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THE METHODS OF THE CULTIVATION OF FRESHWATER BRYOZOANS (BRYOZOA, PHYLACTOLAEMATA)

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The methods of the field collection and the termination of the diapause and dormant stage in the statoblasts of Phylactolaemata are described. The conditions ensuring the viability of statoblasts for several years and their germination are described as well. A method of the prolong maintenance of Phylactolaemata in laboratory is proposed.

The success of the bryzoological studies depends to a great extend on the methods of Bryozoa cultivation. Research of some aspects of phylactolema biology is impossible in natural environment. Moreover, phylactolemas have been used recently as test-objects while determining the contents of chemicals and heavy metals in water reservoirs and this demands their laboratory cultivation for similar tests. In addition, our methods will make a great contribution to the study of these rather extraordinary colonial animals.

At present the organs of asexual bryozoan reproduction, or statoblasts, are regarded as specialized structures which have been formed in the process of adaptiogenesis to extreme environmental conditions. Statoblasts are cryptyobiotic patterns and their dormacy mechanism physiologically is similar to that of cysts, eggs and larvae of other invertebrates. Phylactolema statoblasts have two types of dormacy: the true diapause, or dormacy, and facultative dormacy (Oda, 1959). Statoblast in diapause will germinate no sooner than after one month dormacy even under favourable conditions. Statoblasts in dormant state do germinate under neccessary conditions.

Basing on numerous literature data on adaptiogenesis of phylactolema's reproductive structures under the impact of various ecological factors (Bergin et al., Brown, 1933; Bushnell, Rao, 1974; Mukai, 1974; Oda, 1959, 1966, 1972, 1974, 1976, 1979, 1980; Rogick, 1941), the optimal conditions for prolong maintenance of reproductive material of phylactolemas were chosen and a regime for termination of

diapause and dormacy was worked out. As a result, the methods of cultivation of animals under optimal temperature, light and feeding conditions have been achieved.

Bryozoans *P. fungosa* were collected in autumn from the cooling reservoir of the Bereza Power Plant. For mass collection of statoblasts we used large tanks (for example, polyethylene bath), then collected dead colonies were rubbed through hands in order to release reproductive structures of Bryozoa. Drifting in the upper layer, statoblasts were concentrated along bath perimeter, so they were collected with a pellet and put into weighing cups.

After collection statoblasts need to go through diapause, so they should be dried on filtering paper and kept in a freezer in weighing cup without water. One-month maintenance of statoblasts in a dried and frozen state ensures full diapause for them. Then they should be removed from a freezer and kept at the temperature of 2-5°C. From this you can get a laboratory culture of bryozoans at all seasons of the year as soon as optimal light, temperature and feeding conditions are provided. Statoblasts' dormacy state terminates under the influence of a signal factor, namely photoperiod.

Statoblasts were dispersed by brush over the slides. Several slides were put into each Petry cup. To fix them on the slide, statoblasts were kept dry for some time, then were put into previously settled water and kept in thermostat at 25°C. After exposure to light of 150 wt in the photoperiod regime of 8 L(light) : 16 D(darkness), statoblasts germinated in 3-4 days. Then you can save at least one germinated statoblast per slide for further research. Fresh, properly kept statoblasts have 90%-germination ability which decreases with aging of the material. Thus, material obtained can be used for germination tests during several years, if constantly stored in a fridge.

The slides with germinated ststoblasts and first zooids were removed from Perty cups and put into 800 ml glasses. Bryozoans can be fed on blue-greed algae Chlorella (low concentration) or Infusoria culture, or natural seston, if it is field study. For successful cultivation of bryozoans in laboratory conditions, it is very important to change water and food every day.

During the process of *P. Fungosa* cultivation we came upon the problem of rotifers which settled in the bryozoan colonies. Their eggs have been probably brought into the culture together with statoblasts from the cooling reservoirs. The rotifers *Philodina sp., Colurella sp., Rotaria sp., Leparella sp., Mytilina sp., Lecane sp., Habrotrocha bidens* settled near zooid lophophore. They could kill the colony very soon because of their high reproduction rate. To avoid this, rotifer eggs were blown away by microdropper several times a day.

The slides with germinated zooids have to be fixed in the glasses in the inclined position so as to avoid self-contamination by feces with growth and to keep colony at a lower part of glass surface. The optimal growth temperature of thermostat is 25-27°C. Under all abovementioned conditions during 35 days we were receiving colonies which had germinated from one statoblast and had up to 400 zooids (photo). That was the maximal density achieved in 800 ml volume because of possible further density press impact. In large volumes you can probably get larger mass colonies. With growing density press in zooids in the volumes used, the conditions for somatic colony growth changed for unfavorable and the colonies responded by producing reproductive structures: floatoblasts and sessoblasts, up to 1000 and 40 specimens, respectively.

Study of astogenesis and reproductive somatic relatioships in colonies proves to be possible only during laboratory cultivation process. If phylactolema zooid

ontogenesis is more or less studied, then the study of physiological stages of colony development and their definitions has only begun (Mikhaevich, 1994; Mikhaevich, 1994, 1994a, 1994b). The advantage of laboratory cultivation of Phylactolaemata is that you can investigate various levels of colony development with its growth from zooid ontogenesis to colony astogenesis.

If we observe the abovementioned conditions for termination of Phylactolaemata statoblast diapause and dormacy states and for maintenance of the regime favorable for their viability and germination, that will ensure prolong maintenance of bryozoans in laboratory.

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