Journal of Experimental Marine Biology and Ecology 460 (2014) 177–183

Contents lists available at ScienceDirect

Journal of Experimental Marine Biology and Ecology

journal homepage: www.elsevier.com/locate/jembe

Nutritional effects on the visual system of the rotifer *Brachionus plicatilis* sensu stricto (Rotifera: Monogononta)

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ARTICLE INFO

Article history:
Received 11 April 2014
Received in revised form 8 July 2014
Accepted 9 July 2014
Available online xxxx

Keywords:
Rotifera
Nutrient
Eyespot
Phototaxis
Population growth

ABSTRACT

Rotifers have a light sensor called "eyespot" which is expected to be composed of rhodopsin. Based on the molecular feature of rhodopsin as regenerated with 11-*cis*-retinal, we hypothesized that phototactic behavior should be affected by the nutritional level of food; especially vitamin A availability. This study intended to address the following questions on the nutritional effects of using baker's yeast (*Saccharomyces cerevisiae*) and *Nannochloropsis oculata*: how does diet affect the pigmented area and absorbance of the eyespot, and how do these changes characterize phototactic behavior and population growth in the monogonont rotifer *Brachionus plicatilis* sensu stricto. The pigmented area of the eyespot decreased to 14.7 μm^2 with baker's yeast while it was maintained at the initial size of 82.9 μm^2 with *N. oculata*. Maximum absorbance of the eyespot was observed at a range of 470 to 525 nm in the initial rotifers and it was not significantly changed with diet type and culture day. The value of the maximum absorbance was maintained with *N. oculata*, while it rapidly decreased on day 10 with baker's yeast. Stronger positive phototaxis with *N. oculata* was observed under lower light intensity (0.1 and 0.5 W m^{-2}) at 470 nm. On the other hand, phototaxis with baker's yeast became weak and no phototactic reactions were observed under the same lighting condition. From the genomic DNA database of rotifers, 12 putative opsin-relevant genes were identified. These results corroborate the hypothesis that rhodopsin is the visual pigment in the rotifer eyespot. Lack of vitamin A with baker's yeast should induce reduction of the pigmented area and the sensitivity of the rotifer eyespot resulting in weak phototaxis. The population growth of rotifers showed different patterns related to the food type and light intensity. The lowest population growth (0.33–0.37 day^{-1}) was shown with baker's yeast diet at 0.5 W m^{-2}. This phenomenon may be significantly related to malnutrition on baker's yeast which is deficient not only in vitamin A but also in fatty acids, vitamin B_{12} and its derivatives.

1. Introduction

In the wild, planktonic metazoans including rotifers exhibit diel vertical migration caused by light stimulation (Gerhardt et al., 2006; Jékely et al., 2008; Martynova and Gordeeva, 2010) and many other factors such as temperature, prey–predator relationship and surface current (Stich and Lampert, 1981, 1984; Hill, 1991). Among these factors, light is considered to be the main stimulus which would guide their positioning in the water column (Barcelo and Calkins, 1979). Monogonont rotifers belonging to the genus *Brachionus* possess a red light sensor called eyespot, and show phototactic and photokinetic reactions associated with the sensitivity of the eyespot toward wavelength and intensity (Clément et al., 1983; Cornillac et al., 1983).

Phototaxis of *Brachionus plicatilis* species complex is simultaneously affected by light wavelength and intensity (Kim et al., 2014). Wavelength induced strong positive phototaxis of rotifers was related to maximum absorbance of the pigmented area of the eyespot (Cornillac et al., 1983; Kim et al., 2014). Moreover, rotifer photokinetic movements were also influenced by the light wavelength and intensity (Clément et al., 1983) and these movement patterns were expected to affect their reproduction (Kim et al., 2014). The rotifer eyespot, cerebral eye consists of two types of pigment-bearing cells: one epithelial cell cup containing accessory pigment and one or more sensory neurons (sensory pigments) with membranous structure (Cornillac et al., 1983). These pigments i.e., accessory and sensory pigments have the following function: to perceive the direction of light and to elicit any responses, respectively (Clément, 1980; Clément et al., 1983; Cornillac et al., 1983). Due to the synergistic action of these two pigments, the rotifers show light wavelength and intensity-dependent phototaxis (Clément et al., 1983). Red pigments (accessory pigments, Clément, 1980) are expected to consist of rhodopsin similar to other invertebrates (Wolken, 1971;

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http://dx.doi.org/10.1016/j.jembe.2014.07.005
0022-0981/

Clément, 1980). Rhodopsin is composed of opsin protein covalently linked to 11-*cis*-retinal which is a derivate of vitamin A (Palczewski et al., 2000; Zhong et al., 2012).

Harris et al. (1977) reported the correlation between visual system performance and nutrient level of food as follows: deprivation of dietary vitamin A causes a reduction of visual sensitivity and photopigment concentration in both vertebrates and invertebrates. In this study the hypothesis that the nutritional value of diet affects the visual system of rotifers is investigated under controlled light conditions. The following questions associated with the nutritional level of two diets, baker's yeast (*Saccharomyces cerevisiae*) and *Nannochloropsis oculata*, are addressed: (1) how the diet affects the area and absorbance of the rotifer eyespot and (2) how changes in these parameters influence behavior (phototaxis) and population growth.

2. Materials and methods

2.1. Area and absorbance of eyespot

This study employed the monogonont rotifer *B. plicatilis* sensu stricto (Makishima strain) which does not undergo mixis (Hagiwara et al., 2007). The culture medium (22 practical salinity unit, psu) was made by diluting natural seawater with Milli-Q water (Millipore 0.22 μm) followed by GF/C filtration and sterilization (at 121 °C for 20 min). The rotifers were stock-cultured at 25 °C in total darkness with daily feeding of *N. oculata* at 7×10^6 cells mL^{-1}. *N. oculata* was cultured in modified Erd-Schreiber medium (Hagiwara et al., 1994) under continuous light with gentle aeration. Prior to feeding *N. oculata* were centrifuged at 3968 ×*g* for 10 min and re-suspended in the rotifer culture medium.

Rotifers for feeding trials were started from parthenogenetic eggs collected from amictic females of the stock culture. To obtain these eggs, we pipetted out 500 rotifers carrying female eggs and transferred them into a 30-mL screw-capped bottle containing 10-mL of the same saline water as the stock culture and then agitated them to shake off the eggs. Separated eggs were collected with a Pasteur pipette and incubated in a laboratory dish (90 mm ø) in a 40-mL stock medium (22 psu of saline water). Hatchlings (<3 h) from those eggs were inoculated into a 100 mL of glass flask containing 100 mL of 22 psu sterilized saline water at 1 ind mL^{-1} and cultured for 30 days in triplicates. The cultures were fed with two types of foods: *N. oculata* (at 7×10^6 cells mL^{-1}) and baker's yeast *S. cerevisiae* (Oriental Yeast Co., Ltd., Japan, at 2.5×10^6 cells mL^{-1}) every 12 h to provide the same dry weight of both foods. Weak aeration (at 10 mL min^{-1}) was provided only in rotifer cultures with baker's yeast to prevent precipitation and maintain dissolved oxygen concentration. Three rotifers from each culture were sampled every 10 days to determine the area and absorbance of the eyespot. The animals used for measuring the pigmented area with digital imaging software (Axio Vision Rel. 4.8, Zeiss) were fixed in formalin. The pigmented area calculation was based on an approximated elliptical shape corresponding to the length of the minor and major axes. Three other specimens from each culture were prepared for estimating the absorbance of the pigmented area. Prior to this procedure, each specimen was transferred onto a slide glass and then trapped under a cover glass without anesthesia. Light absorbance was measured using the microspectrophotometer system composed of spectrophotometer 308 PV (Craic Technologies, USA) and BX 61 (Olympus, Japan) compound microscope and calculated according to the equation:

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

I0: the light intensity of radiant energy striking the sample
I: the light intensity of energy emerging from the sample.

2.2. Phototaxis

On the last day of the 30-day culture period, the phototaxis of rotifers on different feeding regimes was investigated in 20 females that were randomly selected from the same cultures as described in the previous section of this study. Selected individuals were immediately inoculated into the middle compartment of the experimental container (15 × 3 × 3 cm) which was divided into three compartments by two sliding partitions (Fig. 1A). The container was constructed manually using reflective black plastic plank (0.3 mm of thickness); it contained 20 mL of the stock culture medium (22 psu) resulting <4 mm of water depth to limit vertical movements of rotifers. Inoculated rotifers experienced dark adaptation for 5 min and then were illuminated with two different light emitting diodes (LEDs: blue with a peak at 470 nm and red at 660 nm; IS-mini, CCS Inc., Japan) one at a time from the side for 15 min without partitions removed (Fig. 1B). The light intensity was adjusted to 0.1, 0.5 and 15.0 W m^{-2} using a light meter (LI-1400, LI-COR Inc., Japan). After irradiation, the partitions were replaced (Fig. 1C) and the number of rotifers in each compartment was counted under a stereomicroscope (SZX-ILLD2-100, Olympus, Japan). For the controls, these steps were performed in complete darkness followed by immediate replacement of the partitions under weak room light (<0.07 W m^{-2}). The same batch of rotifers under each feeding regime was used to investigate the wavelength (blue and red) effects on rotifer phototaxis related to nutrients. The mean value of five replicates was used to calculate rotifer distribution patterns.

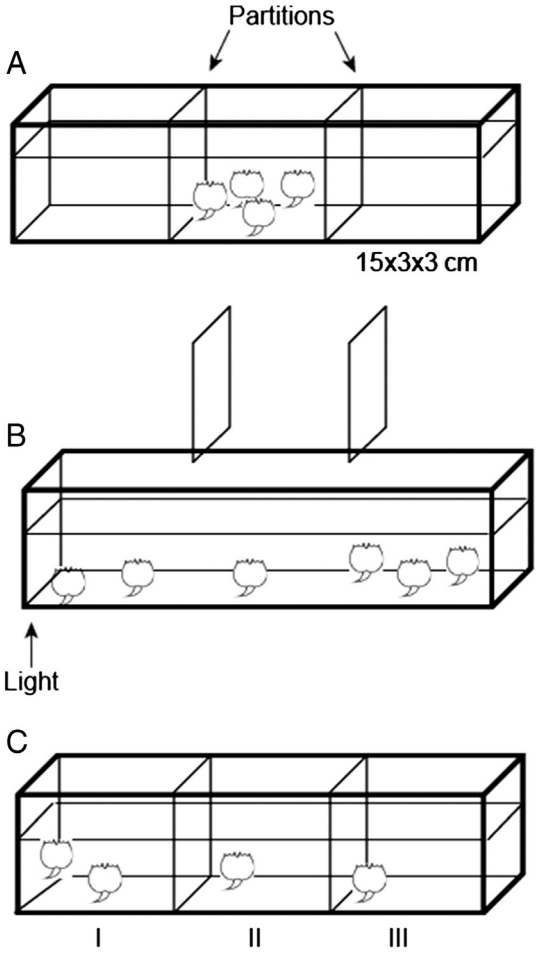

Fig. 1. Experimental procedure for the rotifer phototaxis. (A), dark adaptation for 5 min (B), light irradiation for 15 min without two partitions and (C), rotifer count after the replacement of those partitions.