

Research Article

Biological Characteristics and Propagative Technologies of *Rhodeus sinensis*

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Abstract: Aim of study on biological characteristics and propagative technologies of *Rhodeus sinensis* is of great significance to rationally utilizing its resource, protecting the species, improving the benefit of aquaculture. Research contents comprise biological characteristics, living habits, propagative habits, the source of its parent fish, rearing of parent fish and spawning via artificial estrus and incubation. It is discovered that the main point for propagating *Rhodeus sinensis* lies in identification of the parent fish of *Rhodeus sinensis*, appropriate proportion among males and females, employment of odinagogue, appropriate selection of categories and specifications of freshwater mussels.

Keywords: Biological characteristics, freshwater mussel, propagative techniques, *Rhodeus sinensis*

INTRODUCTION

Rhodeus sinensis which is a kind of famous protogenic aquarium fish in China belongs to the *Rhodeus* category, Acheilognathinae subfamily, Cyprinidae of Cypriniformes, it is a kind of widely distributed and tiny fish among respective river systems in China, especially along the Yangtze River system. This kind of fish is featured with beautiful colors and graceful body contour, thus can be cultivated for ornamentation and has become a kind of well-known protogenetic ornamental fish in China. With living conditions being improved continuously, more and more people are demanding for ornamental fishes, people's demand for *Rhodeus sinensis* is also steadily increasing, whereas, it is relatively difficult to meet such demand by way of fishing from the wild, which makes it imperative to cultivate *Rhodeus sinensis* through artificial breeding, therefore, propagative techniques have become the foremost task to be researched and solved.

This study summarized the research of the reproductive biology and artificial breeding of *Rhodeus sinensis*. It to be used as reference for the extensive group of bitterling breeding enthusiasts.

MATERIALS AND METHODS

The collection and feeding of material fish: The experiment was conducted in 1.0×0.6×0.5 m glass aquarium with no mud in tank bottom, still water under normal temperature, regular water exchange to keep well quality, feeding water as Yangtze River water, pH 7.2~7.6, hardness of water is about 120 mg/L and

Dissolved Oxygen (DO) content is more than 6 mg/L. The *Rhodeus sinensis* with body mass of (1.13±0.32) g that belongs to natural population captured from riverway of Taizhou City, LiXiahe Area of Jiangsu province. The early temporary culture adopted natural food chironomidae larvae (red worm) and the tubificidae, then gradually changed into basic mixed feed, siphon faeces, food residue etc. The healthy individuals shall be selected as the experimental fishes.

Morphology index measurement and fecundity determination: Adopt digital calliper to measure the morphometrics of *Rhodeus sinensis* and then analyzed its feeding habits. Observe the batches of oviposition, fecundity per batch and total fecundity according to one female to one male paired. The batches of oviposition was shortened to one time with every extension on the ovipositor of female fishes, then examine and verify the egg output, record for one time. According to the number of fish eggs and fry spitted by mussel or the number of eggs counted by dissecting the mussel, the number of eggs per batch can be ascertained to calculate the total number of eggs.

The selectivity of mussel: Three kinds of mussels: *Anodonta woodiana*, *Unio douglasiae* and *Cristaria plicata* captured from the riverway in Xiahe Area of Jiangsu province be put into the aquarium at the same time. After the completion of every batch of oviposition, inspect to ascertain whether exist egg in those three mussels, then record its number of eggs. Further, to judge the selectivity of *Rhodeus sinensis*'s egg on those three common mussels as well as other ones with different sizes.

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Reproductive behavior observation: To treat the experimental fish in the following methods for observation at the coming of mating season:

- One female, one male, put into mussel
- One female, 2~6 male, put into mussel
- Two female, two male, do not put into mussel
- Two female, no male, put into mussel
- Two females, no male, do not put into mussel

The observation on swimming time of fry from mussel: The mussel with *Rhodeus sinensis* egg was fed singly, record the oviposition data and the mussel's swimming time, then observe the growth characteristics of fry at the time of swimming. The experimental observation lasted from October to the following October. Record the various physicochemical indexes and data every day during the experimental period.

RESULTS AND DISCUSSION

Biological characteristics of *Rhodeus sinensis*:

Primary characteristics: *Rhodeus sinensis* is featured with compressiform, tiny head, with mouth terminal, without barbel, incomplete lateral line, there are 3-4 scales in its front side, it has one row of pharyngeal teeth, 5/5; the teeth surface is smooth, there are dimples on the surface of the pharyngeal teeth; its bladder has two compartments, the rear one is relatively larger than the other one, 9-11 fin rays are distributed along the entire dorsal fin area, 10-12 fin rays are distributed along the entire anal fin area, zigzagging number of its intestines is around 2.2~2.8, number for its gill raker is around 6-8; length of the digestive tract is about 1.0~1.58 times of its body length. Body size of this fish is usually small, the largest body size is usually not more than 8 cm, such silver blue longitudinal band along its body center extends as far as the rear side of the dorsal fin origin.

Body color of male *Rhodeus sinensis* with in the mating season is relatively more bright than usual, the red halo around the eyes and the red spots around the tail are darker, silver blue dots appear around the rear upper side of its spiracle, those longitudinal strips along the tail fin center present orange red, besides, both the front outer fringe of the dorsal fin and the anal fin present red, no fin rays are generated around the pelvic fin area, the entire fin shows oyster white color, black color is dotted at the outer edge of the anal fin, beard spots can be observed on the rostral side and supraorbital area. Whereas, female *Rhodeus sinensis* is featured by tiny body, bumped belly, black dots can be observed on the dorsal fin of a female *Rhodeus sinensis* with sexual maturity, body color of females is lighter

Table 1: Analysis of feeding habits of *Rhodeus sinensis*

Food category	Frequency for food occurrence
Aquatic higher plant debris	+++
<i>Oscillatoria</i> sp.	++
Crustacean	+
<i>Microcystis</i> sp.	+
<i>Zygnema</i> sp.	+
<i>Spirogyra</i> sp.	+++
<i>Synedra</i> sp.	+
<i>Amphora</i> sp.	++
<i>Scenedesmus</i> sp.	+++
<i>Tribonema</i> sp.	+++
<i>Cosmarium</i> sp.	++
<i>Cladophora</i> sp.	++

Visual inspect the congesting degree of its intestines and stomach, classification standard with 4 grades will be adopted for discrimination of satiation degree; Add water in the contents contained within the digestive tracts and made them into smears; Put such smears under microscope in order to discriminate the food category; Occurrence frequency should also be recorded

comparing with males, ovipositor can be observed to be reaching out of its abdomen.

Living habits: *Rhodeus sinensis* is broadly distributed in a lot of water areas, such as Amur River, Yellow River, Yangtz River and the Pearl River. This kind of fish inhabits at the underlayer of the shallow water area of lakes, reservoir and rivers, they prefer swarm swimming within water body with relatively slow fluidity and lush aquatic plants. *Rhodeus sinensis* is a kind of omnivore fish, mainly living on algae and organic detritus (Table 1), sometimes on aquatic plants and higher plants (Zhang *et al.*, 2010, 2001). This kind of fish usually gather together into a group within their alevin stage, most of them live a kind of swimming life by resting at the edges of aquatic plants near the river banks or at the upper water area without aquatic plant near the river banks; at the end of the alevin stage, those fishes would perform various kinds of swimming along the aquatic plant area, plain swimming, slanting swimming, rotating swimming and sideway jumping swimming can be witnessed, which are beneficial for escaping from enemies and acting; juvenile fishes and adult fishes prefer to live at the underlayer of the water area. Water area with temperature limited within 4~35°C, dissolving oxygen capacity limited in 4.0~8.3 mg/L and pH value limited within 6.8~7.5 is suitable for habitation of *Rhodeus sinensis*.

Breeding habits: The *Rhodeus sinensis* reaches sexual maturity when they are growing to 1 year old, most of the male and female *Rhodeus sinensis* with sexual maturity are 3-5 cm in body length and weigh 1.4-2.1 g. Breeding period is limited within February to October with water temperature around 12.6~22.3°C, starting and ending of their spawning are probably caused by rising water temperature in Spring time and shortening of photoperiod in Autumn time (Asahina and Hanyu, 1983). Spawning high season is usually in April to June

Table 2: Descriptions of three different eggs of *Rhodeus sinensis*

Category	Shape	Diameter/mm	Length/mm	Accumulation of yelk	Distribution	Color
1	Ball shape	0.2~0.5		No accumulation	Within the ovary	Grey white
2	Ball shape	0.6~0.8		No accumulation	Within the ovary	Orange yellow
3	Cucurbit shape	0.2	2.1~2.6	With accumulation	At the bottom of the ovary	Orange yellow
	Cucurbit shape	0.8~1.0				

of each year, with eggs laid in different batches, such eggs are in ball shape or cucurbit shape with grey white or orange yellow color (Table 2). If without any outer intervention, spawning activities usually last from the morning until the evening, which are relatively more frequent in the morning time. The average brood amount of each fish ovum is 127, the absolute brood amount for the minimum ovum is 74, the absolute brood amount for the maximum ovum is 238, fecundity for each batch is not so high with an average of 10 eggs (Wang *et al.*, 2013). Intervals among respective breeding periods are short and regular within the middle term of the propagative season (May to June), which usually lasts 3-6 days, whereas, in mid summer season (July to August), short-time spawning would happen, making such intervals be extended to 16-42 days. Relative fecundity of *Rhodeus sinensis* is 120-500 grain of eggs per gram, corresponding relative average fecundity is 270 grains of eggs per gram, obviously less than crucian carps and pseudorasbora parva (Gozlan *et al.*, 2002). *Rhodeus sinensis* does not possess such a capability in protecting their larvae through both parents, but in laying eggs in the gill cavity of clams for incubation, those fish larvae would stay within the clams after they were hatched for a certain duration until they are capable to ingest by themselves. Such special place environment for spawning, not only supplies sufficient dissolved oxygen, but also helps to protect them from being buried by sand and preyed by the predators, thus is extremely beneficial for increasing survival rate of the fertilized eggs and larvae, this has compensated such weakness of low fertility in a certain degree (Lelck, 1987).

Within the non-breeding season, *Rhodeus sinensis* prefers to move in group with other bitterling. Once the breeding season comes, they begin to move in different groups. When female fishes are ready for spawning, they begin to show their special interests towards the fresh water mussels, their ovipositors will reach out farther than ends of their tail fins, corresponding variation scope for the length of their ovipositors is between 2~31 mm, color of the same turn from dark red to light red. Male fishes show extreme excitations within the breeding period and maintain quite hostile attitudes towards their companies of the same gender. Male fishes would search for appropriate mussels and occupy its surrounding area, shivering their fins all over their bodies especially their tail fins, in order to induce mature female fishes and drive away individuals out of same gender who come nearby or other kinds of fishes.

Once they find any female comes near, such female will be induced to his selected mussel through multiple fierce chasings. Those female fishes who have stayed would change their body postures and angles frequently, pecking the osculum of the mussel with their jaws for a long time until the mussel cancels its alarm and opens its osculum, once water is ejected from the osculum, the females would intensively shrink their muscles, shiver respective fins, rush abruptly from downside to the upper side, so that they can insert their ovipositors into the osculum of the mussel, then their ovipositors will enter the prosopyle from the osculum and then reach deeply into the internal body of the mussel, spawning can be performed in time, upon completions of which, their ovipositors will be instantly be withdrawn, such females will then swim away after all the above procedures are completed. At the same time, this awaiting male will promptly rush to the same mussel and inject sperms towards the prosopyle of the mussel and drive away any other female fish that swim near the mussel in order to ejaculate again. The same mussel will then be witnessed being evaded with white sperms and thus will inhale large amount sperms to make those laid eggs fertilized. After one complete breeding activity is closed, such male fish may give up on this mussel, or may choose to stay and guard for this mussel in order to chase after female fishes for next round of spawning.

It is also possible sometimes, that if activity of the *Rhodeus sinensis* is as disturbing as touching the mussel, making such mussel close its both shells tightly and clamps the ovipositor of the female fish and cause damages to the same, luckily, damaged ovipositor will grow into new one again without affecting next round of spawning activity. *Rhodeus sinensis* a kind of fish that lay eggs in batches, even eggs out of the same batch could probably be produced in several times, corresponding intervals for the same can last from around ten minutes to several hours, therefore, the entire producing process will last for several days. Eggs out of the same batch can be laid within one mussel, or can also be laid into 2 or more mussels. After producing, ovipositor of the female fish will shrink, color of such ovipositor will become darker, which is an visible indication for avoiding escaping from male fishes. Such spawning in batches is especially advantageous under unstable environment, not only helping them in saving their descendants from being completely killed under harsh climatic conditions, but also in relatively increasing their individual fertility

(Nikolsky, 1963). Even without mussels or stimulations from male fishes, ovipositors of those mature female fishes will still extend or shorten, but their spawning activities will visibly sacrificed or inhibited, which is in similar situation with European bitterling (Haranghy *et al.*, 1977). Under extreme panics or intensive stimulations such as fishing or extruding, etc., mature female *Rhodeus sinensis* will also undergo abortions. Due to their long ovipositors, it is frequently discovered that their ovipositors are filled with many aborted eggs in yellow color.

Propagative techniques for *Rhodeus sinensis*:

Origin of the parent fish: *Rhodeus sinensis* is widely distributed among respective water areas in China, due to their intensive stress reactions, those parent fishes would probably be hurt or killed after being captured and therefore are not suitable to be adopted as parent fish, whereas, juvenile fishes are more resilient to totally new man-made environment and are able to rut and breed, therefore, it is suggested to cultivate parent fish from juvenile fishes. There are usually two different methods for obtaining juvenile fishes, one of which is when the *Rhodeus sinensis* is in breeding season, capture fresh water mussels from reproductive water areas and cultivate juvenile fishes through those mussels until parent fishes are cultivated; The second way, is within June to August, juvenile of *Rhodeus sinensis* usually gather at the upper layer of the water body or at the funning water outlet in order to search for food, under which circumstance, those juvenile fishes can be captured with dip net, those juvenile fishes with typical black dotted dorsal fins, symmetrical body figures, relatively higher body figures, fine scale color, without external injuries and body length at around 2 cm will be selected for further cultivation.

Cultivation of juvenile fishes: Fertilized pond cultivation: it is required that water retention capability of the pond be in good condition, it convenient for discharging and injecting of water, the pond bottom should be flat and smooth, thickness of sludge is around 10-20 cm, area of such pond should be appropriately set at around 100-600 m², water resource should be sufficient, without being contaminated, the site should be equipped with convenient transportations, supporting power supply and with micropore aeration system (Chen *et al.*, 2013a). Half a month prior to stocking of juvenile fishes, the pond should be spayed with quick lime in order to clean the pond. Five days later upon such cleaning, water injection into the pond should be conducted, with water depth within the pond being maintained around 0.4 m, 10 kg manure should be spayed for each square meter within the pond in order to cultivate plankton and thus supply *Rhodeus sinensis* with sufficient baits. When water injection is being conducted, bolting-silk net should be used to filter any

other wild or miscellaneous harmful organisms from entering the pond.

Cultivation through aquarium: Juvenile fishes have to live within an environment with clean water rich in oxygen and with fluid water, the bottom of the aquarium should be spayed with granules and equipped with water pump, breeding density should be well controlled, it is optimum to cultivate around 12-18 fishes within an aquarium with an area of 80×60×30 cm, so that mutual chasing and attacking activities will be controlled at a minimum degree. The power pump is only required to be started for 1-2 h, illumination time within each day should be guaranteed with more than 1 h. Aquatic plants such as *Hydrilla verticillata* and *Vallisneria natans* etc., should be planted in order to provide hiding place for *Rhodeus sinensis*, what should be noted is, that frequent change of water is not allowed, because over frequent change of water will panic *Rhodeus sinensis* and disadvantageous for cultivation of fresh water mussels. Baits for juvenile fishes mainly come from cyclop, based on activities situation of those juvenile fishes, 2-5 times of feeding per day, with each fed baits being completely eaten within 0.5 h is preferred. Man-made complex feed can be fed when the juvenile fishes grow longer than 3 cm, earthworm syrup and paste of mussel meat can be used as phagostimulant together with man-made feed, proportion of phagostimulant can be reduced day by day until complete compound feed is fed. It is expected to cultivate separately between male and female fishes when *Rhodeus sinensis* juvenile fishes are cultivated as old as 4 months.

Cultivation of parent *Rhodeus sinensis*: *Rhodeus sinensis* shows aggressivity even in their juvenile period, then those juvenile fishes grow as old as 4 months, their sexual characters begin to appear (ovipositors of female fishes begin to reach out of their body through the cloaca, metallic colors begin to appear on the dorsal fins of male fishes), differences between male and female fishes in terms of their body contours grow increasingly, male fishes begin to chase after those individual fishes near them, it is initially not beneficial for female fishes in food obtaining process, therefore, male and female fishes are separated for cultivation. With growing, body length of male fishes become more intensive in terms of their aggressive behaviors, therefore, stocking density can be correspondingly reduced with introducing of stone moroko, etc., in order to distract them.

Proper algae or aquatic plants should be provided in the parent cultivation process, periodical testing towards water quality, such as water temperature, dissolved oxygen capacity, nitrite, hydrogen sulfate, ammonia nitrogen and pH value should be conducted (Chen *et al.*, 2013b). Water quality modifying agent

should be periodically utilized, bioactivator, such as Photosynthetic bacteria, Effective microorganisms, composite bacillus, etc., should be adopted every half or one month, in order to modify water quality and maintain good circulation of water body. During the breeding test period, the pH maintain at about 7-8.5, ammonia nitrogen ($\text{NH}_3\text{-N}$) <0.02 mg/L, nitrite (NO_2^- -N) <0.1 mg/L, sulfide <0.2 mg/L, dissolved oxygen above 5 mg/L. Water testing method can be refer to 'Water and Wastewater Monitoring and Analysis Method '4th Edn., (China State Environmental Protection Administration (CSEPA), 2002). *Rhodeus sinensis* intake food all the year round, it would get worse appetite and less movements when water temperature is lower than 4°C, but feeding behaviors will still carry on. Their appetite is in the best condition when the water temperature is within 18~21°C. Once water temperature is maintained at more than 15°C constantly, *Rhodeus sinensis* will step into their breeding season, within which period, parent fishes should be selected. Those *Rhodeus sinensis* with their body length longer than 3 cm and bigger body figures should be chosen as reservation of parent fishes.

The provisional pond for maturation of those selected *Rhodeus sinensis* parent fishes should be sterilized with potassium permanganate at a concentration of 5 mg/L for 10 min prior to transferring them into this pond, density of fishes within the pond should be controlled at 500 g/m³. Every 4-m² water surface within the provisional pond should be placed with one toad which weighs around 50 g, for the purpose of preventing from fungal disease. The provisional cultivation water should be sufficient in oxygen, algae, such as scenedesmus and chlorella should be trans-planted, natural sunlight should be provided with corresponding transparency controlled within 40-50 cm. small grain broken debris should be adopted as baits, with comprehensive nutrition within the feeder and more than 30% of protein content. Daily feeding volume should be gradually increased based on their feeding situation and should be controlled within 5-10% of their body weight. Developmental situations should be observed frequently in the parent cultivating process, once water temperature ramps over 17°C, occurrence rate for females' ovipositors is beyond 90%, length of partial females' ovipositors could extend as far as end of their tail fins, indicating, artificial spawning period is entered.

Artificial spawning of parent *Rhodeus sinensis*: Sexual maturity of male fishes is earlier than females, which can be easily identified by means of secondary sexual characteristics. The male shows yellow pearl organs on the jaws and opercula during breeding season. Under such situation, maturity of male fishes can be tested by means of fresh water mussels, when male fishes begin to touch mussels and fight for

mussels, it is time for them to breed. Testing method for maturity of female bitterling lies in whether their ovipositors are turning red and their ovipositors are as long as basis points of caudal peduncle. Considering maturity of bitterling eggs is in batches and mature eggs are in small quantity for each batch, ocytotic can therefore be adopted for maturity promotion. One of the method for spawning inducing is injection ocytotic, narcotize the fish with 30 mg/L MS-222 dipping for 2 min to deactivate the parent fish, it is time for injection of ocytotic once there is no intensive shivering, such method is usually conducted in the morning. Another method is through such specific ocytotic made from composite LRH-A+DOM, which should be injected through the abdominal cavity, female bitterling be injected with 0.1 mL/fish, male bitterling be injected with 0.05 mL/fish. Then, it be placed into the spawning pond for ovulating after their recovery. Such injection method is efficient but difficult in practice and consumes relative more time. Feeding method can also be adopted, mix LRH-A+DOM complexing agent into the diet, successful spawning can also be realized.

Matching proportion among male and female bitterling: During the breeding season, male bitterling usually present colorful body in order to attract females. Those males have strong characteristics in territoriality, they are responsible for searching for proper mussels, then they will delineate certain territory and carry out defensive activities for their territories in an effort to drive away the other contenders. In case too many male fishes are living under one identical environment, fierce competitions will occur frequently, their physical power will be consumed, breeding activities will be impacted, therefore, matching proportion between males and females has certain influence on the success rate of breeding. It is proved that a matching proportion within 1 to 1~1 to 3 between males and females is more appropriate. Breeding success rate will be lower while the proportion is more than 1 to 6 because of fights and competitions, whereas, if such proportion is lower than 1 to 1, then fertility rate be sacrificed due to limited number of males. Within the entire breeding period, each female fish is capable to spawn for multiple times, each cycle lasts around 10 days, variation of the same can be reflected by means of morphological changes of their ovipositors, at the same time, male fishes maintain their strong sexual desire, therefore, appropriate proportions are beneficial for improving the fertility rate.

Selection of mussel size by *Rhodeus sinensis*: Breeding of *Rhodeus sinensis* is realized through their laying eggs via ovipositors into the mussels' body, those eggs will be hatched inside the gill of the mussel (*Anodonta woodiana*) and grow to larvae prior to swimming out of the mussel. Different situations in

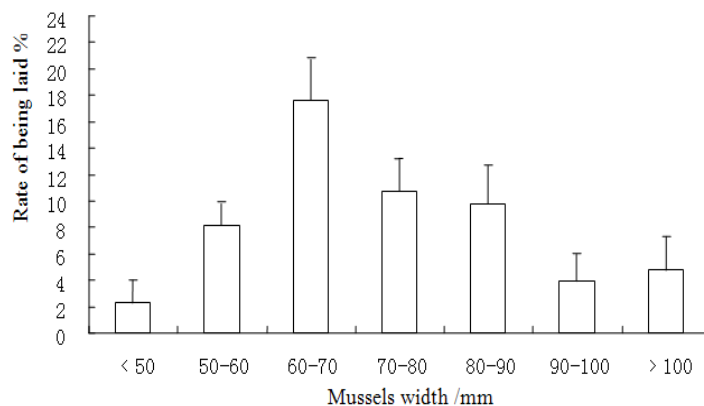


Fig. 1: The choice of Chinese bitterling (*R. sinensis*) spawning in mussel (*A. woodiana*) with different shell width

terms of mussel size selection for spawning by *Rhodeus sinensis* under an environment where various sizes of mussels are cultivated can be referred in Fig. 1. The result showed that if width of mussels is limited within 70 mm, rate for spawning of *Rhodeus sinensis* inside the mussels increases with the increment of volume of the same mussel. Mussels with 60~70 mm width have the highest rate of spawning from the Chinese bitterling, which is visibly higher than the other groups ($p < 0.05$). There is no significant differences between 50~60 mm group and 70~90 mm group ($p > 0.05$), corresponding rate is remarkably higher than groups with widths less than 50 mm and more than 90 mm. The result show that mussels with widths less than 50 mm and more than 90 mm are not optimum options for *Rhodeus sinensis* to lay their eggs.

Specifications of aquarium adopted for this experiment is 150×40×50 cm, capable to hold 240 L water. Seven groups are divided according to the shell width of all mussels, namely, group with width less than 50, 50~60, 60~70, 70~80, 80~90, 90~100 mm, respectively and group with width more than 100 mm. Select randomly from each group 10 mussels then place them into the aquarium. Choose 140 groups of parent *Rhodeus sinensis* with sexual maturity and strong figure (corresponding weight 1.13 ± 0.32 g, body length 51.12 ± 5.15 mm) and place them into the same aquarium. Equip the aquarium with circulating water filtration unit in order to purify the water. Inspect spawning situation every 7 days and make detailed records. Checking method adopted is to open the shell of the mussel with self-made shell opener in order to count the quantity of eggs. Then remove those mussels which have already been laid with eggs and replace them with new mussels to conduct next round of experiment, repeat four times. Rate of being laid is calculated through ratio between number of mussels with eggs and total number of the same group of mussels, rate of being laid with eggs = number of mussels with eggs/10.

The Rosy bitterling will select *Sinanodonta woodiana* and *Unio douglasiae* rather than *Corbicula fluminea* as their target for laying eggs (Xie *et al.*, 2005). The spawning habits of *Rhodeus sinensis* show that in case of insufficient mussels, ovipositors of females will be shortened, intervals will be extended. Through observations towards spawning situations in *Cristaria plicata*, *Sinanodonta woodiana* and *Unio douglasiae*, spawning frequency and quantity in unio douglasiae is more ideal than the other, indicating that spawning of *Rhodeus* has their preferences in the categories of mussels (Shen, 2000). There has some investigations towards bitterling eggs distributed on the mussel flaps (Zeng *et al.*, 2006). Mills and Reynolds (2002, 2003) found that ventilation rate of mussels may be taken as one critical indicator for *Rhodeus sericeus* to select their hosts, Kawabata *et al.* (1992) found that amino acid such as cysteine and serine, etc. has inductive functions towards sexual behaviors of the rose bitterling. Smith *et al.* (2000) found that indicators for *Rhodeus sericeus* to select mussels had strong relations with the content of dissolved oxygen inside water.

Through anatomy, we find that it is less common for mussels with bigger sizes to be laid with eggs, some of those eggs swim among the gill lamella. There are many sensitive tentacles at the blast-holes of mussels and close their blast-holes in case of encountering foreign matters, which is not suitable for bitterling lay eggs. Whereas, the surface of outlet of mussels is relatively smooth without any tentacle, its opening is also relatively bigger, sensitivity to foreign objects comparing with the blast-hole is relatively lower, only when stimulated by intensive stimulations, will the outlet be closed. Prior to breeding, male Chinese bitterling will touch the outlet of mussels for multiple times in order to reduce their sensitivities and make them more appropriate for spawning. The blast-holes and outlets are separated merely through the outer membrane muscle, parts at the back side of the outer edge muscle are connected, once the ovipositor is

inserted into outlet pipe, it will enter the gill cavity of the mussel with the water flow from the blast-holes, so that eggs can be laid. Thickness of such muscle becomes a critical factor in successful spawning, mussels with larger shells usually have thicker outer edge membrane muscles and deeper outlets, adding more difficulties for *Rhodeus sericeus* to insert their ovipositors. Besides, bigger mussels have relatively gill chamber walls, eggs cannot be hung and can be easily discharged outside the mussel's body. Whereas, outer edge membrane muscle of a smaller mussel is relatively thinner, the mussel can be easily irritated due to long ovipositor into its body, causing ovipositor being damaged due to closing reaction of the outlet of the mussel, therefore, this situation is also not good for successful spawning. Therefore, *Rhodeus sericeus* have their selection towards sizes of mussels given that only proper mussel can meet spawning requirements.

Collection and incubation of spawns: Depth of the spawning pond is approximately 40~50 cm, its area is limited into 50 m², water quality is required to be good with green or brown color, transparency is 30~40 cm, the content of dissolved oxygen is above 6 mg/L. Each square meter of water area should be placed with 150 groups of male and female parent fishes and distributed with around 200 *Sinanodonta woodiana* or *Unio douglasiae* with shell width around 60~90 mm for egg collection. Two days later, mussels for laying eggs can be collected. Check spawning situation with specially made egg checking machine, then transfer those mussels which have already been laid with eggs into the incubation pond, compensate the spawning pond with corresponding scale and quantity of mussels. Only around 20 days are necessary for the spawns to be hatched into juvenile fishes.

CONCLUSION

Judging from spawning situation, *Rhodeus sinensis* belongs to continuous spawner. Eggs will be laid in batches based on whether they are mature inside the ovary. Such kind of breeding tactic can be branded as the optimum manner for miniature fish categories such as bitterling, since it's beneficial for improving their fertility, so that generative descendants can be guaranteed to maintain reproductivity of their groups, propagative techniques mainly lie in the natural spawning method, *Sinanodonta woodiana* or *Unio douglasiae* with proper sizes should be selected which shell width around 60~90 mm. For matching among parent fishes, a matching proportion within 1 to 1~1 to 3 between males and females is more appropriate. When such proportion reached 1 to 6, fights behavior will become more visible and lower reproductive success rate, whereas, if such proportion is lower than 1 to 1, fertility rate will be sacrificed due to limited

number of males. Oxygen deficiency issue should be cared for breeding of mussels, since those mussels prefer to take algae selectively, water color should also be adjusted well and algae should be compensated periodically.

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