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<u>NOTES</u>

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RESPONSE OF MNEMIOPSIS LEIDYI LARVAE TO LIGHT INTENSITY CHANGES

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A possible response to light of larvae of Black Sea ctenophore *Mnemiopsis leidyi* of two age groups (first to fourth day and one-two weeks after hatching) was experimentally studied. The larvae were placed in a Petri dish with seawater, in which two areas (light and dark) were created using a light source and a black opaque background. The number of larvae in each area was recorded for an hour after the start of the experiment. It was found that on average 77 % of the early stage larvae (first to fourth day) migrated to the dark area after an hour. We hypothesized that *Mnemiopsis leidyi* early stage larvae have negative phototaxis. Similar response of the older larvae (one-two weeks) was not recorded.

Keywords: Mnemiopsis leidyi, ctenophore, larvae, phototaxis, migration

Ctenophores are planktonic predatory gelatinous marine animals. The species *Mnemiopsis leidyi* belongs to the order Lobata. The animals of this order move using ctenes of cilia, which create water current. Synchronous movement of the cilia is coordinated by the nervous system [6]. The complex of the aboral organ is the main sensory structure of the animal; it controls movement and, possibly, is a light sensor.

The development of *Mnemiopsis leidyi* is direct. However, after hatching, the larva passes the cydippid planktonic stage (body length 0.3 cm (when hatching) to 3 cm); the larva has a pair of long branching tentacles and actively moves in a water column (Fig. 1). During development of *Mnemiopsis* larva, its body structure becomes similar to an adult sexually mature individual (3 to 15 cm): tentacles are reduced, and typical long lobes appear [4].

Modern taxonomists consider that Ctenophora are a "sister taxon" in relation to all other Metazoa; the development of their nervous and muscular systems is unique [3]. Identification of the presence of behavioral reactions to light intensity changes of ctenophore larvae is important for understanding the operation of these systems.



Fig. 1. *Mnemiopsis leidyi* larvae on the first day after hatching

Previously, Schnitzler *et al.* [5] performed a comprehensive analysis of the genes involved in the production and absorption of light in *Mnemiopsis leidyi* larvae; it was shown that the expression of opsin genes, which are involved in light perception, occurs in the apical sensory organ, namely, in nerve ciliary cells. A joint localization of the expression of photoprotein genes and two opsin genes in developing *Mnemiopsis leidyi* photocytes was also revealed. The authors suggested that there is a connection between bioluminescence and phototaxis in ctenophores, and photocytes probably not only emit, but also perceive light [5].

According to the results of the study [1], it was suggested that one of the factors stimulating *Mnemiopsis leidyi* to perform vertical migrations is the avoidance of bright light (over $10 \,\mu\text{E}\cdot\text{m}^{-2}\cdot\text{sec}^{-1}$). It was found that only small animals (up to 22 mm) perform migrations. The authors consider the animals swim to the upper layers following food objects only in the night, while in the daytime they swim down avoiding sunlight.

Information on laboratory experiments aimed at studying the response of ctenophores to the light has not been found in the literature.

The aim of this work is to study the effect of light intensity changes on the behavior of ctenophore larvae.

MATERIAL AND METHODS

Adult *Mnemiopsis leidyi* individuals were collected from the sea from IBSS mooring (Karantinnaya Bay, Sevastopol) in September 2019. The animals were brought to the laboratory, placed in 5-L containers with seawater (5–7 animals per container) and left overnight in a darkened room. In the morning, the appearance of eggs and larvae was observed. On the first day after hatching, the larvae were moved with a pipette into separate 0.5-L glass beakers. The experiments were carried out on larvae in the early stages of development (first to fourth day). They were kept in room temperature seawater (+22 °C); food objects were not introduced. For each subsequent experiment, we selected larvae, which were not involved in previous ones.

A small Petri dish (10 cm in diameter, 1 cm high) filled with filtered seawater was placed on the frosted glass above a light source – a LED element installed at a distance, at which the water in the dish did not heat up. A black opaque background was placed under the dish, so that one its half was dark-ened, and the other one remained completely illuminated (Fig. 2). An impenetrable plastic partition was installed at the light – dark border. Totally, 20 *Mnemiopsis* larvae were placed on the side of the illuminated area, and then the partition was removed. We observed larvae behavior for an hour after the start of the experiment and counted larvae number in dark and illuminated dish areas. The experiments were carried out within four days after larvae hatching, 5 replicates per day; in total, 20 experiments.

During the 110th cruise of RV "Professor Vodyanitsky" (4–20 October, 2019), in the Sea of Azov 1-cm to 2-cm larvae were sampled (the estimated larvae age is one to two weeks). The reactions to light were studied under the same experimental conditions in the laboratory of biodiversity and functional genomics of the World Ocean (hereinafter BiFGWO) onboard the vessel. Totally, 10 experiments were conducted.

RESULTS AND DISCUSSION

Observations of the behavior of early stage larvae within an hour after the start of the experiment showed that within the first 30 minutes, the larvae migrate throughout the entire dish volume, and after 1 hour about 77 % move into the dark area, avoiding bright light (Table 1).



Fig. 2. Scheme of the experiment.

1 - start of the experiment: the Petri dish with seawater is placed above the light source, a black opaque background is placed under one half of the dish, a partition is installed at the light – dark border, *Mnemiopsis* larvae are placed in the light area. 2 - exposure of larvae

in a dish without partition for one hour after the start of the experiment

Table 1. Percentage of larvae in the dark area of the Petri dish within one hour after the start of the experiments

| Age of larvae, days | n | Percentage of larvae in the dark area, % |
|------------------------|----|---|
| 1 | 5 | 85 |
| 2 | 5 | 64 |
| 3 | 5 | 76 |
| 4 | 5 | 83 |
| For all the time | 20 | 77 |

The response of early stage larvae to light intensity change does not manifest itself immediately, but over time. A rapid light intensity change triggers a cascade of internal processes of the animal, which takes a certain time. It is known that phototaxis mechanism of all eukaryotes includes three main stages [2]:

1) light absorption and primary reactions in photoreceptors;

2) transformation of stimuli and transmission of signals to the motor apparatus;

3) change in movement.

Thus, it can be assumed that *Mnemiopsis leidyi* larvae in the early stages of development have negative phototaxis.

For older larvae, it was not possible to reliably establish the presence of a response to light. In some experiments, almost all the larvae migrated to the dark area of the dish within an hour; in others, they occupied the entire dish volume and did not move even two hours after the start of the experiment.

Due to the fact that *Mnemiopsis leidyi* larvae perform vertical migrations in water column, the larger larvae may not have enough water in the Petri dish to move freely. To identify their possible response to light intensity changes, other experimental conditions are necessary.

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РЕАКЦИЯ ЛИЧИНОК MNEMIOPSIS LEIDYI НА ИЗМЕНЕНИЕ ОСВЕЩЁННОСТИ

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Экспериментально изучены возможные реакции на свет личинок черноморского гребневика *Mnemiopsis leidyi* двух возрастных групп (первые-четвёртые сутки и одна-две недели после вылупления). Личинок помещали в ёмкость с морской водой, в которой с помощью источника света и непрозрачного фона создавали две световые зоны (свет и тень); количество личинок регистрировали в каждой зоне в течение часа после начала эксперимента. Показано, что в среднем 77 % личинок, находящихся на ранних стадиях развития (первые-четвёртые сутки), через час мигрируют в тёмную область. Высказано предположение о наличии отрицательного фототаксиса у ранних личинок *Mnemiopsis leidyi*. Подобные реакции у более взрослых личинок (одна-две недели) не обнаружены.

Ключевые слова: *Mnemiopsis leidyi*, гребневик, личинки, фототаксис, миграции