

# Culture of *Cyclops* for Use the First Intermediate Host in Experimental life Cycle of *Spirometra* Species

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## Abstract

**Background:** In natural conditions *Cyclops* are the first intermediate hosts in the life cycle of *Spirometra* species. In this paper we describe simple method of culturing Copepod of the genus *Cyclops* for use the first intermediate host in experimental life cycle of *Spirometra* species.

**Methods:** *Paramecium* was first cultured to be used as food for *Cyclops*. Sample of water was collected from a pond within Sokoine University. About 100 ml of water and pre-boiled wheat grains were transferred in a Petri dish and kept under laboratory conditions for 7 days, a swarm of *Paramecium* was formed. An adult female egg sacked *Cyclops* from a natural water pond in Tarangire National Park, Tanzania was added in a new Petri dish containing tap water, 0.3 ml of *Paramecium* suspension and 4 pre-boiled wheat grains. The mixture was kept under laboratory conditions temperature 26-29°C and observed daily.

**Results:** Eggs from the single *Cyclops* hatched to nauplius. The average time of developing to nauplius I was 1.2 days, nauplius I to copepodite I was 6.9 days, and copepodite I to adult female *Cyclops* was 26.3 days. The average measurements of nauplius I were 120.2µm length and 80.0µm width while the adult female was 846.3µm length and 284.6µm width. The adult female produced 1 to 8 broods (mean 4.3). The life span of *Cyclops* averaged 43.1 days.

**Conclusion:** The *Cyclops* cultured in the laboratory were fed *Paramecium* and used as first intermediate host in the experimental life cycle of Tanzanian *Spirometra* species.

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## Introduction

*Cyclops* are free living forms found everywhere there is water, in damp leaf litter on the ground, ponds, ditches of stagnant water, streams and rivers. They feed on plankton and other small aquatic organisms. The life cycle is adapted to their natural habitat in ponds and other accumulations of stagnant water. Fresh water copepods may act as a biological control for malaria by consuming mosquito larvae.[2] They also serve as intermediate hosts of many animal parasites and parasites of humans. Copepods are worldwide distributed. In Africa, freshwater copepods have been described in Mali by [14]. In the life cycle of *Spirometra* species copepod of the genus *Cyclops* is the first intermediate host. For experimental work in the laboratory, *Cyclops* can be cultured by using different methods. [3] cultured copepod *Eucyclops serrulatus* using *Chilomonas paramecium*, wheat infusion and pre-boiled wheat grain as food. The adult female egg sacked *Cyclops* was used. In order *Cyclops* to survive under laboratory conditions the adult *Cyclops* and nauplius have to be fed *Paramecium* 1970; [5, 6, 11]. In Tanzania there is no records showing a study on culture of *Cyclops*. In the present study, culture of *Cyclops* started with an adult female single egg sac bearing which was fed *Paramecium* as food. Therefore, this paper reports how *Cyclops* were cultured in the Tanzanian laboratory from an adult female egg sacked *Cyclops* fed with live *Paramecium* and stock of *Cyclops* used in the experiment of life cycle of *Spirometra* species.

## Materials and Methods

### Culture of *Paramecium*

Sample of water was collected from a pond located within Sokoine University campus. About 100 ml was transferred in a Petri dish, 12 cm in diameter and 2.5 cm high, added 4 pre-boiled wheat grains. The Petri dish with contents was kept under laboratory conditions, temperature 26-29°C and observed daily. On day 7, there was a swarm of *Paramecium* around wheat grains. About 0.3 ml of the swarm was taken with a Pasteur pipette and transferred to another Petri dish of the same size containing tap water and 4 pre-boiled wheat grains. In this container *Paramecium* formed a swarm. Again same amount of swarm suspension was transferred to

another Petri dish. In order to obtain pure culture of *Paramecium*, the procedure was repeated four times.

### Culture of *Cyclops*

An adult female egg sacked *Cyclops* collected from natural pond in Tarangire National Park, Tanzania was cultured in the laboratory of Sokoine University. The egg sacked female *Cyclops* (Fig.1) was picked with a Pasteur pipette, transferred in a Petri dish 6 cm diameter and 2 cm high, containing tap water 50 ml, cultured *Paramecium* suspension 0.5 ml, 2 grains of pre-boiled wheat grains and trace amount of calcium carbonate powder. On day 3, the eggs hatched to nauplius. A total of six Petri dishes were used for culture, in each six *Cyclops* were transferred. *Cyclops* were maintained in the laboratory at temperature 26-29°C and observed daily under microscope until developed to adult stage. The new adult females developed egg sacs. They were observed until hatching, the time of developing egg sacs to hatching nauplii was recorded. The nauplii were separated into groups each with six, placed in Petri dishes with food contents and observed daily. The time from hatching to death was recorded as longevity. Number of days taken to reach each stage was recorded, and the cluster size was determined. Some gravid females were transferred to new Petri dishes and anaesthetized with carbonated water. They were picked and placed on a slide. Egg sac was teased with a needle to release eggs. The eggs were counted under microscope. Tea strainer sieve was used to separate the adult female *Cyclops* from hatched nauplii according to [12]. Nauplii from 3 Petri dishes were killed by using 5% formalin and counted under microscope (Fig.2). Nauplii in the remaining 3 Petri dishes were allowed to develop to adult stage. The adult female egg sacked *Cyclops* appeared on day 12. The culture was transferred to another Petri dishes where multiplied and released nauplii. The procedure was repeated four times, the final culture of *Cyclops* was used for the experiment (Fig.3). Part of the *Cyclops* culture was maintained by feeding *Paramecium* for 1 year.

## Results

After 7 days of culture there was a swarm of *Paramecium* around pre-boiled wheat grains in Petri dish.

Growth of *Cyclops* cultured in Petri dishes had



Figure 1. Egg sacked *Cyclops* species used in this study.



Figure 2. Nauplius hatched from egg sacked *Cyclops*.



Figure 3. Adult *Cyclops* developed from a nauplius.

an average time of, egg sac to nauplius I was 1.2 days, while from nauplius I to copepodite I was 6.9 days. The time taken from nauplius I to adult female egg sac bearing was 26.3 days. The average measurements of nauplius I were 120.2  $\mu\text{m}$  in length and 80.0  $\mu\text{m}$  in width while the adult female measurements were 846.3  $\mu\text{m}$  in length and 284.6  $\mu\text{m}$  in width. The number of broods produced by an adult female was 1 to 8 (mean, 4.3) and the life span of *Cyclops* in this experiment was 43.1 days.

### Discussion

In the present study, the results shows that *Cyclops* were successfully cultured in the laboratory using *Paramecium* as food. *Paramecium* alone supported the whole life cycle of *Cyclops*. In previous studies copepods were maintained in the laboratory by using different methods. [7] cultured copepods in the laboratory which were used in the experiment of life-cycle of *Spirometra mansonoides*. *Cyclops* were fed microorganisms (molds, bacteria, euglenoid and small ciliates) that developed in hay infusion. [9] cultured copepod by feeding *Chlorella* to nauplii and early copepodite stages, zooplankton containing rotifers and paramecia were fed to copepodite and adults. [4] cultured *Cyclops abyssorum* by feeding *Euglena gracilis* and *Artemia*. [10] cultured *Cyclops visinus* by feeding pure algal diet (*Chlamydomonas reinhardtii* or *Cryptomonas* sp.). [1] reported that *Cyclops* require not only green algae but also large organisms such as ciliates and rotifers as food to complete the whole life cycle. The present study agrees with previous studies that *Paramecium* can be used as a source of food for *Cyclops*. In this study, the optimal temperature of the culture of the *Cyclops* was 26° to 29°C. Many studies have been done in temperate regions where the temperature range was 10° to 20°C. The difference in temperature has not affected the development of *Cyclops*. In the present study, the time for development was shorter than the time in lower temperatures. This agrees with the study done by [5] who reported that the time for development of *Cyclops* at lower temperature is longer than at higher temperature. [8, 13] reported the average period from egg to egg bearing adult female was 29.4 and 30.7 days respectively. These results could be due to the effect of

temperature.

### Conclusion

Laboratory maintenance of *Cyclops* can be achieved by using *Paramecium* as the source of food. The method is simple because the food organism *Paramecium* proliferates in the *Cyclops* culture and supports a rapid growth and reproduction of the *Cyclops*. *Cyclops* is maintained with minimum care because a stable and equilibrium state of micro-ecosystem is established in the Petri dish. This culture method can be used in the study of *Spirometra* life-cycle in which *Cyclops* serve as the first intermediate host. However, another study is needed for the identification of *Cyclops* which exist in Tanzania.

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