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Laboratory propagation of freshwater cyclopoid copepods as an intermediate host for parasitic helminths

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ABSTRACT

Among the freshwater cyclopoids, small and medium-sized species can be propagated in the laboratory with a flagellate *Chilomonas* as the food. By contrast, large species need to be given, in addition to the flagellate, a larger food organism. For this, although a metazoan food is preferable, a ciliate *Stentor* may in part serve as a substitute. Methods for infection experiment involving copepods are given, and photographs of some parasite larvae in the copepod host are shown. Usage of carbonated water is helpful to immobilize cyclopoids and thus recommended for infection experiments.

1. Background

Freshwater planktons belonging to the Family Cyclopidae (Crustacea: Copepoda: Cyclopoida) form an important group as the intermediate host for some parasites, e.g. parasites belonging to Diphyllobothriidae, Dracunculidae, and Gnathostomidae. Reproducing the life cycles of these parasites, either totally or partially, in the laboratory needs infection experiment involving copepods. For this, it would be of great help to establish the method for keeping and propagating the copepods in the laboratory.

2. Objective

Mueller [1] tried to reproduce the life cycle of *Spirometra mansonoides* in the laboratory. Except for this contribution, however, information about the methods involving cyclopoid copepod as the intermediate host has been fragmentary. This manuscript summarizes the author's method of keeping and propagating cyclopoid copepods in the laboratory, and the method of infecting them with some parasites. A portion of this work has been published previously in Japanese [2].

Fig. 1 shows the life cycle of a cyclopoid copepod *Eucyclops serrulatus*. After a female produces clusters of eggs, the larvae called nauplii (*sing.* nauplius) hatch from the eggs. The larva grows through repeated ecdyses into the copepodite stage, which has an elongated posterior part. The adults attained after further ecdyses copulate, and then the females have eggs.

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The whole body length, i.e. the length between the anterior end of the head and the posterior terminus of the furca not including the antennae or setae, is about 1 mm, and the length of the metasome (cephalothorax), i.e. that of the body excluding the tail (abdominal) region, is about 600 μm, in the egg-bearing adult of *E. serrulatus* (Fig. 1). This species is regarded as "medium-sized" in this manuscript. Those species larger than or smaller than this are marked as "large" or "small" respectively. For example, *Cyclops vicinus* and *Macrocyclops albidus* belong to the "large" category, whereas *Tropocyclops prasinus* may be an example of the "small" category. A large species *Mesocyclops woutersi* appears later in this manuscript.

3. Method of culturing freshwater cyclopoids

The following glassware and other items were used.

Petri dish L: 12.0 cm in diameter, and 2.5 cm deep.
Petri dish S: 5.8 cm in diameter and 1.9 cm deep.
Petri dish SS: 3.5 cm in diameter and 1.5 cm deep.
Container H: a container similar to a Petri dish but deeper: 8.8 cm in diameter and 5.9 cm in depth.
Erlenmeyer flask: 200 ml capacity.
Pre-boiled wheat grains: wheat grains, put in boiling water for 30 s shortly before use.
Water: tap water that has been kept in a bottle for more than one day. Otherwise, commercially available drinking water was used. Wheat infusion (see Section 3.1.5) is also suitable as the culture medium for copepods.

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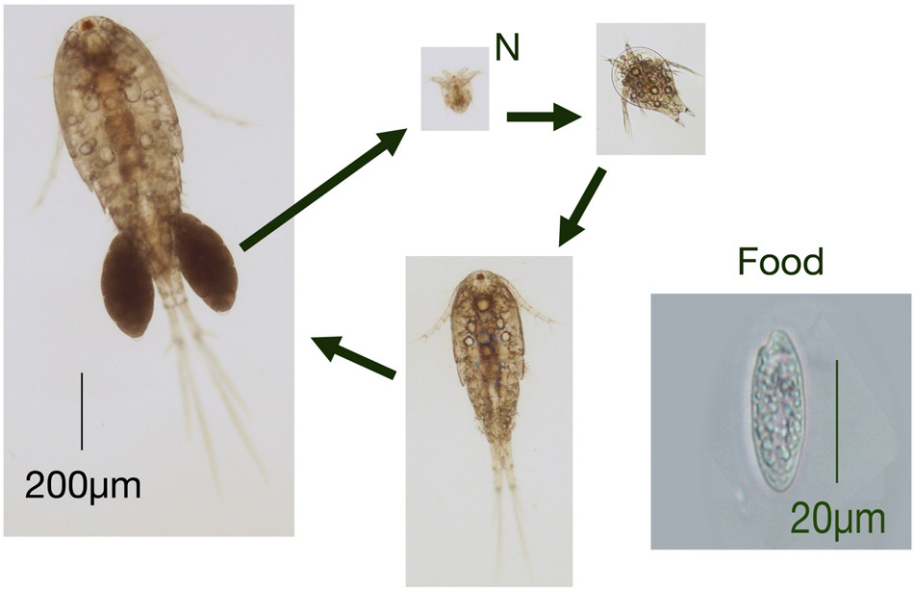

Fig. 1. Life cycle of *Eucyclops serrulatus*. The egg-bearing organism has a pair of egg sacs. The nauplius larva (N) grows through repeated ecdyses into the adult. The food organism *Chilomonas paramecium* is also shown.

The culturing method consists essentially of determining suitable food organisms. Small and medium-sized copepoid species can be cultured by giving a single species of protozoa as the food organisms. On the other hand, it has been postulated that large species need, in addition to the protozoa, a larger food organism to complete the whole life cycle [3]. The method of culturing, therefore, depends on the sizes of that copepod species.

3.1. Culturing small and medium-sized copepods

A method for culturing *E. serrulatus* is described. Cyclopoid copepods of the size similar to or smaller than this species can be cultured in a similar way.

Features characterizing *E. serrulatus* include proportionally long furca, that have rows of spines along their outer edges. See Fig. 1 for the size and other information about this species.

A personal communication from Prof. H. Ueda, a copepod specialist, tells that the so-called *E. serrulatus* has now been divided into six species. In this manuscript, where species identification totally depends on classical taxonomy, more than one species might be involved in the name *E. serrulatus*. Differences among strains have not been detected so far, either for required culture conditions or food organisms.

3.1.1. Methods for collecting cyclopoid copepod in the wild

Approximately 100 ml of water samples are taken from a pond or a river. In the latter case, choose the places where water is not flowing. Water from near the bottom, rocks, stones, or plants is preferable.

a. A hard, transparent, plastic tube of 1 cm outer diameter and about 1 m long may be used. One end of the tube is closed with a finger, and the other end is located at the desired place in the water. When the finger is loosened, the water comes into the tube. Then close the tube again with the finger, and the water in the tube is transferred to the 100-ml plastic bottle. This is repeated until the bottle is full.
b. The collected water samples are transferred into Petri dish L in the laboratory.
c. Observe the water in the Petri dish L under stereomicroscope. If egg-bearing copepods are found, proceed to the step described in Section 3.1.3.
d. If copepods are present but not egg-bearing ones, a pre-boiled wheat grain is put in the water. Then, egg-bearing organisms may appear in a couple of days.

3.1.2. Obtaining food organisms

A flagellate *Chilomonas paramecium* is used as the food organisms (Fig. 1). This is a species with two, unequal anterior flagella (not seen in Fig. 1). *Chilomonas* is morphologically very close to *Cryptomonas*, but does not have the plastid. It grows rapidly feeding on bacteria if the nutrient source for the latter is available.

Chilomonas is obtained in the following manner

a. Collect 100 ml water samples and put them to Petri dish L, in the same manner as described in Section 3.1.1 a.
b. Put 1–2 grains of pre-boiled wheat in the Petri dish.
c. Leave for 3–7 days, then observe under stereomicroscope. *Chilomonas*, if present, proliferates, forming a cloud-like accumulation around the wheat grain.
d. A part of the *Chilomonas* cloud is taken with a Pasteur pipette and put into a Petri dish S containing 20 ml water and a pre-boiled wheat grain.
e. Several days later, when the *Chilomonas* population has grown in this dish, a part of this new population is transferred to still another Petri dish in the same way as described in d. By repeating this procedure, the *Chilomonas* population is maintained as purely as possible.
f. After the *Chilomonas* culture is pure enough, the culture is transferred to a 200 ml Erlenmeyer flask containing 150 ml water and 2–3 pre-boiled wheat grains. The flask is capped with a small piece of aluminum foil and left at room temperature. Afterwards, the *Chilomonas* strain is maintained by transferring the culture to a new flask every 3–4 days.

3.1.3. Establishing a copepod strain

a. If an egg-bearing copepod is found in the water from the wild, it should be isolated. Copepods are strong swimmers that can escape from the sucking power of pipette. In place of the Pasteur pipette, a large Komagome pipette is used. Otherwise, a glass tube of ca. 15 cm long, with the bulb adopted on one end, may be used. At this step, it is tolerable if not only the intended copepod but also other organisms and debris are contained.