# Influence of Food on the Growth and Fecundity of *Lymnaea ollula*, the Intermediate Host of the Liver Fluke

# TADASHI ITAGAKI

#### (Received for publication; May 7, 1986)

### Abstract

The quality of food affected the survival, growth and fecundity of *L. ollula* snails. The snails supplied with the blue-green algae, *Oscillatoria* spp., and the powdered mixed food grew faster and deposited more egg-masses containing more eggs but had a shorter life-span than those fed on the other foods, although the snails supplied with the powdered mixed food deposited egg-masses containing many deformed embryos. The blue-green algae were the best food for breeding the snails in the laboratory, when supplemented with a small amount of soil.

Key words: Lymnaea ollula, food, growth, fecundity

#### Introduction

The development of *Fasciola* larvae in host snails is influenced by water temperature and snail nutrition: water temperature is closely related to the growth of fluke larvae and the production of daughter rediae in mother rediae (Dinnik and Dinnik, 1956, 1963, 1964; Wilson and Draskau, 1976, etc.), and snail nutrition affects the multiplication of *Fasciola* larvae (Kendall, 1949, 1953; Kendall and Ollenshaw, 1963; Boray, 1963). Host snails must be kept in good nutritional state when the susceptibility of lymnaeid snails to the liver fluke is studied in the laboratory. In this study some kinds of food were evaluated by the survival, growth, and fecundity of the snail, *L. ollula*.

### **Materials and Methods**

Laboratory-bred L. ollula snails 1.58-1.87 mm long and 1.09-1.24 mm wide were used. The snails were divided into 8 groups of 12 snails each. The groups were fed on different kinds of food as follows: Group A on boiled lettuce, Group B on the powdered mixed food containing 1 g of commercial fish food, 1 g of wheat germ and 0.6 g of calcium sulfate, Group C on the modified Standen's food (Standen, 1951), Group D on baby food No. 8 manufactured by Meiji Nyugyo Co. Ltd., Group E on baby food No. 10 by the same company, Group F on blue-green algae, *Oscillatoria* spp., Group G on diatoms, and Group H on the bluegreen algae with a small amount of soil.

The modified Standen's food was prepared by the following prescription: rice gruel (Wako Co. Ltd.) 5.0, dried milk G-80 (Morinaga Nyugyo Co. Ltd.) 2.5, chlorella powder 5.0, dehydroacetic acid 1.0, brewer's yeast (Ebios Yakuhinkogyo Co. Ltd.) 2.5, sodium alginate 5.0, lettuce 95.0 and water 400.

Four snails each were kept in petri dishes, 12 cm in diameter, containing 100-150 ml of artificial spring water. The dishes were placed at  $22-26^{\circ}$ C in a light-dark cycle of 12 hours of light from 07:00 to 19:00. Food was supplied and the water was changed every two or three days, when dishes were cleaned. The length and width of surviving snails were measured with a slide caliper every two weeks. The number of surviving snails, egg-masses and eggs per egg-mass were recorded every two or three days. Egg-masses laid were removed to plastic containers, 6 cm in diameter, to

Department of Parasitology, School of Veterinary Medicine, Azabu University, Fuchinobe, Sagamihara 229, Japan.

板垣 匡 (麻布大学獣医学部寄生虫学教室)

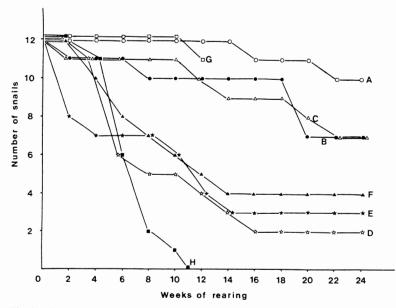


Fig. 1 Survival of snails fed on different foods: A, boiled lettuce; B, powdered mixed food; C, modified Standen's food; D, Meiji baby food No. 8; E, Meiji baby food No. 10; F, blue-green algae; G, diatoms; H, blue-green algae with a small amount of soil.

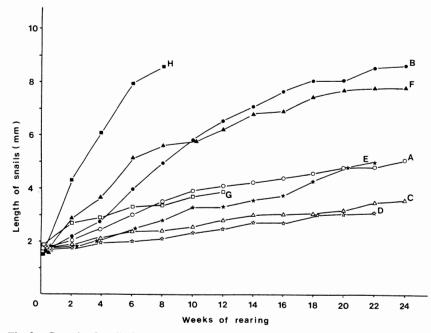


Fig. 2 Growth of snails fed on different foods; A, boiled lettuce; B, powdered mixed food; C, modified Standen's food; D, Meiji baby food No. 8; E, Meiji baby food No. 10; F, blue-green algae; G, diatoms; H, blue-green algae with a small amount of soil.

observe hatchability of eggs. The snails except for those of Group G were reared for a period of 24 weeks.

## Results

#### Survival of snails:

In Groups A, B and C, most snails survived for more than 24 weeks, whereas in Groups D, E and F most of snails died within 10 weeks. The survival rate was 83%, 58%, 58%, 16%, 25% and 33% in Groups A, B, C, D, E and F, respectively after six months. In Group H only one snail died by week 4, but thereafter the number of dead snails increased markedly, and all the snails died by week 11. In Group G most snails survived for more than 12 weeks (Fig. 1).

#### Growth of snails:

In Groups A, C, D, E and G, the growth rate of snails was low. The snails of Groups A and E and Group D grew by some 3.3 mm and 1.4 mm, respectively in 24 weeks. In Groups B, F and especially in Group H, on the other hand, the growth rate was high: the snails of Groups B and F grew by 7.0 mm and 6.2 mm, respectively in 24 weeks, and those of Group H grew by 7.4 mm in 8 weeks (Fig. 2).

## Fecundity of snails:

Oviposition was seen in Groups A, B, F and H, but not in Groups C, D and E. The snails of Group G deposited egg-masses containing no eggs. Group H laid egg-masses earliest of all the groups, and Group A did latest. Average numbers of egg-masses per snail per week and of eggs per egg-mass were greatest in Group H,

Table 1 Oviposition in snail groups fed on different kinds of food

Grou	Day of p oviposition after rearing	Total number of egg masses	Total number of eggs	Mean number of eggs per egg mass	Mean number of egg masses per snail per week	Mean number of eggs per snail per week	Mean length and width of egg masses
А	64	70	374	4.89	0.51	2.70	4.01 × 2.04
В	53	225	3197	14.21	1.38	22.28	7.90 × 3.19
F	41	109	898	9.18	1.28	12.20	5.26 × 2.35
Н	25	108	2111	20.66	4.38	90.52	7.66 × 3.16

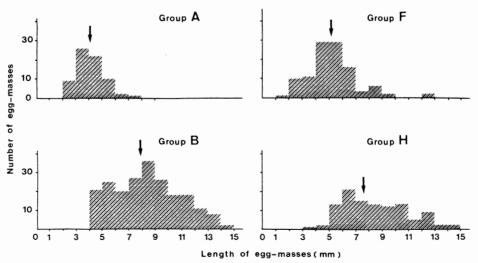


Fig. 3 Relationship between the number of egg-masses and the length of egg-masses. Arrows show means.

followed by Groups B, F and A in order of the numbers (Table 1). As shown in Fig. 3, the mean length of egg-masses was greater in Groups B and H than in Groups A and F, and yet the range of the length was wider in Groups B and H than in Groups A and F. The number of eggs in an egg-mass was mostly 10 or less in Groups A and F, whereas in Groups B and H it widely varied from 0 to some 50, although in Group B, egg-masses with 5 or less eggs were greatest in number (Fig. 4).

# Hatchability of eggs:

The average first-hatching time of eggs was not different between the groups, but the average last-hatching time was twice as late in Group B as in the other groups. The average hatching rate of eggs was highest (93.4%) in Group H and lowest (65.2%) in Group B (Table 2), because eggs laid by Group B contained 15 to 20 percent of underdeveloped or deformed embryos which had the protruded buccal mass or the deformed body or shell (Fig. 5).

# Discussion

The life-span of Lymnaea ollula in the laboratory is 10 to 11 weeks at about  $26^{\circ}C$  (Esaki, 1957; Yoshikawa, 1965) and that of L. truncatula is affected by the growth rate of snails (Kendall, 1953). The growth rate of lymnaeid snails is affected by the amount of food pro-

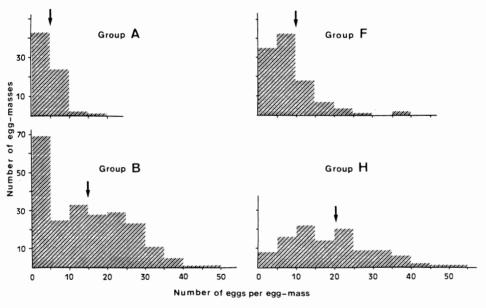


Fig. 4 Frequency distribution of the number of eggs per egg-mass. Arrows show means.

Group	Total number of eggs	Mean day of the first hatch	Mean day of the last hatch	Total number of hatched snails	Percent of hatched snails
A	374	8.2	12.6	298	82.3
В	3,179	8.5	22.4	2,075	65.3
F	922	8.6	12.5	816	88.5
Н	2,129	7.9	11.5	1,989	93.4

 Table 2
 Hatch of eggs laid in snail groups fed on different kinds of food

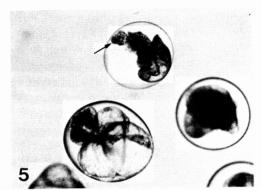


Fig. 5 Deformed embryos. Arrow show buccal mass protruding out of the mouth.

vided (Kendall, 1953; Scheerboon, 1978). The present study showed that the survival and growth rate of snails was different between the snail groups: the life-span of snails was directly affected by the quality of food and yet it was inversely proportional to the growth of snails, and the growth rate was influenced not only by the amount, but also by the quality of food.

Thompson (1984) reared Biomphalaria glabrata successfully with an artificial food supplemented with blue-green algae Spirulina sp. or chlorella or lettuce as nutrient to the Standen's alginate-based media, and concluded that the blue-green algae can supply the snails with nutrition enough to grow. In the present study with L. ollula, blue-green algae, Oscillatoria spp., and the powdered mixed food were quite sufficient for snails to grow. In the case of the blue-green algae, the snails grew best when supplemented with a small amount of soil. Storay (1970) also accelerated the growth of L. pereger by feeding on the diet with soil. Minute sand grains in mud of habitats are necessary for snails to grind food into minute particles in the gizzard, so that snails can utilize many kinds of food as nutrients (Carriker, 1946; Storay, 1970).

The present study made it clear that fecundity of snails is markedly different by supplying different qualities of food. The onset of oviposition was different between the groups of snails: it began 39 days earlier in Group H than in Group A. The snails of Group B, F and H began to oviposit at a shell-length of 5-6 mm. Iwata and Watanabe (1955) also stated that *L. ollula* begins to oviposit at a shell-length of 5-6 mm. Group H with the highest growth rate began to oviposit earliest of all the groups, whereas Group A with the lowest growth rate did latest. So that, the onset of oviposition seems to be much affected by the growth of snails. The snails of Groups C, D and E grew slowly and yet no oviposition was observed in these groups, so that the food given to these groups is insufficient for snails to grow.

The size of egg-masses and the number of eggs in a single egg-mass were different between Groups B and F, although the snails of these groups were the same in size. These differences seem to arise from that in nutrients of each food, and the nutrient necessary for growth will differ from that for reproduction. Steen (1967) reported, on the other hand, that L. stagnalis snails laid egg-masses with much more eggs when fed on lattuce than on the Standen's food, and this is caused not by the foods used but oxygen produced by lettuce; nevertheless it seems that the differences in fecundity is caused by nutrients of food from the results of the present examination.

L. ollula eggs hatch earlier when the water temperature is higher; they hatch in about 9 days at an average temperature of 25°C (Esaki, 1958). In the present study, the eggs laid by Groups A, B, F and H hatched in an average of 7.9-8.6 days, and there was no marked difference in incubation time between these groups. The maximum hatching period, the duration from the first to the last hatching of eggs in an egg-mass, ranged from 11.5 to 12.6 days on average in Groups A, F and H and was 22.4 days in Group B. Hatching rate was 82% to 93% in Groups A, F and H and 65% in Group B. The later time and lower rate of hatching in Group B seem to be caused by the production of many deformed embryos, because most of deformed embryos died before hatching or were delayed in development even if they survived. It is not obvious why Group B produced more eggs with a deformed embryo.

#### References

- Boray, J. C. (1963): The ecology of *Fasciola* hepatica with particular reference to its intermediate host in Australia. Proc. Wld. Vet. Congr., 17th, Sect. Parasitology, 709-715.
- Carriker, M. R. (1946): Observations on the functioning of the alimentary system of the snail Lymnaea stagnalis appressa Say. Biol. Bull., 91, 88-111.
- Dinnik, J. A. and Dinnik, N. N. (1956): Observations on the succession of redial generations of *Fasciola gigantica* Cobbold in a snail host. Z. Tropenmed. Parasitol., 7, 397-419.
- 4) Dinnik, J. A. and Dinnik, N. N. (1963): Effect of the seasonal variations of temperature on the development of *Fasciola gigantica* in the snail host in the Kenya highlands. Bull. Epiz. Dis. Afr., 11, 197-207.
- 5) Dinnik, J. A. and Dinnik, N. N. (1964): The influence of temperature on the succession of redial and cercarial generations of *Fasciola* gigantica in a snail host. Parasitology, 54, 59-65.
- Esaki, Y. (1957): (Study on the ecology of Lymnaea ollula.) J. Jpn. Vet. Med. Assoc., 10, 579-582 (in Japanese).
- Esaki, Y. (1958): (Study on the ecology of Lymnaea ollula.) J. Jpn. Vet. Med. Assoc., 11, 120-123 (in Japanese).
- Iwata, S. and Watanabe, S. (1955): (Observation on the life history of *Lymnaea ollula*.) J. Jpn. Vet. Med. Assoc., 8, 135–138 (in Japanese).
- Kendall, S. B. (1949): Nutritional factors affecting the rate of development of *Fasciola hepatica* in *Limnaea truncatula*. J. Helminthol., 23, 179– 190.
- Kendall, S. B. (1953): The life history of Limnaea truncatula under laboratory conditions.

J. Helminthol., 27, 17-28.

- Kendall, S. B. and Ollenshaw, C. B. (1963): The effect of nutrition on the growth of *Fasciola* hepatica in its snail host. Proc. Nutr. Soc., 22, 41-46.
- 12) Scheerboon, J. E. M. (1978): The influence of food quantity and food quality on assimilation, body growth and egg production in the pond snail Lymnaea stagnalis (L.) with particular reference to the haemolymph-glucose concentration. Proc. Kon. Ned. Akad. Wet., Ser. C, 81, 184-197.
- Standen, O. D. (1951): Some observations upon the maintenance of Australorbis glabratus in the laboratory. Ann. Trop. Med. Parasitol., 45, 80-83.
- 14) Steen, W. J. van der (1967): The influence of environmental factors on the oviposition of Lymnaea stagnalis (L.) under laboratory conditions. Archs. Neerl. Zool., 17, 403-468.
- Storay, R. (1970): The importance of mineral particles in the diet of Lymnaea pereger (Müller). J. Conch., 27, 191-195.
- 16) Thompson, S. N. (1984): Spirulina as a nutrient source in experimental media for maintaining the schistosome vector, Biomphalaria glabrata. Ann. Trop. Med. Parasitol., 78, 547-548.
- 17) Yoshikawa, S. (1965): Studies on the breeding of the pulmonate pond-snail, Lymnaea ollula. Analysis of the factors in the former artificial breeding methods and a simple technic of rearing the snail with a wearing food. Venus (Jpn. J. Malacol.), 24, 72-84 (in Japanese with English summary).
- Wilson, R. A. and Draskau, T. (1976): The stimulation of daughter redia production during the larval development of *Fasciola hepatica*. Parasitology, 72, 245-257.