the speed of motion was about 4.5 mm s^{-1} (17.55 m h^{-1}) or below (Fig. 8).

DISCUSSION

The aim of this study was to investigate the interaction between the host *S. roscoffensis* and symbiont *T. convolutae*, in order to find out whether the host provides an optimal environment for photosynthetic activity of the algae. Regarding the light sources used for the experiment, the blue and red LEDs were suitable for algal photosynthesis, and an oxygen development was detectable. However, the blue light was exceptionally less efficient and the red light is the optimal colour for the green algae photosystems (Butler, 1978; Emerson and Lewis, 1943). Concerning light intensity, *T. convolutae* reaches its optimal electron transfer rate at relatively low light doses. This was also observed in this study for the closely related species *T. striata* and *T. suecia*. Other common zooxanthellae such as diatoms or dinoflagellates reach their peak activity at an illumination level of 500–1500 µmol photons m^{-2} s^{-1}

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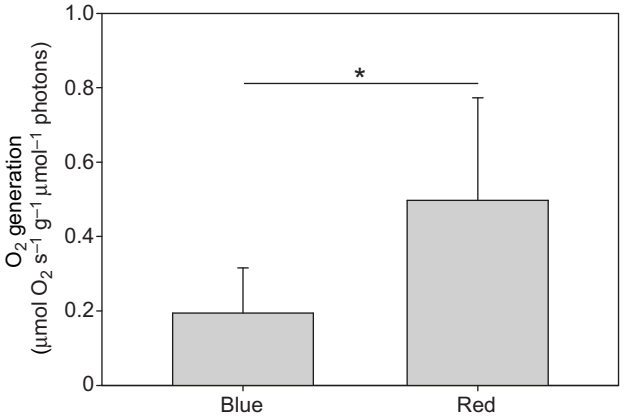

Fig. 2. The measured oxygen generation of free-living *T. convolutae* in red and blue light. O_2 generation is given per second, per gram biomass, per illumination (means+s.d.). The difference is significant (*P=0.043 with t-test, N=5).

(Geel et al., 1997; Guarini and Moritz, 2009) or 600 to 800 μmol photons m^{-2} s^{-1} (Rodríguez-Román and Iglesias-Prieto, 2005), respectively.

The eyes of plathelminthes are very simple organs, built of only one type of receptor cell half shaded by a pigment cup cell. The receptor cells do not have enlargement of the membrane surface like cilia or microvilli (Nilsson, 2009; Yamasu, 1991). These simple eyes are capable of detecting the direction of a light source (Jékely et al., 2008). Nilsson showed by simulations that eyes of this kind are able to find the direction of a light source in water up to a depth of 200 m. Photoreceptors are proposed to originate from a single common ancestor and are all based on the pigment rhodopsin. They

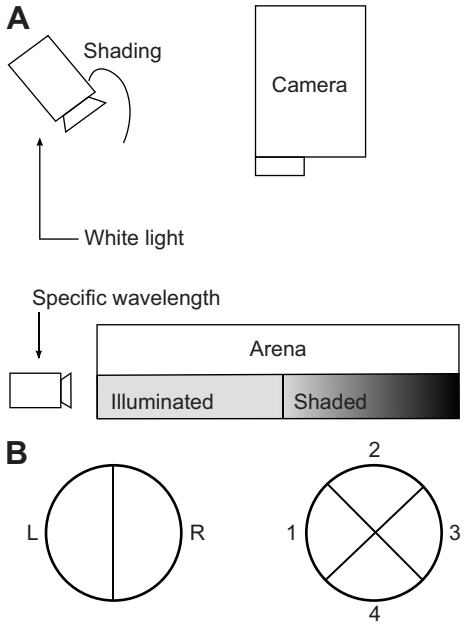

Fig. 3. Sketch of the experimental setup. (A) Side view of the arena, with the light source and video camera indicated. The brightness is indicated below the arena. (B) Partitioning of the arena into two or four sectors. Possible lamp positions are marked with L (left) and R (right) or numbered 1–4 for the two- and four-sector setups, respectively. The two-sector version was used for experiments with one or two light sources (results shown in Figs 4, 6 and 7), the four-sector version was used for experiments with three light sources and a shaded control sector (results shown in Fig. 5).

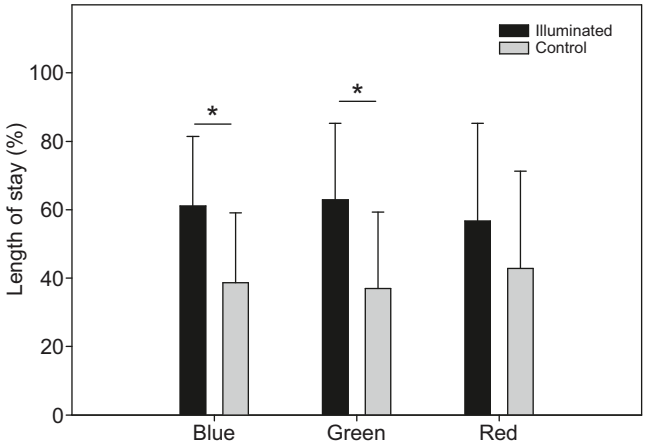

Fig. 4. Allocation preference of *Symsagittifera roscoffensis* in response to blue, green or red light. LEDs were 5 μmol photons m^{-2} s^{-1} (5 PAR) each. Time spent in the illuminated and shaded (control) half of the arena is shown; observation time was 900 s per colour and animal; data are means+s.d. (N=57). Only the allocation preference in the direction of the blue and green light is significant (*P<0.05).

detect one specific wavelength according to rhodopsin's absorption spectrum (Arendt, 2003; Shichida and Matsuyama, 2009). It is very likely that *S. roscoffensis* only expresses one type of receptor and therefore is limited in its viable light spectrum. Depending on the water conditions, blue or green light will reach deeper areas in the sea, which makes light of shorter wavelength more important for phototaxis. *Symsagittifera roscoffensis* lives in a tidal environment in a water depth of about 0–4 m (Doonan and Gooday, 1982). The average light intensity in summer months in 4 m deep water is about 5.47% compared with the surface, but there is a significant difference in the transmission of different wavelengths depending on the water type: the observed light transmissions were 67, 52 and 55% m^{-1} for green, blue and red light, respectively, in summer in a tidal environment (Lüning and Dring, 1979).

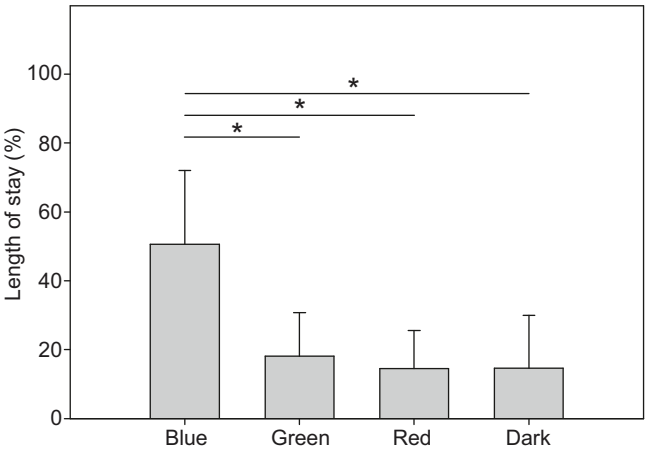

Fig. 5. Allocation preference of *S. roscoffensis* in an arena illuminated by three different light sources. Time spent in the three illuminated sectors is compared with a shaded (control) sector; observation time was 900 s; data are means+s.d. (N=40). The influence of illumination on allocation time in the four sectors is significant (P<0.001, ANOVA on ranks); the allocation time in the blue sector is significantly higher than in all other sectors (P<0.05, Tukey test), while there is no significant difference between the green, red and shaded sectors. Significant differences for a specific pair of sectors are marked with horizontal lines and asterisks.

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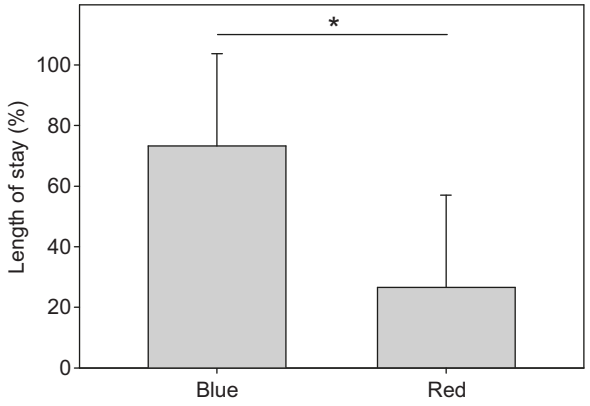

Fig. 6. Allocation preference of *S. roscoffensis* in two arena sectors illuminated by blue light and red light. Blue light photon density, 5 µmol m^{-2} s^{-1}; red light, 44 µmol m^{-2} s^{-1}. Observation time was 900 s; data are means+s.d. (*N*=30). The length of stay in the blue illuminated half of the arena was significantly greater (**P*=0.006, Wilcoxon signed-rank test with continuity correction).

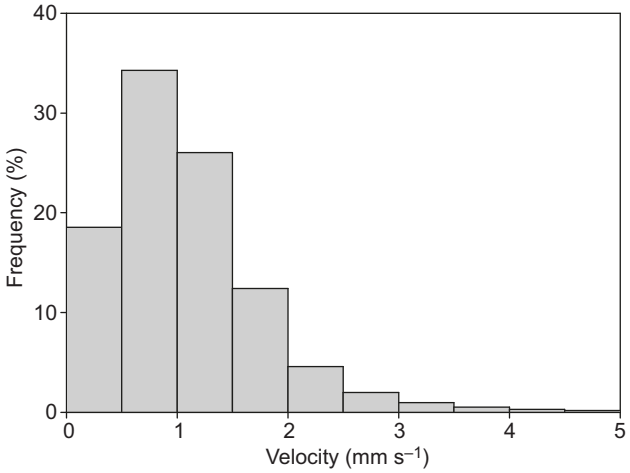

Fig. 8. Frequency distribution of the speed of motion of the animals. Data are in steps of 0.5 mm s^{-1}. In 99% of cases, the speed of motion is up to 4.5 mm s^{-1}.

Our experiment revealed that the host's reaction to light stimuli is not adjusted to the symbionts' needs. *Symsagittifera roscoffensis* did not show a reaction to red light, but did to blue and green light, while the symbiotic algae show the highest photosynthetic rate for red light.

Our understanding is that *S. roscoffensis* does not react to red light, because it is not able to detect it and therefore its eyes are not suitable for measuring the exact photosynthetically active radiation to the benefit of the photosymbionts. Nevertheless, perception of wavelengths that are not dominant in a specific depth may be useful for the detection of fluorescent light, e.g. for communication, which has been observed in different fish species (Michiels et al., 2008; Shcherbakov et al., 2013, 2012).

Considering light intensities, the animals moved in the areas with an illumination higher than optimal for the algae. Possibly, these light intensities may even be harmful for the photosynthetic apparatus, though they may use photoprotectants to avoid oxidative stress (Cruz et al., 2013, 2015; Peers et al., 2009). However, *S. roscoffensis* is very mobile with speeds of up to 17.5 m h^{-1} and would be able to reach water depths providing *T. convolutae* with optimal light by phototaxis. A previously published study by Serôdio et al. suggests an active regulation of the photosynthetic activity (Serôdio et al., 2011). However, in their setup, the light came from below with the gradient generated by a linear attenuation filter. Therefore, the animals trying to approach the light source would estimate it as being near the centre of the arena, maybe with a shift from the dark to bright side of the arena. This could in itself explain the result presented by Serôdio et al. (2011) without any active photosynthesis regulation. We avoided this problem with our system, which is closer to the situation in nature, with our light gradient generated with a light source illuminating the arena at an angle of 70 deg. In our setup, the animals did not avoid high illumination, but further studies are necessary to determine whether the host's behaviour may even harm the photosynthetic symbionts in case of a very high illumination level on bright summer days. The saturation of the ETR was observed as for Serôdio et al. (2011) for individual animals, with their results in the same range as our measurements with the separated algae.

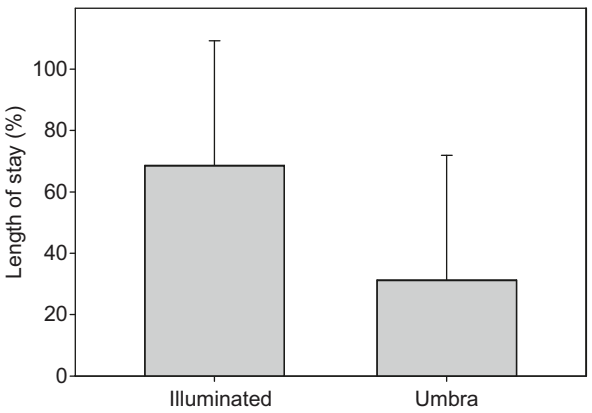

Fig. 7. Allocation preference of *S. roscoffensis* in a light gradient. Time spent in the full illuminated half of the arena versus the half with the light gradient (umbra) is shown; observation time was 1800 s; data are means+s.d. (*N*=18). The length of stay in the illuminated half of the arena is by tendency higher (*P*=0.06, Exact Wilcoxon signed rank test).

Avoidance of high levels of illumination is known for other symbiotic animals: for example, negative and positive phototaxis is known in symbiotic sea anemones (*Actiniaria* spp.) depending on light intensities, e.g. negative phototaxis at a high illumination of 700 foot candle and positive phototaxis at 250 foot candle, which is about 140 PAR and 50 PAR, respectively (Pearse, 1974). Pearse noted that non-symbiotic anemones showed negative or indefinite reactions to light stimuli, which implies a relationship between host behaviour and photosynthesis of the symbiotic algae, but did not give any data about photosynthesis of the symbiotic algae. According to measurements made by other authors, the high illumination is below the photosynthetic optimum of common zooxanthellae (Geel et al., 1997; Guarini and Moritz, 2009; Rodríguez-Román and Iglesias-Prieto, 2005). We speculate that this allows a safety margin in light exposure to be kept for the anemone's symbionts. Such behaviour was not seen in *S. roscoffensis*.

Doonan and Gooday (1982) reported a decreasing population of *S. roscoffensis* in summer months and speculated that this may be the result of high illumination levels, which can be up to 1200 µmol photons m^{-2} s^{-1} on summer days (Migné et al., 2004). An active regulation of the light exposure suggested by Serôdio et al. (2011) would avoid such a decline in the population. With respect the animal's inability to react to red light that we observed in this study,

we think that the position of Doonan and Gooday (1982) is more likely, but further studies are necessary to determine whether there is a harmful effect of high illumination levels in summer months.

MATERIALS AND METHODS

Animal and algae culture

The specimens of *S. roscoffensis* were obtained from the Station Biologique de Roscoff (Roscoff, France) and cultured with artificial seawater provided by the zoological and botanical garden Wilhelma Stuttgart (Stuttgart, Germany). Cultures were kept in Petri dishes (Schott, Mainz, Germany) of different sizes in a climate chamber at 7°C and a low level of illumination for 12 h day^{-1}, starting at 08:00 h (PAR, 7.9 µmol photons m^{-2} s^{-1}). For the experiments, the animals were transferred to single wells of 24-well plates, and acclimated in a climate chamber at 16°C for at least 1 day before measurements were made. The illumination was 16.7 µmol photons m^{-2} s^{-1} for 12 h day^{-1}, starting at 08:00 h. Despite the rather short time for acclimatization, the animals showed a normal behaviour, which was expected as large temperature differences of up to 10°C within a day are common in tidal environments (Morris and Taylor, 1983). All experiments were performed during standard laboratory work time from 09:00 h to 17:00 h.

Symbiotic algae were extracted from naturally deceased worms by pipette and transferred to 50 ml Erlenmeyer flasks (Schott, Mainz, Germany) with Seawater Medium (SWES) according the protocol (version 10.2008) of the SAG (Culture Collection of Algae, Göttingen, Germany). For chlorophyll fluorescence measurements of the algae, fine biofilms were grown in 9 cm Petri dishes on 1% agarose in SWES solution.

Light sources for illumination experiments

The intensities of the different light sources (PAR) were measured with a quantum sensor (QS, Delta-T Devices, Cambridge, UK). LEDs of different colours were used to provide different wavelengths: red (LED CQY 40 L, Osram, Munich, Germany; λ_{max}=660 nm, spectral line half-width $\Delta\lambda_{1/2}$=40 nm), green (LED CQY 72 L, Osram; λ_{max}=560 nm, $\Delta\lambda_{1/2}$=20 nm) and blue (LED L-53MBDL, Kingbright Electronic, Taipei, Taiwan; λ_{max}=466 nm, $\Delta\lambda_{1/2}$=60 nm). Each LED was run with a battery of its nominal voltage and controlled by a 1 kΩ potentiometer for adjusting an intensity of 5 PAR. LEDs were mounted in an interior reflector to provide a homogeneously illuminated field that allowed positioning with a well-defined border between illuminated and shaded parts of the arena (SMZ1089, Signal-Construct, Niefern, Germany). Because of scattered light, the average brightness in the shaded sector reaches 63% of the average brightness in the illuminated sector, which was a good compromise for getting enough light for video recording and a behavioural response of the animals. The response of the animals to different light intensities was tested in a light gradient. An adjustable light source from a binocular loupe (GSZ loupe, Zeiss, Jena, Germany) was fixed with an angle of 70 deg at a height of 20 cm, with a piece of aluminium foil on the upper half of the lamp for shading. The brightness in this gradient started at 24.92 PAR; peak value was 459.17 PAR within a Petri dish of 37 mm diameter.

Fluorescence measurements and oxygen evolution in response to different light regimes

The ETR of photosystem II of isolated algae was measured at varying actinic light intensities using a PAM 2000 fluorimeter with the default settings 'run 9, actinic light intensity series' (Heinz Walz, Effeltrich, Germany). The sensor had a distance of 1 cm to the surface of the algal biofilm.

Oxygen generation by algae at different wavelengths

Oxygen evolution by suspensions of algae isolated from the symbionts was measured with a Clark-type electrode (Type S1, Hansatech, King's Lynn, UK). The rate of photosynthesis following illumination by blue and red diodes was determined as the increase of the oxygen concentration in the suspension per dry algal biomass, measured after drying the algae for 2 days in a SpeedVac concentrator (Savant, Fisher Scientific, Schwerte, Germany). Five replicates (0.5 ml each) of algal suspension were tested at a photon flux density of 25 µmol photons m^{-2} s^{-1} (blue) and 38 µmol photons m^{-2} s^{-1} (red).

Videotracking of *S. roscoffensis*

The behaviour of *S. roscoffensis* was recorded with a standard camcorder (DCR-PC 109 E, Sony, Tokyo, Japan) using the software Virtualdub v1.8.6 (www.virtualdub.org) (Fig. 3). The video frame rate for recording and analysis was one picture per second. The location coordinates of *S. roscoffensis* were evaluated as the allocation time in different sectors of the arena by the program BioMotionTrack D.S. (Shcherbakov et al., 2010) and stored in an Access (Microsoft, Redmond, USA) database. The response to the different wavelengths was tested for 900 s at an illumination of 5 PAR in Petri dishes with a diameter of 15.5 mm and a water depth of 5 mm seawater. The measurements within the light gradient were performed in Petri dishes of 37 mm diameter and 5 mm water depth for 1800 s. The behaviour in response to different wavelengths was tested in a series of experiments by comparing the length of stay within the illuminated and not illuminated half of the arena. In a second series, experiments were performed with sectors representing each of the three wavelengths plus a fourth not illuminated, shaded sector.

For testing the effect of light intensity, the blue light source was adjusted to a photon flux density of 5 µmol photons m^{-2} s^{-1}, while the red LED (660 nm) was operated at 44 µmol photons m^{-2} s^{-1}. The total irradiance for the blue LED was 1.3 mW m^{-2} and for the red light source it was 7.6 mW m^{-2}. The conversion from photon flux density to radiation power was calculated for the dominating wavelength (Schröder and Treiber, 2007). During the behavioural experiments, the light sources were used in random order. To characterize motivation for active orientation behaviour, the animals' speed of motion was additionally calculated.

Statistics

Statistical analysis of allocation time data was performed using either the Exact Wilcoxon signed rank test with continuity correction for pairwise analysis or an ANOVA on ranks. Statistical significance of differences in oxygen evolution was tested using a *t*-test. Significance was assumed for $P<0.05$ in all statistical tests. The data for ETR were fitted with Eqn 1. All statistics were performed with Sigmastat. Values are given as means±s.d.

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Competing interests

The authors declare no competing or financial interests.

Author contributions

M.N. conceived, designed and performed the experiments; M.N., D.S. and A.H. analysed the data; M.N., D.S., A.H., F.B. and R.O.S. contributed reagents/materials/analysis tools; M.N., A.H. and R.O.S. wrote the paper; R.O.S. supervised the project.

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References

Arendt, D. (2003). Evolution of eyes and photoreceptor cell types. *Int. J. Dev. Biol.* **47**, 563-572.

Bhattacharya, D., Pelletreau, K. N., Price, D. C., Sarver, K. E. and Rumpho, M. E. (2013). Genome analysis of Elysia chlorotica egg DNA provides no evidence for horizontal gene transfer into the germ line of this kleptoplastic mollusc. *Mol. Biol. Evol.* **30**, 1843-1852.

Butler, W. L. (1978). Energy distribution in the photochemical apparatus of photosynthesis. *Annu. Rev. Plant Physiol.* **29**, 345-378.

Cavalier-Smith, T. (2006). Cell evolution and Earth history: stasis and revolution. *Philos. Trans. R. Soc. B Biol. Sci.* **361**, 969-1006.

Cruz, S., Calado, R., Serôdio, J. and Cartaxana, P. (2013). Crawling leaves: photosynthesis in sacoglossan sea slugs. *J. Exp. Bot.* **64**, 3999-4009.

Cruz, S., Cartaxana, P., Newcomer, R., Dionísio, G., Calado, R., Serôdio, J., Pelletreau, K. N. and Rumpho, M. E. (2015). Photoprotection in sequestered plastids of sea slugs and respective algal sources. *Sci. Rep.* **5**, 7904.

Doonan, S. A. and Gooday, G. W. (1982). Ecological studies of symbiosis in *Convoluta roscoffensis*. *Mar. Ecol. Prog. Ser.* **8**, 69-73.
Emerson, R. and Lewis, C. M (1943). The dependence of the quantum yield of chlorella photosynthesis on wave lenghth of light. *Am. J. Bot.* **30**, 165-178.
Geel, C., Versluis, W. and Snel, J. F. H. (1997). Estimation of oxygen evolution by marine phytoplankton from measurement of the efficiency of Photosystem II electron flow. *Photosynth. Res.* **51**, 61-70.
Gooday, G. W. (1970). A physiological comparison of the symbiotic alga *Platymonas Convolutae* and its free-living relatives. *J. Mar. Biol. Assoc. UK* **50**, 199-208.
Guarini, J.-M. and Moritz, C. (2009). Modelling the dynamics of the electron transport rate measured by PAM fluorimetry during Rapid Light Curve experiments. *Photosynthetica* **47**, 206-214.
Händeler, K., Grzymbowski, Y. P., Krug, P. J. and Wägele, H. (2009). Functional chloroplasts in metazoan cells - a unique evolutionary strategy in animal life. *BMC Front. Zool.* **6**.
Jékely, G., Colombelli, J., Hausen, H., Guy, K. Stelzer, E., Nédélec, F. and Arendt, D. (2008). Mechanism of phototaxis in marine zooplankton. *Nature* **456**, 395-399.
Johnson, M. D. (2011). The acquisition of phototrophy: adaptive strategies of hosting endosymbionts and organelles. *Photosynth. Res.* **107**, 117-132.
Keeble, F. (1910). *Plant-Animals; A Study in Symbiosis*. Cambridge: Cambridge University Press.
Keeble, F. and Gamble, F. W. (1907). Memoirs: the origin and nature of the green cells of *Convoluta roscoffensis*. *Q. J. Microsc. Sci.* **s2-51**, 167-220.
Kremer, B. P. (1976). Algen und Plastiden als Endosymbionten wirbelloser Tiere. *Biol. Unserer Zeit* **6**, 83-90.
Kremer, B. P. (1977). Rotalgen-Chloroplasten als funktionelle Endosymbionten in einem marinen Opisthobranchier. *Naturwissenschaften* **64**, 147-148.
Lüning, K. and Dring, M. J. (1979). Continuous underwater light measurement near Helgoland (North Sea) and its significance for characteristic light limits in the sublittoral region. *Helgol. Wiss. Meeresunters.* **32**, 403-424.
Michiels, N. K., Anthes, N., Hart, N. S., Herler, J., Meixner, A., Schleifenbaum, F., Schulte, G., Siebeck, U. E., Sprenger, D. and Wucherer, M. (2008). Red fluorescence in reef fish: a novel signalling mechanism? *BMC Ecol.* **8**, 16.
Migné, A., Spilmont, N. and Davoult, D. (2004). In situ measurements of benthic primary production during emersion: seasonal variations and annual production in the Bay of Somme (eastern English Channel, France). *Continental Shelf Res.* **24**, 1437-1449.
Morris, S. and Taylor, A. C. (1983). Diurnal and seasonal variation in physico-chemical conditions within intertidal rock pools. *Estuarine Coastal Shelf Sci.* **17**, 339-355.
Muscatine, L., Boyle, J. E. and Smith, D. C. (1974). Symbiosis of the Acoel Flatworm *Convoluta roscoffensis* with the Alga *Platymonas convolutae*. *Proc. R. Soc. Lond. B Biol. Sci.* **187**, 221-234.
Nilsson, D.-E. (2009). The evolution of eyes and visually guided behaviour. *Philos. Trans. R. Soc. B Biol. Sci.* **364**, 2833-2847.
Norris, R., Hori, T. and Chihara, M. (1980). Revision of the genus Tetraselmis (Class Prasinophyceae). *J. Plant. Res.* **93**, 317-339.
Parke, M. and Manton, I. (1967). The specific identity of the algal symbiont in *Convoluta roscoffensis*. *J. Mar. Biol. Assoc. UK* **47**, 445-464.
Pearse, V. B. (1974). Modification of sea anemone behavior by symbiotic zooxanthellae: phototaxis. *Biol. Bull.* **147**, 630-640.
Peers, G., Truong, T. B., Ostendorf, E., Busch, A., Elrad, D., Grossman, A. R., Hippler, M. and Niyogi, K. K. (2009). An ancient light-harvesting protein is critical for the regulation of algal photosynthesis. *Nature* **462**, 518-521.
Rodríguez-Román, and Iglesias-Prieto, R. (2005). Regulation of photochemical activity in cultured symbiotic dinoflagellates under nitrate limitation and deprivation. *Mar. Biol.* **146**, 1063-1073.
Rumpho, M. E., Worful, J. M., Lee, J., Kannan, K., Tyler, M. S., Bhattacharya, D., Moustafa, A. and Manhart, J. R. (2008). Horizontal gene transfer of the algal nuclear gene psbO to the photosynthetic sea slug *Elysia chlorotica*. *Proc. Natl. Acad. Sci. USA* **105**, 17867-17871.
Schröder, G. and Treiber, H. K. (2007). *Technische Optik*. Würzburg, Deutschland: Vogel Buchverlag.
Schwartz, J. A., Curtis, N. E. and Pierce, S. K. (2014). FISH labeling reveals a horizontally transferred algal (*Vaucheria litorea*) nuclear gene on a sea slug (Elysia chlorotica) chromosome. *Biol. Bull.* **227**, 300-312.
Serôdio, J., Cruz, S., Cartaxana, P. and Calado, R. (2014). Photophysiology of kleptoplasts: photosynthetic use of light by chloroplasts living in animal cells. *Philos. Trans. R. Soc. B Biol. Sci.* **369**, 20130242.
Serôdio, J., Silva, R., Ezequiel, J. and Calado, R. (2011). Photobiology of the symbiotic acoel flatworm *Symsagittifera roscoffensis*: algal symbiont photoacclimation and host photobehaviour. *J. Mar. Biol. Assoc. UK* **91**, 163-171.
Shcherbakov, D., Schill, R. O., Brümmer, F. and Blum, M. (2010). Movement behaviour and video tracking of *Milnesium tardigradum* Doyère, 1840 (Eutardigrada, Apochela). *Contrib. Zool.* **79**, 33-38.
Shcherbakov, D., Knörzer, A., Hilbig, R., Haas, U. and Blum, M. (2012). Near-infrared orientation of Mozambique tilapia *Oreochromis mossambicus*. *Zoology* **115**, 233-238.
Shcherbakov, D., Knörzer, A., Espenhahn, S., Hilbig, R., Haas, U. and Blum, M. (2013). Sensitivity differences in fish offer near-infrared vision as an adaptable evolutionary trait. *PLoS ONE* **8**, e64429.
Shichida, Y. and Matsuyama, T. (2009). Evolution of opsins and phototransduction. *Philos. Trans. R. Soc. B Biol. Sci.* **364**, 2881-2895.
Taylor, D. L. (1973). Algal symbionts of invertebrates. *Annu. Rev. Microbiol.* **27**, 171-188.
Trench, R. K. (1979). The cell biology of plant-animal symbiosis. *Annu. Rev. Plant Biol.* **30**, 485-531.
Trench, R. K. (1993). Microalgal-invertebrate symbioses: a review. *Endocytobiosis Cell Res.* **9**, 135-175.
Venn, A. A., Loram, J. E. and Douglas, A. E. (2008). Photosynthetic symbioses in animals. *J. Exp. Bot.* **59**, 1069-1080.
Wägele, H., Deusch, O., Händeler, K., Martin, R., Schmitt, V., Christa, G., Pinzger, B., Gould, S. B., Dagan, T., Klussmann-Kolb, A. et al. (2010). Transcriptomic evidence that longevity of acquired plastids in the photosynthetic slugs *Elysia timida* and *Plakobranchus ocellatus* does not entail lateral transfer of algal nuclear genes. *Mol. Biol. Evol.* **28**, 699-706.
Yamasu, T. (1991). Fine structure and function of ocelli and sagittocysts of acoel flatworms. *Hydrobiologia* **227**, 273-282.