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Laboratory maintenance of field-collected *Lymnaea viridis* for use as an intermediate host for *Fasciola hepatica*

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야외에서 채집한 간질의 중간숙주인 애기물달팽이의 실험실 사육

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초 록 : 전남대학교 수의과대학에서는 조류 blue-green algae를 먹이로 하여 간질의 중간숙주인 애기물달팽이를 실험실에서 유지시켜 왔으나 조류의 배양에 어려움이 많아서 보다 간편하면서 달팽이의 번식에도 좋은 방법을 고안하고자 노력하였다. 28.5×17×18cm 크기의 유리수조에 모래를 1.5cm 높이로 깔고 10개의 꼬막껍질을 그 위에 올려놓은 다음 4리터의 증류수를 부어넣었다. 야외에서 채집한 달팽이의 난괴를 부화시켜 얻은 0.63cm 크기의 어린 달팽이 45마리를 세 그룹으로 나누어 세 개의 수조에서 20℃의 온도를 유지하면서 사육하였고 먹이로 상추잎을 넣어주었다. 매주 수조바닥의 물 2ℓ를 흡인하여 갈아주면서 상추잎을 바꿔넣었고, 계속해서 공기를 통과시켜 산소를 공급하였다. 이와 동일한 방법으로 실험실에서 조류로 사육하던 17대의 달팽이를 사육하여 그 성적을 비교하였던 바 야외에서 채집한 달팽이의 성장률이 실험실에 적응된 것들에 비하여 약간 떨어졌으나 생존율이나 산란율에서는 큰 차이가 인정되지 않았다. 그래서 1년이 넘도록 이 방법으로 달팽이를 사육하면서 간질의 피낭유충을 생산하고 있다. 꼬막껍질은 서서히 분해하면서 달팽이의 각을 형성하는 물질을 제공하여 생존율을 높게 한 것으로 생각된다. 그리고 수조를 햇볕이 드는 창문 가까이에 놓아두었기 때문에 조류가 자생하여 적은 양이나마 달팽이의 먹이가 되었을 것으로 보인다.

Key words : culture, *Lymnaea viridis, Fasciola hepatica*, aquarium.

Introduction

The intermediate hosts of the liver fluke *Fasciola hepatica* are lymnaeid snails which are amphibious or semi-aquatic. Their natural habitat is the land-water interface where algae, the principal food of the snail, grow when exposed to sun and air[1]. Thus, attempts have been made to

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reproduce the natural habitat of the snails to maintain them in the laboratory[2].

The mud slope culture method initiated by Taylor and Mozley[2] and followed by many workers later has problems associated with the excretory end products although the system simulates conditions of the natural snail habitat. In certain parts of the world, large numbers of algal cultures can be prepared at the end of the summer and stored for use in the winter[3]. Different species of algae are frequently obtained from one habitat[4], and after a few cultures, active growth of unsuitable algae or mosses sometimes leads to the failure of the snails to thrive[5].

In our laboratory it has not been difficult to maintain *Lymnaea viridis*, the intermediate host of *F hepatica*, in culture, but has been difficult to provide them with consistently good conditions. The established culture vessels were not always suitable for the snails. Having met with these problems with the mud slope culture method, we proceeded with an attempt to modify the method. In the present study, attempts were made to maintain the field-collected *L viridis* in the laboratory by the modified culture technique. The laboratory-adapted *L viridis*[6] were cultured by the same method for comparison.

Materials and Methods

Glass aquarium : A commercially available glass aquarium 28.5×17×18cm was used. In setting up the aquarium, washed sand was placed at the bottom to occupy about 1.5cm on the bottom of the aquarium. Ten old, decomposing shells of *Tegillarca granosa*(ark shells) were left on the sand. The aquarium was then filled with four litres of distilled water and continuous aeration provided[7]. Water depth was approximately 9.5cm. A glass plate was placed on top, leaving a small passage for the aerator tube. The aquarium was positioned near a window.

Snails : Snails 6-8mm in shell length were collected from rice-fields. Egg-masses from these snails and the 17th generation of laboratory-adapted *L viridis* snail, which had been reared on algae[6], were incubated at 22℃, and the shell length of the newly hatched snails were measured under the microscope(40X). The young snails about 0.63mm in shell length were transferred to the aquarium.

Snail culture : A total of 45 young snail from the field-collected adults were divided into three groups and cultured in three aquaria. Same number of young snails from the laboratory-reared adults were cultured in an identical manner. Washed green lettuce leaves were fed in adequate quantities. About two litres of water from the bottom of the aquarium was removed every week by aspiration, together with excreta of the snails, and replaced with distilled water[8]. Fresh lettuce leaves were replaced once every week.

The snail was cultured at the temperature range of 20-23℃. The rate of growth was measured by shell length with a caliper(Mitutoyo Venier, Japan). The survivorship and the time of first egg-laying of the snails were observed.

Statistics : Differences in the rate of growth between laboratory-adapted(LA) and field-collected(FC) snails were tested by Student's *t*-test.

Results

The growth rate of the snails is summarized in Table 1. The LA snails grew faster than FC snails throughout the experiment; the differences in the shell length of the snails, however, were significant($p<0.01$) only at day 14 and day 54 on after hatching(a.h.). No differences were found in the growth rate among the groups of both LA and FC snails. The survival rates of the LA and FC snails at day 74 a.h. were 91 and 80%, respectively.

In LA snails the first egg-laying commenced at day 34 a.h., and the largest snail of them was 7.3mm in shell length.

Table 1. Shell length(mm) of *Lymnaea viridis* cultured in the shallow aquarium system(mean±SD)

Days after hatching	Laboratory-adapted	Field-collected
14	3.1±0.7(45)	2.3±0.3(45)
24	4.3±1.1(44)	4.2±0.7(41)
34	5.2±1.1(44)	4.8±0.7(41)
44	6.6±1.0(42)	6.3±1.0(38)
54	8.1±0.7(42)	7.2±1.1(38)
64	9.0±0.9(41)	7.8±0.8(36)
74	9.9±0.8(41)	8.3±0.8(36)

Numbers in the parentheses are the numbers of snails survived.

From day 44 a.h. on, most of the snails laid eggs. The FC snails initiated egg-laying at day 39 a.h., and most of the snails laid eggs from day 64 a.h. on. The snails preferentially laid eggs on the sand and the ark shells, and rarely on the sides of the aquarium. They were observed continuously crawling on the sides of the aquarium below the water level.

Discussion

It is well known that the lymnaeid snails, the intermediate host of *F hepatica* are semi-aquatic. To maintain the snails in the laboratory, the mud slope culture method has been widely adopted as the method simulates conditions of the natural snail habitat best[9]. The culture method, however, poses some problems[3-5] and various attempts have been made to overcome the problems[10-12].

A culture method for *Fossaria cubensis* was described by Isseroff and Read[13], and was modified later by Isseroff and Smith[12]. The modified culture technique employs a shallow aquarium system for various freshwater, amphibious snail species[8]. Boyce *et al*[14], by using the technique, monitored the egg production of *Pseudosuccinea columella* and *F cubensis* in the laboratory; they were able to maintain the snails in the laboratory for over one year and have reared large numbers of snails with a minimum cost.

The snail culture method described in the present study is similar to the modified technique of Isseroff and Smith[12]. It was proved to be a practical alternative to the breeding of laboratory-adapted strain of *L viridis*. The results in the present communication, together with those of Boyce *et al*[14], confirmed that the amphibious snail species, whose natural environment is mudbanks, can be maintained in the laboratory in shallow aquarium systems[15]. The method enabled us to reduce the time and effort in maintaining large numbers of *L viridis* in our laboratory.

Compared to the snails fed on the laboratory-cultured blue-green algae[6,16], the growth rate and egg-laying of the snails in this study were considerably delayed. These results support previous findings in which the growth rate and fecundity of snails were significantly affected by food[5].

The experiments in this communication were stopped when the snails were 74 days old. At that age the snails were laying more than enough eggmasses for breeding. the eggs hatched in 10~12 days. The individual snails thus reared proved to be suitable intermediate host for *F hepatica* where cercarial shedding occures about 35 days after exposure to miracidia[17]. The snails have been maintained in our laboratory for eight months under these conditions

The high survival rate of the snails in this study has been attributed to the decomposing ark shells, which provided materials for shell-forming. Young snails are usually very brittle when thier shell lengths are measured. Continuous crawling of the snails on the sides of the aquarium below the water level suggests that algae grew on the glass during the experiments.

Conclusion

A method of breeding and maintaining *Lymnaea viridis* in the laboratory is described. The snails were cultured in glass aquaria with continuous aeration; washed sand was placed at the bottom with ark shells. The aquarium was filled with distilled water, and the snails were fed fresh lettuce leaves. The technique was suitable for maintaining field-collected *L viridis* in the laboratory and provided a simple, economical method of rearing large numbers of snails with a minimum expenditure of time and effort.

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