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Occurrence of *Haematococcus pluvialis* Flotow emend. Wille in a small artificial pool on the university campus of the Collegium Biologicum in Poznań (Poland)

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Abstract: *Haematoccocus pluvialis* is a widely distributed unicellular green alga belonging to the order Volvocales and family Haematococcaceae. Its natural habitats include small, often temporary, continental and coastal rock pools, water holes and other small natural or artificial water habitats. It has been reported world-wide, but in Poland only a few cases of its occurrence have been noted in southern Poland. However, *H. pluvialis* was identified in a small artificial pool at Adam Mickiewicz University in Poznań during very hot and sunny days in July 2005. Because of these severe conditions only the resting hematocyst stage of this species was initially observed. This stage contained the red carotenoid pigment astaxanthin. Using confocal microscopy, large amounts of astaxanthin were observed in the central area of the resting cells. After transferring the hematocysts to culture media the flagellate macrozooid and palmella stages developed. The presence of *H. pluvialis* in this pool in the north-western part of Poland supports the capability of this species to expand its distribution range over long distances.

Key words: *Haematoccocus pluvialis*, astaxanthin, hematocyst

1. Introduction

Many of the unicellular chlorophytes are known to have a broad range of distribution. Their rapid reproduction rates, small cell size, and capability of forming resting stages when exposed to adverse conditions are factors that have aided in expanding their spatial distribution. However, many of these organisms are rarely noted, resulting in incomplete records of their geographical range. An example of this situation in Poland are records of the chlorophyte *Haematococcus pluvialis* Flotow emend. Wille. This alga which belongs to the order Volvocales and family Haematococcaceae, was reported in Poland in the late 19th century near Wadowice, Makowo (Gutwiński 1897), Kraków, Czarna (Gutwiński 1884), Jelenia Góra, Karkonosze regions (Cohn 1857, 1883), Tatra Mountains (Rostafiński 1881a, 1881b) and from Grodkowo near the Opole (Schroeder 1925). The absence of recent records of this taxa in phycological lists from other limnological investigations in Poland may be explained by the fact that when present it occurs in generally very low concentrations, and due to its multiple life stages is not easily identified.

The natural habitat for this species includes small inland and coastal rock pools, water holes and other small natural, or artificial water bodies world-wide (Kol 1925; Messikomer 1946; Skuja 1948, 1956; Pringsheim 1966; Kisselev 1969; Stein 1975; Wawrik 1980). These habitats are characterized by their unstable, often temporary conditions, that may include extreme differences in temperature, light intensity, and UV illumination, which are often accompanied by changes in water level.

2. Material and methods

Samples for phycological analysis were collected in mid-July from an artificial pool on the university campus. The pool is shallow (ca.1 m deep), with surface

Fig. 1. *Haematococcus pluvialis* in a small artificial pool on the university campus of the Collegium Biologicum in Poznań

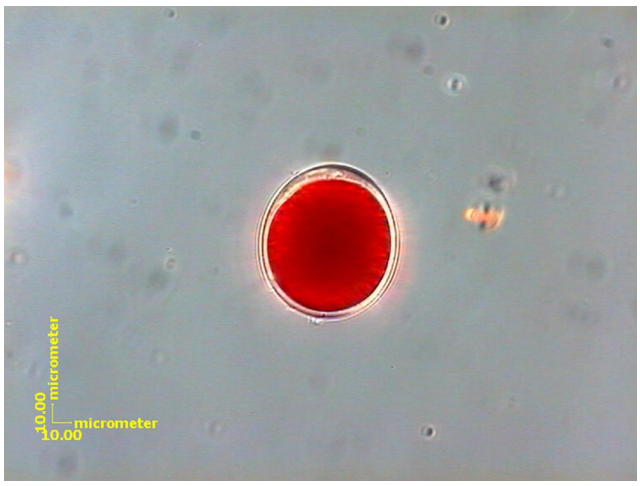

Fig. 2. Resting hematocyst stage of *Haematococcus pluvialis*

dimensions of 4 x 5 metres. Surface layers of algae attached to the walls of pool were removed and placed in a glass container. Species identification was determined using light microscopy (magnification 1000x) with Nomarskii contrast, according to Rothmaler et al. (1994). Photographs were taken and saved for documentation using Zeiss software KS 300. Pigment analysis and their distribution within the cells were examined using a conphocal microscope. The surface structure of the resting cell wall was determined using a scanning electron microscope. The resting stages of *H. pluvialis* were cultured using WC media and incubated under a 12:12 light and dark regime at 23°C.

3. Results

The noticeable red coloration within the algal cells that covered the walls of the pool indicated the developmental stage of *Haematoccocus pluvialis* (Fig. 1). Microscopic analysis showed that the resting hematocyst stage of *H. pluvialis* was responsible for the red coloration. These hematocysts had an oval shape, a thickened cell wall and no flagella (Fig. 2). It is during this stage that the cells hematocysts contain large amounts of astaxanthin.

The resting cells (hematocysts) were transferred into WC medium and incubated under stable light and temperature conditions in the laboratory. After two days flagellate forms (the macrozoids) were present in the cultures. They were green, possessed two flagella and were surrounded by a transparent gelatinous sheet (Fig. 3). In addition, the cultures contained the palmella stage of this taxon that consisted of green, spherical cells without flagella. This stage can produce other palmella cells or macrozooids, and under less stable conditions can produce hematocysts. Two weeks after this culture was established the hematocysts has developed for the second time.

Examination of these hematocysts with scanning electron microscope indicated their cell wall had a smooth, fine surface structure (Fig. 4). There were no ornamental or additional structures present.

Observations using the conphocal microscope showed the distribution of pigments within the cells. Chlorophyll *a* was localized near the cell wall and astaxanthin concentrated in the central area of the cell (Fig. 5). Movement of the macrozoids was recorded using the light microscope, with their swimming speed determined as 200 m/h.

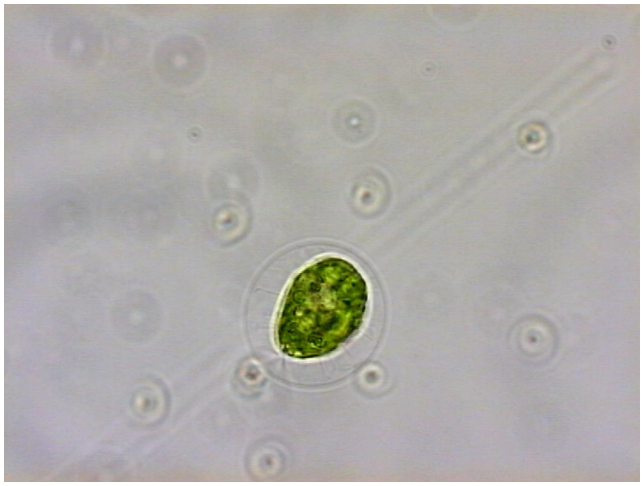

Fig. 3. Flagellate macrozoid stage of *Haematococcus pluvialis*

4. Discussion

The occurrence of *Hematoccocus pluvialis* in the pool in Poznań was a noticeable phenomena due to the alga's red coloration. The color was the consequence of high concentrations of the pigment astaxanthin in the hematocyst stage of this alga. It is a carotenoid that *H. pluvialis* is capable of concentrating in these resting cells during periods of stress. Most crustaceans, including

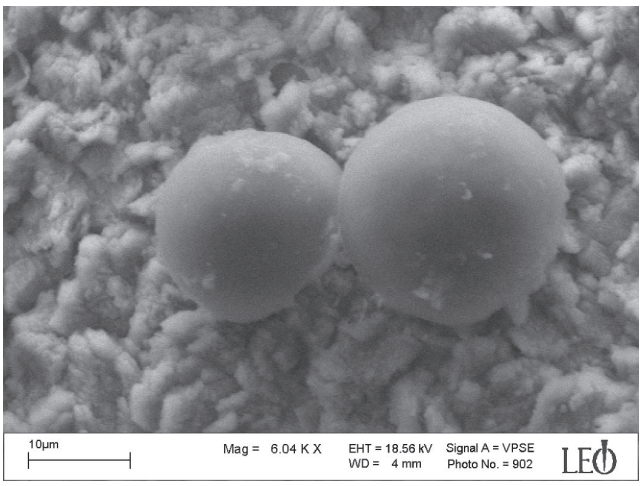

Fig. 4. Scanning electron microscopy photograph of hematocysts

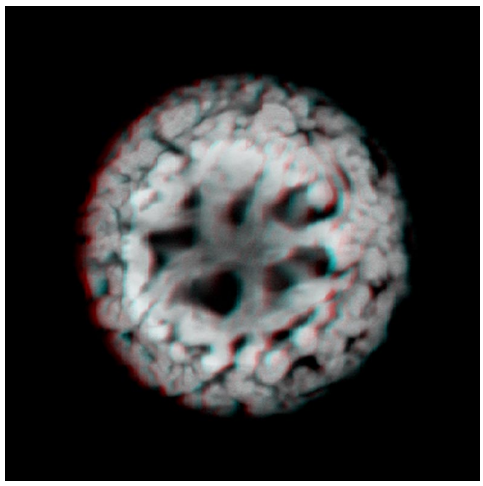

Fig. 5. Distribution of astaxanthin within a cell of *Hametaococcus pluvialis*

shrimp, crawfish, crabs and lobster, are tinted red by as result of accumulation of astaxanthin from their diet. Astaxanthin is also responsible for the pink colour of flesh of wild salmon. In commercial fish and crustacean farms, astaxanthin is commonly added to their diet in order to compensate for the lack of a natural food source of the pigment (Torrissen *et al.* 1989). Commercial production of astaxanthin is often derived from culturing *H. pluvialis* for its subsequent use in the food industry (McCoy 1999).

In nature, astaxanthin is produced principally by a few species of bacteria, microalgae, fungi and lichens (Johnson & Schroeder 1995; Armstrong 1997). Animals cannot synthesize carotenoids *de novo*, thus ultimately they must obtain these pigments from other sources in their food chains (Britton *et al.* 1995).

This pigment not only contributes to the pigmentation of certain terrestrial and aquatic organisms, but also to other body functioning activities (Torrissen & Christiansen 1995). Astaxanthin has been characterized as a strong antioxidant, which can help protect the retina from oxidative damage (Snodderly 1995). It has been suggested that astaxanthin may also serve as a growth enhancer and fertilization hormone for fish and crustaceans (Sigurgisladottir et al. 1994), and also function as a Vitamin A and B precursor in fish (Torrissen & Christiansen 1995). Recently it has been shown that astaxanthin may reduce cancer development in rats and mice (Tanaka *et al.* 1995).

The astaxanthin production by *H. pluvialis* protects these cells from ultraviolet exposure and oxidative stress. According to Di Mascio *et al.* (1991) astaxanthin was found to be twice as effective antioxidant as beta-carotene and about 80 times more effective than vitamin E. *H. pluvialis* is capable of accumulating the highest proportion of astaxanthin in relation to its dry weight compared to other microorganisms: 1.5 to 5.0% (Johnson & Schroeder 1995; Krishna & Mohanty 1998). The concentration of astaxanthin in the hematocysts of *H. pluvialis* occurring during severe environmental conditions, such as increased light intensity, supports the role of this pigment in preventing cellular damage from ultraviolet exposure. During less stressful conditions the hematocysts are transformed to the motile macrozooids. They can loose flagella as soon as conditions became more severe and transform into palmella cells. This stage can return to macrozooids stage when condition are improved or turn into hematocysts when conditions became harsh, as during nutrient depletion or high light intensity.

The site of occurrence of *H. pluvialis* in the small pond in Morasko is very distant from its previously reported locations in Poland, indicating the capability and potential for this alga to expand its spatial distribution. Further investigations throughout Poland may indicate a much wider distribution of this species.

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