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Experimental Mass Breeding of *Oncomelania quadrasi*, the Vector Snail of Schistosomiasis in the Philippines

NAOKO NIHEI[1)], HIROSHI ITAGAKI[2)], YASUHIDE SAITOH[2)], MASAMI KANEKO[2)], SHIRO CHINONE[2)] AND TAMOTSU KANAZAWA[1)]

[1)]*Department of Parasitology, National Institute of Health, 1-23-1 Toyama, Shinjuku-ku, Tokyo 162, Japan.*
[2)]*Department of Parasitology, Azabu University School of Veterinary Medicine, Fuchinobe, Sagamihara, Kanagawa 229, Japan.*
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Abstract

Mass breeding of *Oncomelania quadrasi*, the vector snail of schistosomiasis in the Philippines, was attempted in the laboratory and the conditions for snails to propagate or grow well, such as optimum population density, composition of bed substratum and temperature, were examined. In order to breed juvenile snails, water-circulating aquaria provided with the bed composed of crushed oystershell were used. 337 to 1000 juveniles per aquarium with a size of 18.5 cm × 32.5 cm and 23.5 cm deep, were raised to adults during a period of 8 to 12 weeks, showing no crowding effect. The aerated closed-aquaria as well as the circulating were favorable for young snails to grow although a crowding effect appeared when more than 200 snails were kept in one aquarium. Shallow unglazed clay pots with a soil bed were suitable for the snails to reproduce as well as in the case of *O. nosophora*. The optimum temperature for young snails to grow was 25 or 30°C. At 20°C snails matured although the growth was retarded. At 15°C, however, only half of the snails grew to adults at a slow pace. Parent snails could reproduce at 20, 25 and 27°C although the hatching of eggs was delayed at 20°C. The culture bed composed of crushed oystershell alone was suitable for young snails to grow but unsuitable for parent snails to reproduce. The bed composed of crushed oystershell mixed with Kofu soil was suitable for the reproduction of parent snails. However, Kofu soil or gray paddy soil inhabited by *O. nosophora* in Japan was the best for *O. quadrasi*.

Key words: mass breeding; *Oncomelania quadrasi*; oystershell; reproduction; schistosomiasis.

Introduction

Schistosoma japonicum infection has been a serious public health problem in the central and southern Philippines. Ten years ago it was estimated that 700,000 out of 4 million people at risk were infected in 22 provinces. The prevalence was 6.6% in 1990 (Jordan *et al.*, 1993; WHO, 1993). The authors have investigated geographical distribution and habitat conditions of the host snail *Oncomelania quadrasi* on Leyte, Bohol and Mindoro islands of the Philippines (Nihei, 1971; Nihei *et al.*, 1993, 1994; Saitoh *et al.*, 1994).

To eliminate the endemic disease and to develop efficacious drugs, successful maintenance of the parasite and vector snails in the laboratory is necessary.

The rearing methods of *O. quadrasi* in the laboratory have been devised by Pesigan *et al.* (1958), Chi and Wagner (1957), van der Schalie and Davis (1968), and Wagner and Wong (1956). Those carried out by these authors were on a small scale.

This study was undertaken to establish the mass breeding of *O. quadrasi* in the laboratory and to elucidate the best conditions for young and parent snails to grow and reproduce, respectively.

Correspondence: Naoko Nihei
二瓶直子[1]，板垣　博[2]，斉藤康秀[2]，金子理実[2]，茅根士郎[2]，金澤　保[1]（[1]国立予防衛生研究所寄生動物部，[2]麻布大学獣医学部寄生虫学講座）

Materials and Methods

Snails

Snails used were collected from Bohol Island of the Philippines and maintained in the laboratory. Mature male and female snails, 3.0–4.0 mm and 4.0–5.0 mm in shell length, respectively, and just hatched juvenile snails, 0.5–1.0 mm, occasionally up to 2.0 mm in shell length, were used in the reproduction and breeding experiments, respectively.

Soil and oystershell

The soil used as the bed substratum of culture was collected in the paddy field inhabited by *O. nosophora* in the western area of the Kofu Basin in Yamanashi Prefecture. Hereafter it is referred to as Kofu soil and was used as the control soil in the experiments. All the soil used was air-dried and sifted through a sieve of 2 mm-meshes after collection.

Oystershell used as culture bed material was prepared by crushing oystershell used as chicken feed, and the crushed shell was washed with tap water and further sieved before use if necessary.

Water

Tap water dechlorinated by leaving overnight or distilled water was used for the experiments.

Rearing system

To examine the optimum temperatures for juvenile snails to grow, deep petri dishes were used (Nihei, 1978b). Deep petri dishes, 9 cm in diameter, were provided with a lid and the bed substratum of Kofu soil was placed on the bottom about 5 mm thick. About 200 ml water was then added to the dishes in which 20 juvenile snails each were transferred and fed with the dried food. All the snails were recovered and measured for shell length every 2 weeks.

For mass breeding experiment of young snails, the water-circulating and the aerated closed-aquaria were used.

The circulating aquaria were made using a tropical fish aquarium, 18.5 cm × 32.5 cm and 23.5 cm high, and set up with a filter bed, about 4.5 cm thick, composed of crushed oystershell piled up on the sublayer of pebbles and glasswool on the bottom. The water level was regulated to about 4 cm deep by overflow through a tube which was set up on the side wall at that depth. Water pumped up to the reservoir was dropped into the aquaria at a rate of 3–4 ml per minute. The water in the aquaria was filtered through the bed and carried back to the reservoir which was supplied with water once a week.

The aerated closed-aquaria were made using a fish aquarium, 27.5 cm × 17.0 cm and 17.0 cm deep, equipped with a bed substratum about 5 mm thick, composed of crushed and cleaned oystershell after minute particles were sieved out, and water was poured to about 10 cm deep. The aquaria were aerated by bubbling.

To examine the reproduction of snails, parent snails were kept in shallow unglazed clay pots, 9 cm and 4 cm in diameter and depth, respectively, with soil, about 1 cm deep, on the bottom. Cultures were covered with a lid and placed in a large shallow plastic tray with some water to supply humidity. To each culture 3 male and 5 female snails were transferred. In the experiments on temperature, cultures were placed in the bioincubator (BIOMULTI-INCUBATOR, Model LH-30-8CT, NK System, Nihon Ikakikai Seisakusho, Osaka, Japan) at 15, 20, 25, 27 and 30°C, respectively, while they were placed in a room at 25°C in the experiments on bed substratum. In both experiments cultures were illuminated by room light from 06:00 to 18:00. One month later, parent snails were removed and another month later, hatched young snails were counted under a stereoscopic microscope.

Food of snails

Snails in deep petri dishes and both kinds of aquaria were supplied with dried food made by Standen's modified method (Saitoh and Itagaki, 1980), and additionally with 2 circular sheets of filterpaper attached on the inside wall, but only in the closed-aquaria in oder to supply humidity and as food.

Snails maintained in clay pots were fed with 30 mg of powdered food weekly (Matsuda, 1969).

Results

Influence of temperature and population density on growth of juvenile snails

The growth of snails was influenced by the temperature (Table 1). Snails reared at 25 and 30°C were significantly larger than those at 15 and 20°C at weeks 2 and 3. Snails reared at 25°C were the largest at weeks 4, 5 and 6. Snails reared at 20°C, however, were not different in shell length from those kept at 30°C in week 6 and matured in week 7. At 15°C, however, only half of the snails matured.

To determine the optimum number of snails per culture, 337 to 1000 juvenile snails each were kept in 4 circulating aquaria and recovered every 4 weeks and measured for shell length. The recovery rate of snails ranged from 56.8 to 90.2% and 80.6 to 87.8%, 4 and 8 weeks later respectively. Young snails which were 1.69 to 1.79 mm at the start of experiment attained a shell length of 2.70 to 3.52 mm and 3.69 to 3.99 mm, 4 and 8 weeks later respectively. From these results no crowding effect was observed on the growth of snails because little differences were observed in the shell length and the recovery rate of snails when less than 1000 snails were maintained in one aquarium (Table 2).

The snails maintained in 8 aerated closed-aquaria ranged from 80 to 370 and 1.54 to 1.92 mm in number and mean shell length, respectively. The number of snails recovered and the mean shell length increased to a range from 62 to 343 and from 2.38 to 3.37 mm, respectively, 5 weeks later, and from 63 to 305 and from 3.04 to 4.15 mm, respectively, 8 weeks later. An average shell length was smaller by about 1 mm in 343 snails kept in an aquarium than in 62 snails in the same aquarium 5 weeks later. Snails attained a shell length of about 4 mm in aquaria containing less than 100 snails each, whereas they attained about 4 mm in those containing less than 100 snails 8 weeks later. These results showed that in the closed-aquaria, a crowding effect was observed in the growth of snails (Table 3).

Table 1 Growth of young *O. quadrasi* in deep petri dishes at different temperatures*

Temperature (°C)	Mean shell length ± SD (mm)					
	1†	2†	3†	4†	5†	6†
15	1.60±0.17	1.84±0.32	2.13±0.41	2.34±0.34	2.47±0.39	2.72±0.49
20	1.63±0.20	1.67±0.27	2.00±0.52	2.51±0.62	3.05±0.62	3.42±0.86
25	1.90±0.41	2.17±0.42	3.00±0.31	3.47±0.30	3.60±0.42	3.94±0.43
30	1.84±0.29	2.23±0.41	3.12±0.44	3.14±0.48	3.30±0.52	3.87±0.46

*Twenty just hatched snails each were kept in petri dishes.
†Weeks after start of experiments.

Table 2 Growth of juvenile *O. quadrasi* in water-circulating aquaria

Aquarium No.	0*		4*			8*		
	No. of snails reared	Mean shell length (mm)	No. of snails recovered	Recovery rate of snails (%)	Mean† shell length (mm)	No. of snails recovered	Recovery rate of snails (%)	Mean† shell length (mm)
1	337	1.73±0.41	304	90.2	3.52±0.58	296	87.8	3.99±0.42
2	484	1.72±0.48	428	88.4	3.49±0.60	390	80.6	3.97±0.48
3	720	1.79±0.65	409	56.8	2.70±0.79	610	84.7	3.69±0.54
4	1000	1.69±0.39	865	86.5	3.48±0.63	812	81.2	3.81±0.48

*Weeks after start of experiments.
†All the snails which could be recovered were measured.

Influence of temperatures on reproduction of snails

All the experiments were carried out at a room temperature of 25°C. Fecundity of snails was compared among cultures maintained at different temperatures ranging from 15 to 30°C. At 20 and 25°C, 54 and 82 juveniles on average were produced respectively in Experiment 1 and 34 and 87 young snails respectively in Exp. 2, and at 27°C an average number of 72 snails were produced, whereas no snails were obtained at 15 and 30°C in both experiments because the parent snails did not feed at 15°C and most snails climbed up the side wall of the aquaria and died out at 30°C (Table 4).

Influence of the materials of culture bed substratum on reproduction of snails

When crushed oystershell with different particle sizes was used as the culture bed, a far smaller number of young snails were produced than those in cultures provided with Kofu soil, although a fair number of juveniles were obtained in cultures with the bed of the crushed oystershell, less than 63 μm

Table 3 Growth of juvenile *O. quadrasi* in aerated closed-aquaria

Aquarium No.	0*		5*			8*		
	No. of snails reared	Mean shell length (mm)	No. of snails recovered	Recovery rate of snails (%)	Mean† shell length (mm)	No. of snails recovered	Recovery rate of snails (%)	Mean† shell length (mm)
1	370	1.58±0.50	343	92.7	2.38±0.78	305	82.5	3.05±0.76
2	274	1.79±0.42	241	88.0	2.56±0.52	231	84.3	3.04±0.54
3	245	1.84±0.53	203	82.9	2.81±0.17	198	80.8	3.14±0.67
4	218	1.56±0.42	171	78.4	2.83±0.58	167	76.6	3.88±0.56
5	194	1.92±0.44	159	82.0	3.08±0.66	149	76.8	3.53±0.48
6	117	1.54±0.35	83	70.9	2.76±0.55	99	84.6	4.14±0.61
7	104	1.75±0.37	87	83.7	3.35±0.59	75	72.1	4.15±0.43
8	80	1.79±0.46	62	77.5	3.37±0.65	63	78.8	3.94±0.51

*Weeks after start of experiments.
†All the snails which could be recovered were measured.

Table 4 Reproduction of parent *O. quadrasi* kept in clay pots at different temperatures*

Temperature (°C)	Number of snails hatched				95% confidence limit
	1†	2†	3†	Mean	
(Exp. 1)					
15	0	0	0	0	
20	59	54	49	54	60–48
25	130	61	56	82	129–36
30	0	0	0	0	
(Exp. 2)					
15	0	0	0	0	
20	52	50	1	34	67– 2
25	93	92	76	87	98–76
27	108	61	47	72	108–36
30	0	0	0	0	

*Three males and 3 females each were kept in clay pots.
†Trial No.

in particle size (Table 5).

Cultures with the bed composed of crushed oystershell mixed with Kofu soil in different ratios were compared with each other on the fecundity of snails. Cultures with the bed of crushed oystershell mixed with Kofu soil in a ratio of 50:50 or 65:35 produced many more snails than those with the soil only. In the case of the bed composed of oystershell with different particle sizes and the soil, a greater number of young snails were produced when coarser particles of the shell were used (Table 6).

Kofu soil and crushed oystershell were autoclaved at 110°C for 30 min. When the bed was composed of sterilized oystershell mixed with sterilized Kofu soil

Table 5 Influence of particle size of crushed oystershell in bed on reproduction of *O. quadrasi**

Particle size (μm)	Number of snails hatched						95% confidence limit
	1†	2†	3†	4†	5†	Mean	
420–212	3	1	0	–	–	1	3–0
212–63	4	0	0	–	–	1	7–0
63–37	7	6	6	4	0	5	8–1
63>	24	16	5	–	–	15	55–9
37>	37	2	2	–	–	14	64–0
212–37	43	20	10	–	–	24	66–0
Kofu soil	194	119	77	50	50	98	173–23

*Three males and 5 females each were kept in clay pots.
†Trial No.

Table 6 Influence of mixed bed materials on reproduction of *O. quadrasi**

Materials and mixture ratio	Number of snails hatched							95% confidence limit
	1†	2†	3†	4†	5†	6†	Mean	
(Exp. 1)								
Oystershell	1	0	0	–	–	–	0	2–0
Kofu soil (50)+ oystershell (50)	170	125	111	110	106	73	116	149–83
Kofu soil (65) + oystershell (35)	97	81	64	–	–	–	81	122–40
Kofu soil	62	51	29	–	–	–	47	89–6
(Exp. 2)								
Kofu soil (50) + oystershell (212–63 μm) (50)‡	98	74	70	–	–	–	81	98–64
Kofu soil (50) + oystershell (37 μm>) (50)‡	68	62	17	–	–	–	49	81–18
Kofu soil	194	119	77	50	50	–	98	173–23

*Three males and 5 females each were kept in clay pots.
†Trial No.
‡Particle size of powdered oystershell is shown in parentheses.

Table 7 Influence of autoclave of bed materials on fecundity of *O. quadrasi**

Bed materials: Soil and mixing ratio	Number of snails hatched						95% confidence limit
	1†	2†	3†	4†	5†	Mean	
Kofu soil	134	129	119	115	95	118	131–105
Kofu soil (50) + oystershell (50)	235	198	176	130	123	172	213–131
Kofu soil (air-dried)	229	192	117	–	–	179	244–115

*Three males and 5 females each were kept in clay pots.
†Trial No.

at a ratio of 50:50 or the sterilized soil alone, almost the same number of juveniles were produced as in those with unsterilized Kofu soil. This shows that sterilization of the materials caused no adverse effects on the fecundity of the snails (Table 7).

Discussion

Many kinds of cultures for *Oncomelania* snails have been devised, such as a fish aquarium provided with a sloped bed, a plastic tray, a petri-dish, an unglazed large or middle sized clay pot and others (van der Schalie and Davis, 1968). In the present experiments, parent and juvenile snails were reared in different kinds of breeding systems because the parents are mainly terrestrial, while juveniles are aquatic in behavior. Parent snails were maintained in shallow unglazed clay pots with the soil bed, whereas juveniles were placed in aquaria including some amount of water on the bed composed of crushed oystershell instead of soil that was used in the breeding of *O. nosophora*. The main component of oystershell is calcium compounds, especially calcium carbonate. Oystershell has been used to supplement foods with calcium as calcinated powder, to purify water and to cultivate "nori" or a kind of edible seaweed because it has an antimicrobial activity and provides the weed with some object on which to attach itself (Ouba, 1988; Miura, 1992). In the present experiments many snails attached themselves to crushed oystershell placed in a culture on the bottom, and actively fed on the artificial food and grew rapidly.

Dropping of water from the reservoir into cultures increased dissolved oxygen in the water, prevented the water in cultures from contamination, stabilized the conditions of cultures and lessened the difference in growth of snails. Crushed oystershell made the surface of bed expand and so reduce the crowding effect.

In cultures of *O. nosophora*, filter paper is supplied as food, but in the present circulating aquaria no filter paper was used because the paper was ripped into pieces and consequently the water was contaminated. In the aerated closed-aquaria juvenile snails climbing up on the side wall fed on the filter paper attached to the wall, but when circulation of water was disturbed by the paper set in an unsuitable position or by its swinging by aeration, or the water was contaminated by the rotting of the paper, the habitat conditions of snails were occasionally upset to result in retardation of the growth of young snails.

Just hatched young *O. quadrasi* snails, 0.49 mm in shell length, attained 2.04 mm at 8 weeks of rearing (Chi and Wagner, 1957). A great number of juvenile snails kept in the present closed-aquaria attained 3 mm in shell length 8 weeks later. In the circulating aquaria, young snails grew to adults at weeks 8 to 12 of rearing and no crowding effect was observed similar to the condition in the closed-aquaria containing less than 100 parent snails. In this case the growth of young snails may have been accelerated by supplement of food with filter paper attached to the side wall of the aquaria.

Reproduction of snails is influenced by the temperature at rearing. A temperature of 26°C is optimum for rearing the snails among the temperatures

of 20, 26 and 32°C tested, being followed by 20°C, and at the optimum temperature parent snails begin to spawn earlier and produce a larger number of eggs and juveniles (Wagner and Wong, 1956). In the present experiments also, 25°C was the optimum. At 20°C it took a longer time for the start of oviposition, but almost the same number of young snails as at 25°C were obtained 8 weeks later. Snails grew well at temperatures from 25 to 30°C, although they grew to adults even at 20°C. From these results snails can be best maintained at 25°C throughout their life span.

In the water circulating rearing system, 4 aquaria were simultaneously supplied with water dropping from one reservoir, so that about 4000 snails could be maintained at the same time in the present system, because about 1000 snails could be maintained in one aquarium. The reservoir can supply water to more than 4 aquaria, so that more snails can be maintained at one time in the present system.

Even when the same Kofu soil was used, eggs and young snails were produced in different numbers. This is not considered to be a result from the difference in the term of experiments but from that in the age distribution of snails used or in the condition of the culture.

The soil from paddy fields inhabited by *O. nosophora* was best for the snail to spawn and hatch (Nihei, 1978a). Crushed oystershell used in the present study as a clean bed of cultures of *O. quadrasi* resulted in good growth of young snails, but in poor fecundity of parent snails.

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