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Cultivation of Chinemys Reevesii (Chinese Three Keeled Turtle) in Greenhouse System

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Abstract

Chinemys reevesii (Chinese three keeled turtle) is developed in china's turtle farms for production in pet trade market and chinese medicine. The aim of this study was to calculate growth rate and survival rate of juvenile *C. reevesii* under the greenhouse based overwinter breeding technology to survive and not to get weight loss. 2000 stocking density were cultured in each twenty tank and then randomly collected 10 turtles from each tank during January to may 2019 to calculate growth rate. The results showed that the temperature was maintained within the range of 30 ±2°C and ambient air was controlled by 32±2°C. After five months cultivation, final weight reached average 278.44 ±57.61 g with 125.13±99.73 final weight gain by high survival rate of 89.8± 2.73% and low feed conversion rate 1.8±0.2. Moreover, resulting dissolved oxygen 3.8 to 9 mg/l, pH between 7 to 8, ammonia and nitrite at 0.005 to 0.02 mg/l showed suitability of this species cultivation. As a result, this study will be hopeful artificial husbandry to increase healthy growth rate during overwintering turtle in greenhouse system.

Keywords: *chinese three keeled turtle; greenhouse; growth rate; survival rate, FCR, temperature, water quality*

Introduction

For many centuries, turtles have been used as food, pets and in traditional medicine in different regions of the world [1]. Freshwater turtles are being developed for traditional medicine, meat, eggs and pet trade. Because of this, turtle farmings are boomed in aquaculture industries in the past two decades [2]. As farm bred turtles took advantage of good numbers in the last 20 years, the market share has raised significantly. Asia especially china is stood at the top of world turtle consumption as food and medicine. Despite having used in turtle products for thousands of years, turtles command a high price and this is the reason to develop farms in china [2]. To organize large scale commercial turtle farms some companies have invested larged sums of money. Although early efforts focused almost exclusively on the Chinese soft shell Pelodiscus sinensis [1], the farm output of this species has now satiated demand. Thus, farms have started to rear more valuable hard shelled turtles.

Chinemys reevesii, Reeve turtle (or Chinese three keeled turtle) (family Geoemydide) is widely distributed in eastern asia, including Central and Eastern contenital china, Taiwan, Kimmen island, Southern and central japan and korea [3] This species is one of the most commercially important turtles for aquaculture and is widely cultured in China [4, 5]. Shell from C.reevesii are the most valuable in the high volume Traditional Chinese Medicine market in Taiwan [1]. In china, especially Anhui provience, the estimate production rate is flucturated from 2012 to 2018. More than 80% of this species occur in china [6]. This species has been classified as endangered in the IUCN Red List of Threatened species [1, 6] and Chinese Red List of Threatened species [7] owing to overhunting and habitat destruction in the wild.

As turtle are reptiles, hard shell turtles cannot control their internal body temperature so that they are greatly affected by temperature. The best way for warming themselves is to move to be warmer place during the winter. In central china such as Anhui Province, hard shell turtle usually hatches during June to September and juveniles are too weak to grow through winter time. Most hard shell turtle farmers always use greenhouse system to hibernate for them during winter and for early start up. In the low range of temperature, they can get weight loss. In the greenhouse, air and water temperature are needed to control constantly between 30 and 32°C (Li et al, 2009). There are many kind of greenhouse designs in markets so that farm owners can choice as they like. Turtles can adapt to natural or artificial environments well, but in greenhouse, turtles, when reared in high density and poor environment are prone to disease. To minimize such occurrences, culture techniques and several culture models, have been developed and introduced as standard processes for turtle culture. Hence, the main objective of this paper was threefolds (1) to utilize greenhouse based breeding program (2) to use effective microbe in monitoring water quality under greenhouse (3) to develope economic

model of hard shell turtle by describing weight increment for current and future turtle farming. This study will be able to get advantages to reduce farmers hesitate using greenhouse.

Materials and Methods

Experimental Culture Site, Greenhouse Design and Construction: The green house was located in Luan city in western Anhui Province. Its administrative area spans 31 3002.15 N Latitude and 116 36 06.35 E Longitude. Luan has a monsoon influenced, humid subtropical climate. Winter are cold and damp, the January 24 hours average temperature is 2.6 C (36.7F). Summer is typically hot and humid, with a July average of 27.8°C (82.0°**F).Because of long time low temperature** conditions, it was not suitable for the growth of juveniles three keeled turtle and could hardly survive. In this study, the cultivation period in greenhouse was 150 days from January 2019 to may 2019.

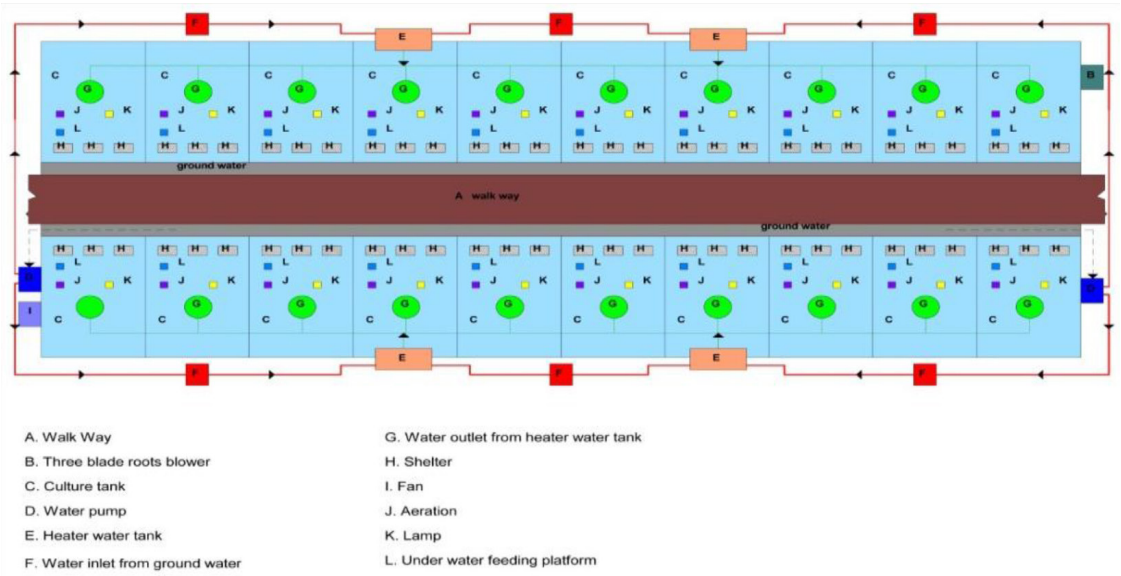

Figure 1: Plan view of greenhouse.

Figure 2: Construction of Greenhouse in Luan city in western Anhui Province, China.

Figure 3: Juvenile *Chinemys reevesii*.

The greenhouse design of chinemys reevesii were considered for the suitability of its behavior and physiological parameters and effects on the external environment and construction cost. Its design for juvenile chinemys reevesii were (1) to gain healthy turtles with good weight gain, (2) to control for a suitable and healthy culture environment, such as optimal air and water temperature and good water quality, (3) use of effective microbes to reach high productivity.

Figure 4: Juvenile turtle pellet.

The cardinal whole factory was 1130m^2 and made rooms for two greenhouses, two hatching rooms, one storage rooms and the other facilities such as oxygen supply equipment, four oxygen generators, pipeline oxygen supply and drainage facilities. Each greenhouse has twently 14.17 m^2 culture tanks. All tanks were made of concrete brick. For each culture tank, there were included shelter, underwater feeding platform, water inlet and outlet drainage system, temperature control system. As turtle are timid animal and hide behind shelters without feeding, they need shelters to take a rest, activity, hidden from noise and sudden circumstance. Those shelters, 1.2m squared polyethylene knotless small mesh (0.5cm square) were parallel with a distance about 80cm to each other at the depth of 30cm water. For feeding, feeding platform were used in each culture tank. Each asbestos platforms (100 x 120 cm) was places under water with 40cm depth. There were foam insulation cotton on the roof. Total project budget for one greenhouse were about 130,000 RMB (approximately 18571 USD). Construction began in March 2016 and was completed by May 2016.

Aeration system, water supply and discharge system: In the greenhouse, a three blade roots blower (2.2kw) was used for aeration system. When the compressed air was turned on, an upwelling flow with microbubbles was generated. Nine air stones for each culture tank were aerated constantly.

Two 4 mL x 0.62mH x 1.3m W heater water tanks was filled from the ground water with water pump. These tanks ran to culture tanks with water outlet pipe line system. Two 4cm diameter water supply inlets were set through the tank wall to adjust water level through valves. Chinese three keeled turtle are not good swimmer so that the water depth should be kept relatively shallow [8]. The reason to keep shallow water was that it was easy to swim and reduce energy and also save production cost. Moreover, according to the turtle respiration system, they have to come water surface to breath air for a while. Therefore, water level was controlled at 0.8m.

For discharge system, the tank bottom was built in the form of wok shaped sloping toward the central drain. The bottom type drainage caliber of greenhouse culture tank was 40cm width. As uneaten feed and fees settled down at the bottom as the organic debis, they can easily siphoned to the seawage ponds for filtration system. Each culture tank had their own discharge system because their own discharge system can prevent the cross contamination and infection. Water was drained one tenth of the amount of water in each culture tank once a week. The underground 5 cm diameter pipe was connected to L standing pipe outside the culture tank.

Temperature control system: The framework pipes was made up of stainless steel to support the arc shaped ceiling. The framework was so strong that this can not only withstand snow falling in winter but also light insulation. There was sharp slope on both sides of the roof not to stay snow and to fall off. The highest point of the celling was 1.8m. Welling and ceiling were built with thermal insulation layers.

As with temperate species, Chinese three keeled turtles are absolutely affetcted by temperature. The only recourse they have for warming themselves is to remove warmer place for winter period. Temperature gradients should be provided for the water, ambient air and basking area [9, 10]. To provide a warm environment in turtle greenhouse, air need to heat first and secondly water is warmed through air to water heat transduction and aeration. Firstly water heating system may cause evaporation and inside greenhouse foggy and poor visibility. Moreover, moist air can be a favour to grow pathogenic microbes. Water should be maintained within the range of 30 to 32**°C,** the ambient air between 32–34°C. A basking area where the turtle can leave the water completely, with a good basking light to help the turtle thermo regulate, is an absolute must. A good heat emitting light should always be provided over the basking area. For basking area there were four 32 Watt in each culture tank.

Stocking: The culture tanks had size 420 cm x 600cm x 68cm and water level was 0.8m depth as the water depth should never be less than about 1.5 times the length of the turtle shell. Before, transferring the turtle into the culture tank, they are needed to disinfect with calcium oxide solution at 50–100 mgl^{-1} for 3–5 days. Stocking density in the culture tank was 2000 turtles m^{-2}. The average weight of the turtles were 123.68g.

Useage of effective microbe: Effective microbe, Lactobacillus acidophilus and Candida prion producing strain were used. The effectiveness of this product can proliferate beneficial intestinal flora, control the harmful bacteria to the intestine, stomach, and pancreas of the hard shell turtle, so as to achieve the purpose of producing bacteria by bacteria reduce the occurrence of disease [11, 12]. The beneficial compound bacteria contained in this product can attach to animal feces, decompose the excreted feces twice reduce the pollution of feces to water body from the source, control the function of sticking, heat, eutrophication of water body and harmful algae breeding. Aquatic

feed is a high protein and nutrient substances so that long term stacking will breed and contaminate will breed and contaminate with a set of bacteria [13]. This product has the function of keeping fresh preventing feed mildew and removing the clamor for mildewed feed.

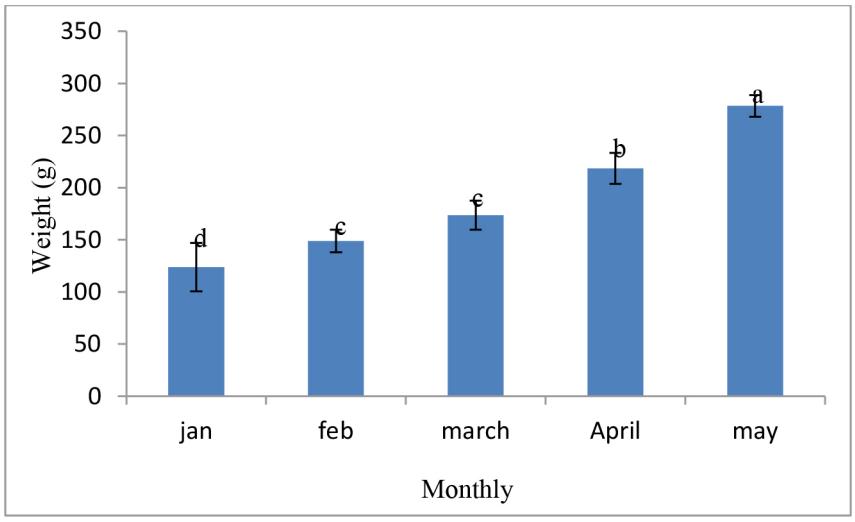

Figure 5: Expression of weight increment of juvenile *chinemys reevesii.* Bar indicate the standard error of the mean (n=30). Different Latin letters on top of the columns denote statistically significant differences (P <0.05).

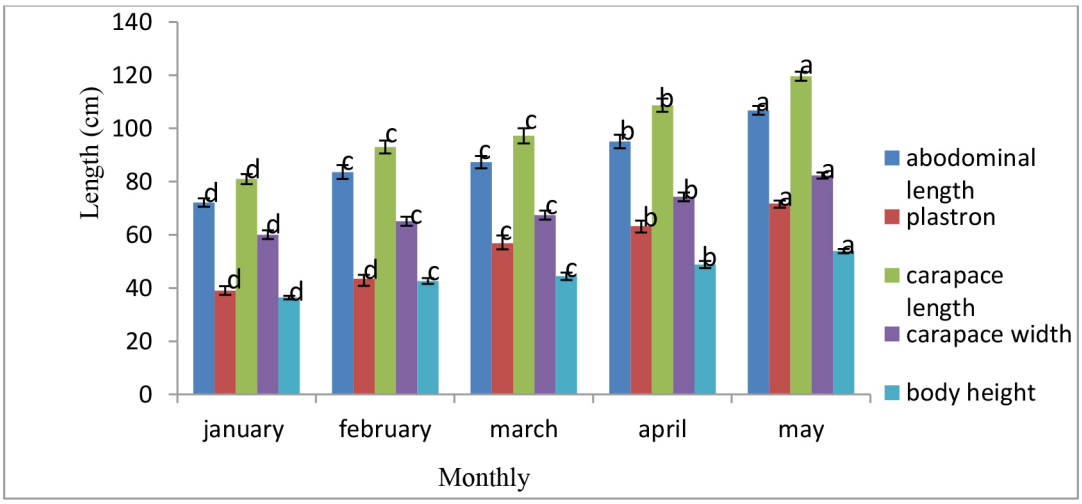

Figure 5: Expression of length increment of juvenile *chinemys reevesii.* Bar indicate the standard error of the mean (n = 30). Different Latin letters on top of the columns denote statistically significant differences (P<0.05).

Water management: Although the uneaten feed less and feces waste from culture tanks were routinely drained out to the settling tank, the dissolved organics from the waste can still remain and accumulate in the culture tank [12]. It will be able to become favourable for microbe propagation. Because of this, the water quality will be declined. This situation was especially evidenced in intensive type of aquaculture [14]. It was reported that bacteria can get rid of pollutants by digesting them [15]. EM sprayed to culture water body at the concentration of 5gm-3culture water once a month in the first 3 months and 10 gm-3 of culture water at fortnightly. Water was collected monthly around 10:00 Am for each pond and water temperature, PH, dissolved oxygen, ammonia and nitrite were analyzed by standard methods using spectrophotometer.

Feeding: Reeve turtle are omnivores and there is a wide variety of commercial turtle food available on the market and most have been formulated to provide optimum nutrition for aquatic turtles at all stage of growth. Juvenile hard shell turtles were fed juvenile feed from Fujian Zheng Yuan Feed Co, Ltd. Feeding time was at 7:00 Am and at 18:00 Pm per days for winter. In this experiment, starting weight for turtles was average 123.68g. Breeding density was 2000 turtles per pond. The proximate chemical composition of turtle feed was moisture ‹10%, crude protein ≥42%, crude fat ≥5%, crude fiber ≥5.0%, crude ash ≤16.0% ,lysine ≥2%, Calcium ≤4.5%, total phosphous ≥1.2% and water ≤ 11.0%. We added vitamin C for extra immunity protection. The maximum amount of feed per day have been 40kg.

Moreover, Effective Microbes (EM) was fed when it first came out of shell. According to the manufacturer's specifications, Lactobacillus acidophilus, the main microbe in its EM, could secret acidolin, acidophilin and aetocidon and enhanced antagonistic function against entire pathogens in the intestine and to improve the immunity. EM needs 1000g for the whole greenhouse. It was fed every half month.

Total ratio of feed rate was about 3% body weight at the first month and then altered monthly because this ration was determined monthly observations and experiment. If the uneaten feed was left, it was cleaned through the central drain.

Result

Survival and Growth rate: The juvenile initial weight was mean 123.68 ± g and final at 278.44± 57.61 g. The final survival rate got 89.8 ± 2.73% after five months period. Furthermore, no bite mark, no bleeding, no pathogen had been checked. It was shown that this stocking density, 2000 turtle/tank was suitable for this period. Temperature is the major environmental factor for survival and growth weight.

Feed and feeding: In aquaculture, one of an important parameter was Feed Conversion Ratio (FCR) because upto about 60% of the total production cost was represented by feed cost. The lower the feed conversion ratio, the higher the economic return. Feed conversion ratio in this trail was 1.8 ± 0.2 SD (Table 1). The suitable nutrition and crude protein contant 42% became the good diet. In this trail, turtle was already finished within one hour after observation. Moreover, the usage of solid underwater feeding platform gave the favorite feeding site of juvenile turtle.

Table 1: Survival Rate and Growth Rate of juvenile *C.reevesii.*

	Final weight	Final survival number	Survival Rate	Weight Gain	FCR
Mean	278.44	1796	89.8	125.13	1.8
Standard deviation	57.61	82.26	2.73	99.73	0.2

Water quality: Every tank was tested water monitoring system. Changes of the monthly average temperature, ammonia, nitrite and dissolved oxygen in culture tanks from January 2019 to may 2019 are shown in Figures (6, 7& 8). All water quality parameter was met well water quality standard for fisheries (people republic of china national standard GB 11607–89). Water temperature was maintained within the range of 30±2°C which was the most favourable temperature for juvenile turtle's intake and growth. Geothermal heat pump system effectively controlled the narrow variation of temperature balance.

The monthly ammonia and nitrite were in the range of 0.005 to 0.02mg/l. In this experiment, good water quality could maintain by two factors. Firstly, routinely discharge of organic debris by central drainage greatly reduced the loading in the culture tank. Second, the use of EM may facilitate the assimilation of ammonia into microbe and reduced ammonia [12]. The dissolved oxygen level was maintained 3.8 to 9 mg/l and pH between 7 to 8.

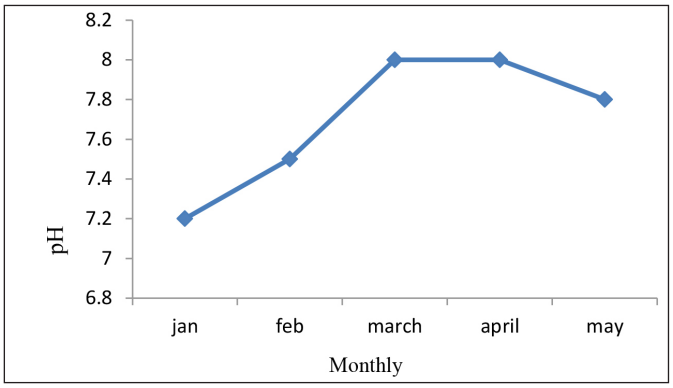

Figure 6: Average monthly pH of greenhouse culture pond from january to may.

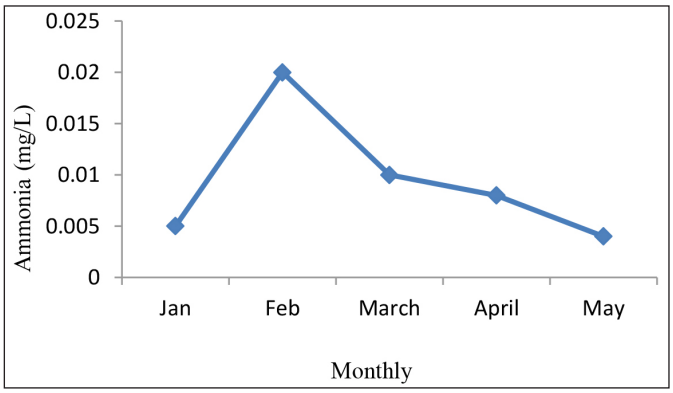

Figure 7: Average monthly Ammonia of greenhouse culture pond from january to may.

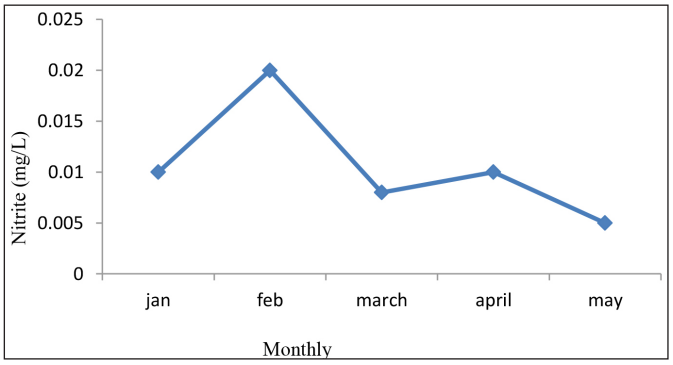

Figure 8: Average monthly Nitrite of greenhouse culture pond from january to may.

Ecological analysis: The cost of one greenhouse was 130,000RMB including slope shape greenhouse frame and plastic cover, twenty culture tanks and basic equipments. Electric fee was about 3369.63 RMB per month for one greenhouse. Labor cost was on the basis of 1500 RMB per one month and the feed cost was 13320 RMB per month. Benefit of production rate was 7500 RMB per ton.

Discussion

The cultivation of juvenile Chinese three keeled turtle in greenhouse with suitable environment was important in green aquaculture system because this species was endangered species according to IUCN red list and also stood highly in medicine and pet trade market. Moreover, turtle cultivation in greenhouse were caused high mortality and not suitable for environment. In this paper, by using green house

can show that there was no serious disease and pathogen event when checking any turtle health. Furthermore, the green house was designed to provide suitable and stable living environment [16]. The water temperature was steady under the control of ground source heat pump system. Stocking density influences survival, growth, health and feeding. Thus determination of stocking density for cultured animals is essential for optimizing production, profitability and sustainability. Hard shell turtle can fight and bite each other for food and space when getting high stocking density [17–29]. Generally, high stocking density can get high production rate but it will not always be true for economic benefit. In this study, the whole period survival rate and final stocking density number were 89.8±2.2% and 1796±84.23% respectively (Table 1). After monthly checking these species, no disease, no bite each other and no destructive each other were shown that this stocking density and culture tanks' capacity gave safety production. The reason for high increasing nitrite and ammonia concentration was due to high stocking density with dead turtle, feed residue and feces. But this study demonstrated that water quality of ammonia and nitrite was safe range because of good stocking density, usage of EM microbe and wok shaped tanked bottom design during this five month period. As shown in table 1, FCR 1.8± **0.2 SD and protein content 42**% was also favourable because this study used underwater feeding platform so that it can easily calculate feed conversion ratio after checking and calculating.. The ammonia and nitrite were in the safe range because the usage of EM. It was shown that water quality environment was suitable for the growth of juvenile turtle. The final weight, survival rate and FCR were also favourable after checking and calculating during these five months period.

Conclusion

This study could report that the greenhouse cultivation was more suitable than outer pond in winter period. Because of shelter, it can reduce the fighting and mortality so that it can get high survival rate. Moreover, underwater feeding platform system gave good feed and feeding facilities. Furthermore, the wok shaped tank bottom design was effective in great drainage system and maintained water to be cleaned. Usage of effective microbes could provide not only water quality but also feed rate. High survival rate 898±2.73%, good feed conversion ratio 1.8±0.2%, good water quality was concluded that it was excellent business by cultivation of juvenile turtle in greenhouse during over winter period. Moreover, the future of turtle farming is not only important for the economy of the region but also has potential to serve as the foundation for conservation and recovery of wild turtle population.

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