

## A New Breeding System of *Oncomelania hupensis nosophora* in the Laboratory

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Because of a successful snail control in Japan, it has become difficult to obtain a great number of *Oncomelania hupensis nosophora*, the snail host of *Schistosoma japonicum*, from the field. Many trials of laboratory breeding of the snail have been reported, and Petri dishes, clay pots, plastic trays, butterfly jars, aquaria for tropical fish and others have been used as the vivaria for adult and young snails. We have devised to establish a new breeding system and to maintain snail colonies in the laboratory.

### Natural environmental conditions in the habitats of *O. hupensis* *nosophora*

Vivaria of freshwater snails should have environmental conditions simulated as nearly as possible to those of their habitats in the field. We observed the natural environmental conditions of the habitats of the *Oncomelania* snail at Ryo in Kofu City of Yamanashi Prefecture and at the bank of Tone River in Narita City of Chiba Prefecture. Most young snails inhabited the bottom of shallow waters, while most of adult crawled on moist muddy sites by the water. The substrate of the habitats was composed of a few layers of different components of soil. The surface layer princi-

pally consisted of mud, silt and fine vegetative debris, the lower one of sand, and the lowest one of sand and gravel. The surface was occasionally provided with sparse vegetation and supplied with the water that oozed up through the substrate. These natural environmental conditions of the habitats gave us an idea to make a new aquaterrarium for *Oncomelania* snails.

### The breeding system

The present system mainly consists of an aquaterrarium (at) for adult and an aquarium (aq) for juvenile snails (Fig. 1). The reason why an aquaterrarium and an aquarium are prepared for adult and young respectively is that *Oncomelania* snails change habitats from aquatic to amphibious with growth, as observed in the habitats of the field.

The aquaterrarium (at) provides adult snails with environmental conditions similar to those of natural habitats. A plastic container, 60, 45 and 20 cm in width, depth and height respectively, was used for the aquaterrarium. The substrate of the aquaterrarium is piled up on a plastic plate, which is placed 2 to 3 cm above the bottom, with many small holes and a vertical plastic pipe (d), but the bottom filter for tropical

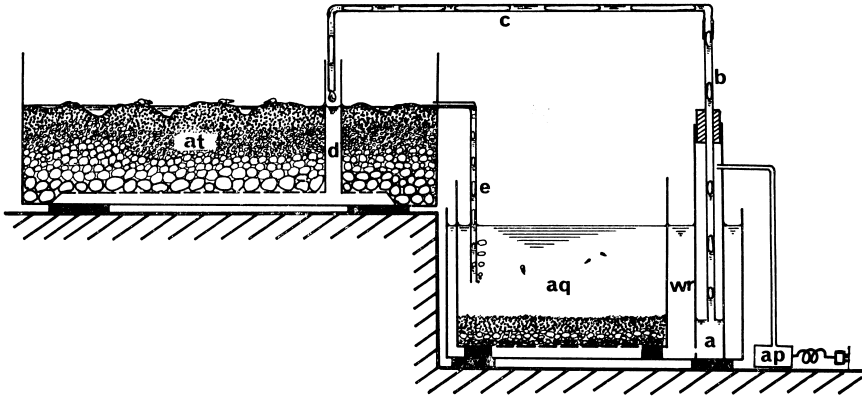


Fig. 1 New breeding system

- |  |                                   |
|--|-----------------------------------|
| a: Pump cylinder (polyvinyl chloride pipe) | c: Duct tube                      |
| ap: Air pump                               | d: Vertical pipe of aquaterrarium |
| aq: Aquarium                               | e: Drainpipe                      |
| at: Aquaterrarium                          | wr: Water reservoir               |
| b: Pumping tube                            |                                   |

fish can be utilized for that plastic plate. The substrate is composed of a few layers of mud and silt, sand and gravel. The surface of substrate, or the habitat of snails, is composed of mud and silt, where shallow winding channels are dug, which lead to the drainpipe (e). The water level in the aquaterrarium can be varied by changing the level of the drainpipe. In the case of amphibious *Oncomelania* adult snails the level should be kept for the water not to flood all the surface.

The water pump consists of three parts: the cylinder (a) which is a thicker polyvinyl chloride pipe about 25 mm in inner diameter and about 30 cm in length, a rubber cork and the pumping tube (b), a thinner glass or vinyl tube 5 to 10 mm in diameter. The length and diameter of the cylinder is varied according to the depth of water in the reservoir (Fig. 2).

The water pump sends water from the reservoir (wr) to the aquaterrarium by utilizing the force of air bubbles rushing up in the pumping tube (b). The efficiency of the pump is directly proportional to the difference between the water level of the

water reservoir and that of the lower end of the pumping tube. When the tube is 10 mm in inner diameter and the difference is more than 25 cm, the amount of water

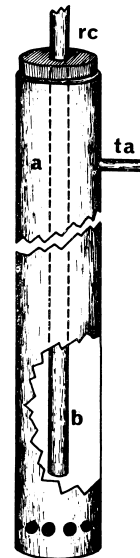


Fig. 2 Water pump. Showing the lower end of the pumping tube and small holes near the lower end of the cylinder.

- |                     |                 |
|---------------------|-----------------|
| a: Pumping cylinder | rc: Rubber cork |
| b: Pumping tube     | ta: Air tube    |

pumped up to the levels of 50 and 100 cm above was 150 and 70 ml per minute respectively. If the lower end of the pumping tube is not cut horizontally, air bubbles rush up through the tube but water is not pumped up. The water in the reservoir, furthermore, should be more than 5 cm in depth for the pump to function effectively.

The air pump and the water pump must be got balanced each other in capacity: when they are not so, exchange the pumping tube for another one with different diameter or the air pump for another with different capacity or change the distance between the water surface and the lower end of the pumping tube.

The open end of the duct tube should be kept free in vertical pipe (d) and also above the water level of the aquaterrarium. If not so, the water in the vertical pipe overflows and its outside gets wet, when snails climb up the side and go into the pipe. And further, when the upper end of the pipe is kept less than 10 cm above the water level, the same situation as above will result. If the tubes (b, c), especially at the joint between them, become clogged with fine soil particles or a sheet of green algae on the inner surface through being used for a long time, then the tubes should be exchanged for new ones.

The aquarium (aq) for rearing juvenile snails is made of a plastic container with three dimensions of 50, 27 and 30 cm and is set in the water reservoir, 45 by 35 and 25 cm in height. The aquarium is bored with many small holes in the bottom and also acts as the filter of the water that flows down from the aquaterrarium through the drainpipe (e). The filter layer of the aquarium consists of glass- or nylon-wool, gravel and sand and is piled up on the bottom in this order. The drainpipe of the aquaterrarium is a plastic tube about 7 mm in inner diameter and its lower end should be kept submerged in the aquarium. The aquaterrarium should be better placed

about 50 cm above the aquarium. When the difference in level between the aquaterrarium and aquarium is less than 50 cm, or the drainpipe is more than 10 mm in diameter, the water is not aerated sufficiently when flowing down through the pipe. But when the drainpipe is less than 5 mm in diameter, it is liable to be choked with adult snails and debris carried out of the aquaterrarium.

Soils used for the aquaterrarium and the filter bed of the aquarium were obtained from Ryuo in Kofu City of Yamanashi Prefecture and from the campus of our university. The soil was dried completely or sterilized by heating to kill small animals, especially earth worms in it, and thereafter, was sieved into silt and sand, and pebbles of different sizes.

#### **The routine maintenance of the system**

Snails were fed with the artificial food once a week. The food was prepared after a modified Standen's formula (1951). We substituted fresh lettuce for dried lettuce in his formula and added a flaked baby meal, dried chlorella and dehydroacetic acid as an antiseptic. The recipe is as follows:

Powdered wheat germ "Ebios"*	2.5 g
Flaked baby meal "Maimeal"†	5 g
Dried whole milk	2.5 g
Fresh lettuce	95 g
Dried chlorella	5 g
Sodium alginate	5 g
Dehydroacetic acid	1 g

Fresh lettuce is homogenized in a 400 ml of water. All the ingredients are added successively with sodium alginate last while being mixed together. The resultant soupy liquid is poured as a very thin layer into

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\* Manufactured by Ebios Yakuhin Kogyo Co. Ltd.;  
† by Meiji Nyugyo Co. Ltd.

shallow dishes such as used for photographic development. The dishes are then flooded with 2% calcium chloride solution. When the soupy liquid sets to gel, the solution is recovered and can be used repeatedly. The resultant insoluble gel of calcium alginate is cut into strips, which are then removed on a paper. The strips should be turned up several times not to stick to the paper until dried up completely, and are reserved in a can. The proper amount of food given once a week is different according to the number of snail present, but should be adequate for the snails to consume within 4 or 5 days.

On feeding snails once a week, the water in the reservoir is checked, the snails that have climbed up on the sides of the aquaterrarium are knocked down, and adult snails that have escaped into the aquarium are put back to the aquaterrarium.

On recovering young snails, the drain-pipe is closed every two weeks to flood the aquaterrarium and then the water is abruptly poured into the aquarium in order to wash away eggs and juvenile snails together with excreta of snails and accumulated waste, which foul the habitat and upset the balance. Young snails more than 3 mm in shell height are removed from the aquarium to the aquaterrarium or another vivarium.

When dried soil is used as the substrate, some kinds of vegetation usually grow on the habitat. If the vegetation is moderate in amount, it gives a favorable environmental condition to the snails, whereas if it is too thick, it would arrest the growth of snail. The overgrowth of green filamentous algae in the water of the habitat often inhibits the growth and breeding of snail if the sunlight is too much intense.

#### Cultivation of snails

*O. hupensis nosophora* was reared in the breeding system to clarify the effects of population density, food and illumination on the productivity and growth of snails.

*Oncomelania* snails were collected from the two habitats at Ryuo in Kofu City and the bank of River Tone in Narita City. Tap water was used without dechlorination and the water had been circulated in the system for a few days before experiments started. Experiments were undertaken from the spring in 1972 to the spring in 1979.

One series of experiment was performed in the greenhouse where the temperature was controlled at 20 to 30 C during the winter months only. The other series of experiment was undertaken at a temperature of 25 to 30 C under 20W 60 cm vegetable fluorescent tubes. Illumination on the habitat was controlled by changing the number of tubes and the distance between the tube and the habitat. The distance was varied 10, 15, 20 and 37 cm, and only in the case of a distance of 10 cm, the tubes were varies 1 to 4 in number. A light-dark cycle consisted of 13 hours of light alternating with 11 hours of dark.

The Kofu and the Tone River strains of the *Oncomelania* species have been maintained in the greenhouse since May and October of 1972 respectively, and the Kofu strain has produced 18 successive generations. The highest productivity was gained at an inhabited area of about 40 cm<sup>2</sup> per snail (Table 1). Young snails were produced from the end of March to the beginning of October, and a seasonal reproductive periodicity was clearly observed. The highest growth and recovery rate of young were obtained at an inhabited area of about 10 cm<sup>2</sup> per snail in the aquarium. Young snails grew to adults in about three months (Table 2). Productivity and growth of snails were much accelerated by feeding the artificial food of the modified Standen's formula (Table 3).

Under artificial lighting *Oncomelania* snails reproduced only where the cultures were illuminated by one fluorescent tube suspended 10 to 37 cm above, but no young was produced where illuminated by 2 to 4

Table 1 Relation between population density of adult snails and their productivity

Inhabited area per snail (cm <sup>2</sup> /snail)	No. of young per adult snail at different time after experiment started			No. of dead shells
	1.5 months	2 months	3.5 months	
10.9	4.2	3.0	1.8	10
16.1	4.4	1.2	3.3	5
23.3	2.8	3.7	5.8	4
30.3	2.4	11.7	19.8	6
41.8	5.4	43.3	61.1	5
45.0	4.7	15.1	27.0	5
51.9	3.1	9.8	17.8	5
60.0	3.4	9.7	14.8	3
69.7	2.7	12.1	13.3	0
75.0	2.3	12.2	17.0	4
103.9	0.7	4.0	5.7	1

Experiment was made for 3.5 months. Snails of 5 different generations were used. Juvenile snails were put back to aquarium after counting. Adult snails were differentiated from young by marking with manicure liquid.

Table 2 Relation between population density and growth of young snails in aquarium

Inhabited area per snail (cm <sup>2</sup> /snail)	Average shell height (mm) at different time of experiment*			Rate recovery of young at end of experiment (%)†
	At start	1.5 months	3 months	
2.5	1.52	2.42	5.32	37.2
5.0	1.50	2.53	4.63	67.0
10.0	1.52	3.61	6.34	67.4
15.0	1.50	4.23	6.27	56.1
20.0	1.52	3.45	6.34	38.0
30.0	1.27	4.15	6.08	40.7
46.4	1.27	4.20	6.93	44.4
59.7	1.27	3.75	6.25	28.6

Experiment was made for 3 months. Snails of 3 different generations were used.

\* Shell length was determined by measuring 50 snails randomly at start of experiment and 20 snails 1.5 and 3 months after start unless sufficient number of snail for measurement could not be obtained.

† All the young snails were counted with the naked eye.

fluorescent tubes suspended 10 cm above cultures. Young snails were produced in the greatest number where the cultures were lighted by one fluorescent tube 15 cm above the habitat (Table 4). It was not ascertained whether snails reproduced all

the year round or not under the artificial lighting because the experiment was performed only in the period from the winter to the next spring when no juveniles were produced in the greenhouse.

Table 3 Effect of the artificial food on productivity of adult and growth of young snails\*

	No. of adult snails at start of experiment	No. of young snails	No. of young per female	Average shell height of young (mm)†
Feeding	34 (M 11 : F 23)‡	597	25.96	5.75
Not feeding	34 (M 11 : F 23)	283	12.30	2.75

Experiment was made for 84 days. Adult snails were differentiated from young by marking with manicure liquid.

\* The artificial food after a modified Standen's formula

† The shell height was determined by measuring 20 snails randomly.

‡ M: male, F: female

Table 4. Effect of illumination on productivity of snails

	Distance from fluorescent tubes to habitat (cm)							
	10		15		20		37	
No. of tubes*	1	2	3	4	1	1	1	1
No. of young snails	52	0	0	0	230	72	12	12
No. of dead adult shells	5	6	12	18	3	4	8	8

Experiment was made from 22 December to 22 February in 1977. 45 adult snails had been allocated and kept in each aquaterrarium about 2 months before the experiment started.

\* 20 W 60 cm vegetable fluorescent tubes were used.

### Discussion

Many types of vivaria have been reported for rearing and breeding of *Oncomelania* species. Some of them are used for raising a small number of snails for a short period of time (Sandground and Moore, 1955; Komiya *et al.*, 1959; van der Schalie and Davis, 1965; Wagner and Chi, 1959). Recently, a few types of culture have been developed for successive or mass-breeding of the species (Matsuda *et al.*, 1969; Matsuda 1969; Oshima *et al.*, 1971; Iwanaga and Tsuji, 1972; French, 1974; Ku *et al.*, 1975). Matsuda (1969) and Matsuda *et al.* (1969) prepared different types of vivaria for breeding of adult and for raising of young. Oshima *et al.* (1969) developed a technique for mass-cultivation with a new apparatus, which consisted of the rearing box, purifying box and electronic thermostat. Ku *et al.* (1975) used unglazed clay pots for mass-

breeding of *Oncomelania* in the laboratory.

We have devised a new system for successive breeding of *O. hupensis nosophora* without troublesome maintenance. One of the features characteristic of our system is that the habitat of adult is constantly supplied with adequate moisture by the water that oozed up through the substrate. Most of snails inhabited the banks of channels in the aquaterrarium. Oshima *et al.* (1969) stated that the flow of water in the zigzag channel of the "rearing box" prevented the development of bacterial film on the water surface. In the present aquaterrarium the water is always aerated and circulated and flows in the channels, where no bacterial film developed. Another feature is that the substrate of aquaterrarium and the filter bed of the aquarium are supplied with aerated water, and oxidation of waste matters will be made in the filter bed of aquarium and the substrate of aquaterrarium. Vevers (1972) stated on the

function of filter bed of marine aquarium that aerated water could accelerate the oxidation of ammonia to nitrite and nitrate by microorganisms coating the grit particles in the bed. It is troublesome and time-consuming to collect young produced and to remove them to other vivaria, especially when different kinds of culture are prepared for oviposition of adult and raising of young. In the present system this troublesome task is performed by flooding the aquaterrarium every two weeks. This is another feature of our system.

The experiments of the influence of soil on the oviposition and growth of young have broadly yield inconsistent results. Some concluded that a few kinds of soil had an inhibiting effect on oviposition and resulted in retardation of the growth, whereas others had not (Tsuda, 1972; Kawamoto, 1954). Nihei (1978a, b, c) performed precise analytical examinations on soils adequate for oviposition and growth of young. She concluded that the characters of soil gave a remarkable influence on the survival or growth rate of young and that the soils adequate for the breeding of young were gray, gray brown and brown soils with the texture between sand and clay, which could be found along great rivers. In our experiments, soils from the habitats of *Oncomelania* in Kofu City and from the campus of our university were used for the substrate, and no remarkable differences were seen between those two soils in fecundity of snail.

Many kinds of natural or artificial food have been used for cultivation of freshwater snails. Standen (1951) prepared an alginate gel food for rearing a freshwater pulmonate *Australorbis glabratus*. The Standen's formula was modified by Moore *et al.* (1953), Sandground and Moore (1955), and Oshima *et al.* (1969) for *Oncomelania* species. Our formula was a Standen's modified by adding and substituting a few ingredients. One of them was flaked baby meal "Maimeal"

which was first used by Yoshikawa (1965) as the food of *Lymnaea ollula* with success. The artificial food used was attractive for *Oncomelania* too when given submerged in the water and prevented the habitat from being fouled because the food was insoluble in water. In addition to the artificial food, microflora such as diatoms might thrive in the water of the aquaterrarium and supply snails with a natural adequate food. Komiya *et al.* (1960) described that diatoms and other algae should be the main food-stuff of *Oncomelania* snails in their natural habitat. Dazo and Moreno (1962) concluded from field observation that *O. quadrasi* appeared to be a herbivore and its diet consisted mainly of green algae and diatoms, and that the species consumed a wide variety of plant foods and had no special preference. Davis and Werner (1970) further stated that the ability of a soil to sustain proliferation of diatoms dependent on the presence of microchemical constituents, which was an important factor. In the present system the water might be supplied with these constituents when passing through the substrate of aquaterrarium. French (1974) found that the fecundity of *Oncomelania* snails was positively proportional to the soil surface area of vivaria, and stressed the importance of a large mud-surface-area for rearing young to maturity. In our experiment the productivity was highest at an inhabited area of about 40 cm<sup>2</sup> per snail, and it reduced with the increase of the area. This may be caused by incomplete recovery of juvenile snails because of low population density.

A large quantity of filamentous green algae occasionally grew in the aquaterraria and aquaria placed in the greenhouse and inhibited the growth and fecundity of snail when the sun light was too much intense. van der Schalie and Davis (1968) also stated that stronger constant light caused increased rates of death of adult snails, and one of the main factors involved was the rapid

proliferation of algae on the soil. They (1965) and French (1974) described further that excessive algal proliferation hindered the movement of young and caused its death and that dense algal mats tended to limit the soil surface available for snails. In the present aquaterraria, when soils were used after dried up, some amount of emergent vegetation usually grew and covered the habitat, and this prevented green filamentous algae from overgrowth and consequently covering the habitat.

Several kinds of organism have been reported to occur in cultures of freshwater snails. Earthworms were most harmful for maintenance of the present aquaterrarium because they churned up the substrate and the snail habitat was disrupted with piles of casting of earth worms. The invasion of earthworms could be prevented by complete drying or heating of soil used. van der Schalie and Davis (1968) stated that the cultures were heated in an oven at 60 C for 2 hours to eliminate the difficulties caused by earth worms.

Seasonal reproductive periodicity was reported by some authors. Kawamoto (1954) observed oviposition of *O. hupensis nosophora* from the middle of April to the middle of June in the laboratory and Ishii and Tsuda (1951) from May to August. Ku *et al.* (1975) observed the high fecundity of field-collected *O. hupensis* from March to June with the peak in May and no oviposition from August to October in the laboratory. DeWitt (1952), on the other hand, successfully bred four species of *Oncomelania*: *quadrasi*, *nosohora*, *hupensis* and *formosana* in the aquaterrarium without regard to the seasons. van der Schalie and Davis (1968), Davis and Iwamoto (1969) and Matsuda (1971) reported that field-collected or laboratory-reared *O. hupensis nosophora* reproduced throughout the year in the laboratory under constant or cycled light at a conditioned temperature, and the field-collected population showed the peak

output in July. Matsuda (1969) stated on the periodicity of reproduction in the laboratory that though field-collected snails produced many young for 2 to 3 months after collection, thereafter the number of young produced decreased and further that the snails might adapt themselves to the temperature after successive breeding in the laboratory and become to reproduce constantly throughout the year. Our snail colonies reproduced from the end of May to the beginning of October in the greenhouse where only the temperature was controlled at 20 to 30 C during the winter months, whereas they seemed to reproduce throughout the year under the artificial lighting because juveniles were produced in the winter months when no juveniles were produced in the greenhouse. That clear seasonal periodicity of reproduction in the greenhouse might be caused by the influence of seasonal photoperiodicity on snail reproduction and/or by its influence on proliferation of microflora as food, which will result in the deficiency of microflora in non-reproductive months. Wang *et al.* (1956) also observed that the number of eggs increased proportionally to photoperiod. Davis and Werner (1970), moreover, concluded that the diatom diet was correlated with continual development of fully mature oocytes of *Oncomelania* snails. Ku *et al.* (1975), on the other hand, observed no much difference in fecundity between the field-collected and laboratory-bred snails but seasonal variation of reproduction in snail populations bred in different time of year, and stated that it might be possible to secure continuous breeding throughout the year by spacing the egg-laying of mother snails.

The present aquaterrarium can also be used for rearing other freshwater snails than *Oncomelania* if water level is varied by changing the situation of drainpipe. If the water level is more heightened, it can be applied for breeding the aquatic species



such as *Semisulcospira* and *Lymnaea*, and if it is lowered, land snails may be raised.

### Summary

A new breeding system has been established for successive maintenance and mass-breeding of *Oncomelania hupensis nosophora*. The system mainly consists of an aquaterrarium for adult and an aquarium for young snails. The aquaterrarium provides adult with amphibious habitat similar to that in the field, and the aquarium does young with aquatic habitat. The habitat of adult is supplied with adequate moisture by the water oozing up through the substrate of the aquaterrarium.

The Kofu strain of the snail species has been maintained in the laboratory over 18 successive generations in the same aquaterrarium without any troublesome routine maintenance other than supplying food and water once a week and removing grown-up snails from the aquarium to the aquaterrarium every two weeks.

Optimum inhabited area per snail was 40 cm<sup>2</sup> for breeding of adults and 10 cm<sup>2</sup> for growth of juveniles. Feeding the artificial food after the modified Standen's formula accelerated productivity of adults and growth of juveniles.

The snail colonies reared in the greenhouse showed clear seasonal periodicity in reproduction, whereas those under the artificial lighting seemed to reproduce throughout the year.

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## 宮入貝の新飼育装置と飼育実験

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実験室における宮入貝の継代飼育と大量飼育のための装置を考案した。本装置はおもに成貝飼育槽と稚貝飼育槽とからなり、成貝の飼育場所には地層を通してしみ出す水によって適当な湿気を与え、また稚貝の飼育水槽は同時に濾過槽を兼ねている。本装置の特徴は曝気した水を濾過循環させ、成貝と稚貝にそれぞれ好適な生息環境を与え、また、長期の大量飼育に適するように飼育管理の省力化を図ったことである。餌料として人工餌料を用い、成貝の繁殖と稚貝の成長に好結果を得た。卵と孵化した稚貝を稚貝飼育水槽に移すには、成貝飼育槽の排水管を一時的に閉鎖して浸水状態としたのち、急に排水

し、そのときの水の力で卵と稚貝を稚貝飼育水槽に洗い流す方法をとった。

甲府産宮入貝は同一飼育槽で18代の継代飼育に成功し、管理としては週に1度の給餌と蒸発した水の補給、2週に1度の卵と稚貝の洗い流しを行うだけである。貝1個当たりの好適飼育面積は、成貝の繁殖のためには約40cm<sup>2</sup>、稚貝の発育には約10cm<sup>2</sup>であった。

本装置は成貝飼育槽の排水管の取り付け位置を変えることによって水位を調節でき、高くすればカワニナ、モノアラガイなどの水棲貝を飼育することができ、反対に低くすれば陸産貝を飼育できる。