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Development and Reproduction of *Eucyclops serrulatus* (Copepoda: Cyclopoida) in the Laboratory Culture

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실험실에서 배양한 *Eucyclops serrulatus* (검물벼룩목 요각류)의 생식과 성장에 관한 연구

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실험실에서 배양을 하기 위해 담수에서 광범위하게 서식하는 4종류의 검물벼룩목 요각류를 실험하였다. 이 중 *Eucyclops serrulatus*만이 성공적으로 배양되었다. 다음으로 9가지 먹이 조건 중 어떤 것에서 유생발달이 정상적으로 이루어지는 지를 살펴보았다. 이렇게 선정된 실험실 배양 환경하에서 *E. serrulatus*의 생식과 성장을 관찰하였다. *E. serrulatus*는 상대적으로 짧은 세대간격을 가지고 많은 수의 자손을 낳는 것으로 밝혀졌다. 배양 방법이 간단하여 짧은 시간 동안 많은 순수 배양된 개체들을 얻을 수 있다는 점으로 볼 때 *Eucyclops serrulatus*는 담수 생태계의 오염을 측정하는데 bioindicator 생물로서 응용 가능성이 크다고 할 수 있겠다.

Key words : *Eucyclops serrulatus*, Laboratory culture, Test animal, Development, Reproduction

INTRODUCTION

Copepods have many ecological importances. Calanoid and cyclopoid copepods form the first vital link in the food chain that leads from the minute algal cells of the phytoplankton up to the large fish and mammals. These phytoplankton-feeding copepods are by far the most important primary consumers in marine planktonic communities and, as such, form the base of virtually all pelagic food chains. Also, copepods are plenty in freshwater planktonic communities. Members of the families Cyclopoidae in the Cyclopoida, Canthocamptidae in the Harpacticoida, and Diaptomidae in the Calanoida are particularly successful in all kinds of freshwater habitats (Huys and Boxshall, 1991).

Cyclopoids are especially by far the most abundant and successful group of copepods in the freshwater. The family Cyclopoidae contains an enormous number of genera and species from all kinds of freshwater habitats including standing water bodies such as lakes, ponds, ditches, temporary pools and wells, and running water such as streams and rivers. The other free-living cyclopoids are primarily marine in distribution; the family Cyclopoidae being predominantly benthic and the Oithonidae planktonic (Kim and Chang, 1989; Huys and Boxshall, 1991).

Because of the economic value of copepods, their important ecological status, and broad applications in ecotoxicology (Hoffman *et al.*, 1995), an aquaculture of copepods is generally conducted and have developed greatly in Europe and the US. Although the laboratory culture of these species is the basic step to the study of their ecology, there is no information especially on the culture of freshwater copepods. The problems of environmental pollution make us develop the culture method of endemic species for evaluating our environment. The biology of test species sho-

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uld be known first if they are to be used as test species. So laboratory culture is needed to develop the test species.

Purposes of this study was threefold: (a) To screen appropriate species for laboratory culture among four species in family Cyclopoidae; (b) After screening, to determine a method for pure culture of the selected animal; (c) To investigate its development and reproductivity in the laboratory culture.

MATERIALS AND METHODS

1. Culturing and routine techniques

Copepods were sampled from the pond and the dam in Seoul National University, Seoul, Korea (37.27° N, 126.57° E). For collecting egg-sacs, copepods were anaesthetized with carbonated water and eggs-sacs were removed with a needle. Egg-sacs of 10 individuals were transferred to 50 ml beaker with M4 media, the media for Daphnia continuous culture. After hatching, larvae were transferred to 50 ml beakers containing M4 media. The animals were maintained at 20°C, 16L : 8D, and 10,000 Lux. They were fed in excess of daily requirements (Vijerberg, 1989). They were observed every six hours before copepodid stage and every 12 hours from copepodid stage. Among cultured copepods, only one gravid female which moved actively was chosen for continuous pure culture.

2. Experiments

Experiment I : Screening proper species to a laboratory culture

Four species in family Cyclopidae; *Macrocyclops fuscus, Macrocyclops albidus, Tropocyclops prasinus,* and *Eucyclops serrulatus* were tested for culture in the laboratory condition. After the selection of the proper species, an active one of this species was picked and cultured continuously until the number of progeny was enough for the next experiment.

Experiment II : Suitability of various diets for the development of *E. serrulatus*' nauplii

The experimental method of Amarasinghe (1997) was taken to determine suitability of diets for the development of nauplii. From continuous pure culture of *E. serrulatus*, adult females carrying egg sacs were kept and eggs were taken from them and distributed in 100 ml glass beakers (~50 eggs/beaker). The beakers were filled with M4 media and contained one diet per beaker at excess concentration of daily requirements with 5×10^5 cells ml^{-1} (Vijerberg, 1989). Three replicates were carried out for each diet. The food regime is shown in Table 1. After two days, the contents of the beakers were filtered on a 50 μm sieve and rinsed in a Petri dish. Remaining eggs were removed and the hatched nauplii were pipetted into beakers containing fresh food suspension. Every other day, the animals were checked under a microscope and pipetted into beakers with new food suspension. Dead animals were removed. The experiments ran until all animals were dead or grown to adults.

Table 1. The food regime.

Single species
Chlorophyceae flagellates *Chlamydomonas reinhardii*
Chlorophyceae non-flagellates *Scenedesmus falcatus* *Selenastrum capricornum* *Chlorella* sp.
Heterotropic flagellates *Euglena gracilis*
Protozoan ciliates *Paramecium aurelia*
Combination of two species
Chlamydomonas reinhardii + *Paramecium aurelia*
Euglena gracilis + *Paramecium aurelia*
Chlamydomonas reinhardii + *Euglena gracilis*

Experiment III : The development and reproduction of *E. serrulatus* in the laboratory culture

Developmental times were determined by monitoring the extrusion of egg sacs and the time taken for hatching. Newly hatched nauplii were isolated into groups of five to ten animals in beakers and observed every six hours. Food was added daily and media were changed daily. The period from hatching to death was determined as longevity. The number of days required to reach each stage was calculated.

Newly moulted adult males and females were kept together in 50 ml beakers containing M4 media with algal food and were observed daily. To determine the clutch size, several gravid females were removed and anesthetized with car-

bonated water. Eggs were removed with a needle, and counted.

Newly moulted females were isolated as they became gravid and kept singly in nylon mesh sieves in 50 ml beakers. The nylon mesh sieve allowed separation of the female from newly hatched nauplii (Vijerberg, 1989). When the eggs hatched, the females were transferred to other beakers containing fresh media. Every six hours copepods were checked to ascertain the time of their egg release. The culture media were refreshed every day. The copepod nauplii hatched from the released eggs were fixed by adding a drop of 5% formalin before they were counted under a microscope. Egg appearance and hatching were recorded. Embryonic development time was determined as the period between egg appearance and hatching. Fecundity was the mean number of nauplii produced in each clutch.

Fifteen to thirty gravid females were isolated and kept singly in nylon mesh sieves in 50 ml beakers. Every female that liberated a clutch of nauplii was transferred to another 50 ml beaker. Interclutch period was determined as the mean time interval from hatching of one brood to the extrusion into sacs of the next. Brood number was the total number of brood which one female produced during its life time. The reproductive duration was time from carrying the first clutch to the last. The experiment continued till all female copepods died. Animals from the same clutch were reared to maturity. All newly moulted adult females were observed daily and the appearance of first clutches of eggs noted and recorded. The maturation time was time between hatching and carrying the first clutch.

For evaluation of the data, statistical inferences of mean were drawn using SAS Version 4.10. program (SAS Institute Inc.).

RESULTS

Experiment I : Screening proper species to a laboratory culture

After screening proper species for laboratory culture among four species in family Cyclopidae, only one species, *E. serrulatus* could be cultured in the laboratory condition.

Experiment II : Suitability of various diets for the development of *E. serrulatus*' nauplii

Only the flagellate algae, *Chlamydomonas reinhardii* was sufficient as a food source for newborn nauplii (Table 2). Complete development to the adult stage and egg production occurred with this algae. The newborn nauplii in the other diets died after a few days.

Table 2. Suitability of various diets for the development of *E. serrulatus*' nauplii; + indicates successful development of newly hatched nauplii to adults, − indicates no development.

	Development
Chlorophyceae flagellates *Chlamydomonas reinhardii*,	+
Chlorophyceae non−flagellates *Scenedesmus falcatus* *Selenastrum capricornum* *Chlorella* sp.	 − − −
Heterotropic flagellates *Euglena gracilis*	
Protozoan ciliates *Paramecium aurelia*	−
Chlamydomonas reinhardii + *Paramecium aurelia*	−
Euglena gracilis + *Paramecium aurelia*	−
Chlamydomonas reinhardii + *Euglena gracilis*	−

Experiment III : The development and the reproduction of *E. serrulatus* in the laboratory culture

Duration of each developmental stage and longevity

All developmental data are presented in Table 3. Mean duration of nauplius stage was the shortest among the three developmental stages. Copepodid stage is divided in six substages. But because individuals have reproductivity in copepodid 6 stage, in this study, copepodid 6 stage was included in the adults stage. In this copepodid stage, the mean time of copepodid 1 stage and 2 stage were longer than the others (Fig. 1). The lifetime of *E. serrulatus* was longer than two months. The longest was about three months, but the shortest was one month. The life−time of *E. serrulatus* consisted mainly of the copepod stage (Fig. 2).

Brood number and reproductive duration

The mean brood number was 3.8 (Table 4). Mean reproductive duration is also presented in Table 4. It could be calculated from the period

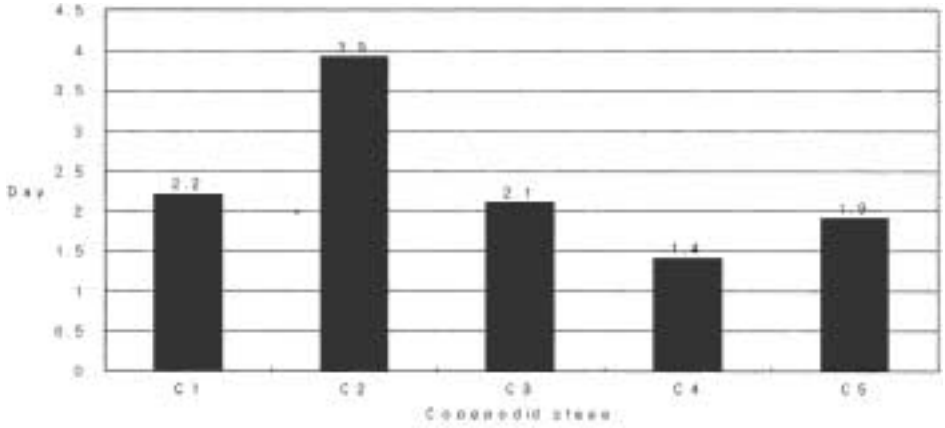

Fig. 1. The time rate of copepodid stage in *E. serrulatus* in the laboratory culture.

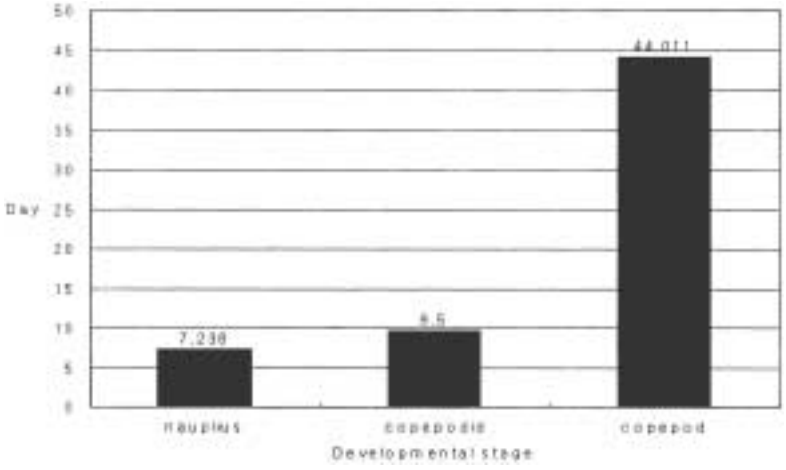

Fig. 2. The time rate of each developmental stage in *E. serrulatus* in the laboratory culture.

from the extrusion of the first egg-sacs to the last egg-sacs.

Clutch size

Because mean brood number is 3.8, clutch sizes were measured only from the first to the fourth egg sacs. Clutch sizes were related to their order (Table 5). On the average, they tend to grow up to the third clutch and decline after the fourth clutch (Fig. 3).

Fecundity and embryonic development time

The duration of embryonic development is given in Table 3.

Fecundity was the mean number of nauplii produced in each clutch. These are shown in Table 6. There is also a same tendency as that of clutch sizes (Fig. 4).

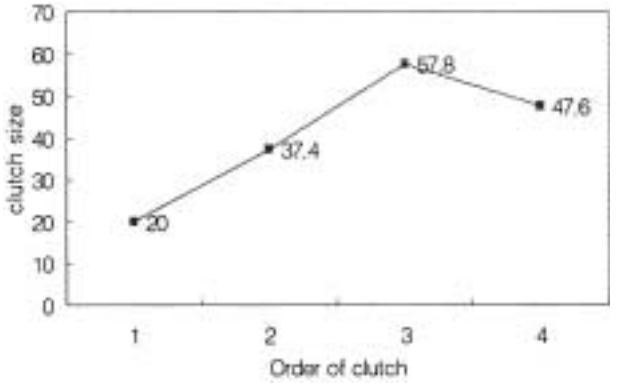

Fig. 3. Mean clutch size in the order of clutch.

Hatching rate of each clutch was the value from the mean number of nauplii produced in each clutch divided by the mean of each clutch

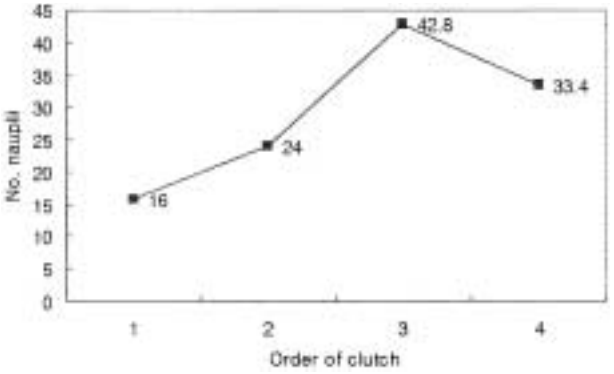

Fig. 4. Mean No. of nauplii produced in the order of clutch.

Table 3. The development of *E. serrulatus* in the laboratory culture; Std Dev = Standard deviation, N = the number of observations, CLM = Confidence Limit of Mean.

	N	Mean (day)	Std Dev	Lower 95.0% CLM	Upper 95.0% CLM
Mean duration of embryonic development	10	1.900	0.738	1.372	2.428
Mean duration of nauplius stage	21	7.238	0.768	6.889	7.588
Mean duration of copepodid stage (C1～C5)	6	9.500	0.837	8.622	10.378
Mean time of C1	6	2.200	0.707	1.458	2.942
Mean time of C2	6	3.900	0.253	3.635	4.165
Mean time of C3	6	2.100	0.469	1.608	2.592
Mean time of C4	6	1.400	0.509	0.865	1.935
Mean time of C5	6	1.900	0.724	1.140	2.660
Mean duration of copepod stage		44.011			
Mean duration of maturation time	6	16.500	1.049	15.399	17.600
Mean longevity	20	60.750	14.764	53.840	67.660

size. In the hatching rate, there was a significant similarity throughout all clutches (Table 7).

Interclutch period

The period between clutches are in Table 4.

Time to first clutch, duration of maturation time

E. serrulatus could produce the first clutch in

Table 4. Mean time interval between hatching of one brood to the extrusion into sacs of the next, mean brood number, and mean reproductive duration; Std Dev = Standard deviation, N = the number of observations, CLM = Confidence Limit of Mean.

	N	Mean	Std Dev	Lower 95.0% CLM	Upper 95.0% CLM
Mean time interval (day) between hatching of one brood to the extrusion into sacs of the next	20	1.400	0.681	1.082	1.719
Mean brood number	20	3.800	0.616	3.512	4.088
Mean reproductive duration (day)		10.64			

Table 5. Clutch sizes of *E. serrulatus* in the laboratory culture; Std Dev = Standard deviation, N = the number of observations, CLM = Confidence Limit of Mean.

	N	Mean	Std Dev	Lower 95.0% CLM	Upper 95.0% CLM
Mean 1st clutch size	5	20.000	3.536	15.610	24.390
Mean 2nd clutch size	5	37.400	9.813	25.215	49.584
Mean 3rd clutch size	5	57.800	14.342	39.992	75.608
Mean 4th clutch size	5	47.600	4.276	29.874	65.326
Total clutch size		162.8			

Table 6. Mean fecundities of *E. serrulatus* in the laboratory culture; Std Dev = Standard deviation, N = the number of observations, CLM = Confidence Limit of Mean.

	N	Mean	Std Dev	Lower 95.0% CLM	Upper 95.0% CLM
Mean number of nauplii produced in 1st clutch	5	16.000	3.391	11.789	20.210
Mean number of nauplii produced in 2nd clutch	5	24.000	7.106	15.176	32.824
Mean number of nauplii produced in 3rd clutch	5	42.800	6.870	34.269	51.330
Mean number of nauplii produced in 4th clutch	5	33.400	5.225	26.912	39.888
Total number of nauplii produced in clutches		116.2			

Table 7. Hatching rates of *E. serrulatus* in the laboratory culture; Std Dev = Standard deviation, N = the number of observations, CLM = Confidence Limit of Mean.

	N	Mean	Std Dev	Lower 95.0% CLM	Upper 95.0% CLM
Hatching rate of 1st clutch		0.800			
Hatching rate of 2nd clutch		0.642			
Hatching rate of 3rd clutch		0.740			
Hatching rate of 4th clutch		0.702			
Mean hatching rate of clutches	4	0.721	0.067	0.615	0.827

the copepodid stage 6. Maturation time was the period needed to mature enough to produce the first egg-sacs (Table 3).

DISCUSSION

The laboratory culture of *E. serrulatus*

Among four cyclopoids, only *E. serrulatus* could be cultured under the laboratory condition in the present study. *E. serrulatus* is probably the most common cyclopoid in both Korea and the world and is well adapted to all kinds of aqueous environments. It is present throughout the year (Kim and Jang, 1989). In spite of the commonness of this species only few data exist on its reproductivity. Also, no data exists on the biology of *E. serrulatus* dwelling in Korea. Therefore laboratory culture of *E. serrulatus*, the most common species in Korean freshwater, is important and is a basic step for the study of it's ecology.

Under the laboratory condition, *E. serrulatus* could easily be reared, could tolerate a wide range of temperature (10～25°C), pH (6～8) and dissolved oxygen and could reproduce itself fairly well (unpublished data). Because they have relatively short generation time and high fecundity, a lot of individuals could be obtained during a short period of time. It was found feasible to maintain *E. serrulatus* on a small scale for a long period under the experimental conditions described in the present study.

The ease of maintenance may indicate that this species has the big probability of application to various fields. Many freshwater invertebrates were already used for the assessment of metal pollution in the freshwater (SETAC, 1992). Because *E. serrulatus* is suitable for the Criteria for selection (ASTM, 1997), it, not previously used as a test animal, could be a candidate for the good test organism to evaluate the potential hazards of toxicants. Additionally, the study about a marine copepod has already proven that a marine copepod species (*Tisbe Battagliai*) is somewhat more sensitive to some endocrine disrupting materials than daphnids. It shows another probability of key application of *E. serrulatus* for assessing endocrine disrupting materials in freshwater (Hutchinson *et al.*, 1999).

Suitability of *Chlamydomonas reinhardii* for the development of *E. serrulatus*' nauplii

Complete development beginning from newborn nauplii to adult male and female was only possible on the diet of *Chlamydomonas reinhardii*. The green-flagellate *Chlamydomonas reinhardii* is among the most frequently used food species in cultures of freshwater zooplankton. *Chlamydomonas reinhardii* is a high quality food and has the advantage of being a mobile organism that stays in suspension much better than non-mobile food organisms. It has been generally accepted that algae are considered to be important food sources for the juvenile stages, while adults are generally thought to be primarily predaceous in planktonic cyclopoid copepods. But in some studies, additional ingestion of algae by the predatory stages has been reported (Toth and Zankai, 1985; Toth *et al.*, 1987; Adrian, 1991), and pure herbivory of cyclopoids has been reported in one study; *Cyclops vicinus* could be cultured with a diet consisting of phytoplankton only (Santer and Bosch, 1994). A purely herbivorous nutrition of *E. serrulatus* has not been described previously. No publication exists which describes the development of cyclopoid copepods using non-motile algae as the sole food source.

The development of *E. serrulatus*

In this study, longevity is the mean of females. Generally, the longevity of female is longer than that of male (Maier, 1990). The effect of temperature on the development of *E. serrulatus* was previously studied by Maier (1990). The results obtained by this author are slightly different but very similar to those given in the present work. In his study, the optimal temperature of the cul-

ture of *E. serrulatus* was narrowed to the range of 10°C to 15°C. Yet because many other studies proved that the optimal temperature was 20°C for cyclopoids in temperate regions, the culture temperature was set in 20°C in this study. These differences may be explained by the difference of the source of *E. serrulatus*. In the present study, all animals studied were the pure breed of the laboratory culture, but Maier used animals sampled in ponds only after acclimation. These differences also may be due to slight differences in experimental conditions or genetical differences among populations.

In copepods, the developmental times were longer at lower temperature and were shorter at higher temperature (Maier, 1990). And they were also affected by food preference (Santer and Bosh, 1994; Amarasinghe *et al.*, 1997; Hopp *et al.*, 1997), and pH (Hansen *et al.*, 1991).

The reproduction of *E. serrulatus*

No literature data on the reproduction of *E. serrulatus* is found. When compared with other littoral cyclopoid species, for example with *Megacyclops viridis*, *Macrocyclops albidus*, and *Acanthocyclops vernalis* (Abdullahi, 1990), the general efficiencies of *E. serrulatus* in reproduction are higher. Abdullahi's study (1990) shows that among three cyclopoids, the largest one has the smallest clutch size and longest interclutch period, but in the smallest one vice versa. The fact that *E. serrulatus* is smaller than other three cyclopoids, could explain it's higher efficiency of reproduction. *E. serrulatus* may adopt to laying much more eggs during short time as its ecological strategy for survival. The reproduction of copepods is dependent on temperature and female size (Abdullahi and Laybourn−Parry, 1985; Abdullahi, 1990; Maier, 1990).

ABSTRACT

Four cyclopoids were tested for laboratory culture. Among these animals, only *Eucyclops serrulatus* was successfully cultured in the laboratory. Under the laboratory culture condition, nine kinds of diets were tested for the suitability of nauplius development. Development and reproduction of *E. serrulatus* were also investigated. *Chlamydomonas reinhardii* was the only one which could induce complete development from nauplii to adults. It was found that *E. serrulatus* had relatively short generation time, could produce lots of progenies, and could be handled easily, allowing to obtain many individuals during a short period. With these characteristics, this species may be a candidate for a good test organism for evaluating freshwater pollution.

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