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Larviculture techniques of Chinese mitten crab *Eriocheir sinensis*

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ABSTRACT

Eriocheir sinensis is considered a luxury aquatic food for Chinese people due to its delicate flavor, and it therefore reaches a high market value. Its hatchery production and farming are being performed almost exclusively in China. Although breakthroughs in seed production and larval rearing techniques of *E. sinensis* have been achieved in the early 1980s, a fast expansion of hatchery production only took off in the 1990s, with the dramatic decline in natural recruitment. Many techniques have only been published as brief descriptions of local farmers' experience and most of these articles were written in Chinese. This paper provides general information on the hatchery techniques in aspects of broodstock maturation, spawning and larval rearing and points out the main bottlenecks of current mitten crab hatchery operations.

1. Introduction

The Chinese mitten crab *Eriocheir sinensis* is an indigenous species in East Asia, with a native distribution along the eastern pacific coast of China, from 24°N northwards to the Korean Peninsula, 42–43°N; from 124°E westwards to 112°E (Gu and Zhao, 2001). Yet it has been recognized as an invasive species in Europe and Northern America, probably translocated through ballast water of ships over the past hundred years (Herborg et al., 2003, 2007; Rudnick et al., 2003; Ruiz et al., 2006). Mitten crabs are considered as a most nutritious and delicious crustacean by Chinese consumers, and thus have high economic value in China.

Unlike Penaeid shrimp and freshwater prawn that are produced in large parts of the tropics and sub-tropics, mitten crab is only cultured in China. Before the 1980s, aquaculture production of mitten crab relied on the provision of wild megalopa and the restocking of captured megalopa in lakes. However, natural recruitment became severely exhausted in the late 1980s due to over-fishing and the construction of dams and irrigation works. The dramatic decline in availability of wild-caught megalopa has greatly stimulated the development of controlled production of seed to stock ponds. A significant improvement in hatchery techniques for mitten crab was achieved in the beginning of the 1980s (Zhao, 1980). The boom in mitten crab aquaculture however only emerged ten years later, especially since Penaeid shrimp culture in China was hit by severe virus outbreaks in the early 1990s. The total production in hatcheries doubled from 3.2 billion megalopa (227 mt) in 2001 to 9 billion megalopa (638 mt) in 2005 (China Fisheries Yearbook, 2007). Meanwhile, the annual yield of market-sized mitten crabs increased from 17 500 mt in 1993 to 570 000 mt in 2005 (Cheng et al., 2008). The development of mitten crab aquaculture can be divided into two stages: from 1993 to 2004 research mainly focused on production improvement; since 2005 the quality (mostly in terms of body size) has been emphasized. Mitten crab aquaculture spread nearly all over china, whilst hatchery production is mainly in the coastal area along Yangtze River and Liao River basin, where the mitten crabs are naturally distributed.

Overall, three major populations are recognized according to the river estuaries where they live: the Liaohe, Yangtze and Ou Rivers. Although a number of studies have been conducted to distinguish the different geographical populations through the analysis of morphological, biochemical and molecular variations (Li et al., 1993; Li and Li, 1996; Xu et al., 1997; Li and Li, 1999; Li and Zou, 1999; Zhao and Li, 1999; Zhou and Gao, 1999), the structure of native mitten crab stocks still remains unclear in terms of population genetic structure (Sui et al., 2009c). Mitten crabs from Yangtze River basin are the favored broodstock resource due to their better growth performance and more delicate taste, though there is little documented evidence to support this preference.

2. Broodstock management

In commercial hatcheries, the selected males and females are maintained separately in freshwater. Before mating, the salinity of the water needs to be elevated in order to achieve the final maturation of both female and male crabs (Zhang and Li, 2002). Brood crabs mate and spawn at an optimal water salinity of 13–17 and temperature of 9–13 °C.

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The ratio of female and male is 2:1–3:1. Mating occurs either in late autumn or early spring when the water temperature is low enough. In the first case, the berried females carry the eggs over winter, which is considered as a way to obtain a higher spawning rate and better egg quality. The window for megalopa production is very limited, i.e. only in the period of April to May in the Yangtze River estuary (Zhang et al., 2002), although the extension of the reproduction period can be archived from January to June by temperature manipulation (Sui et al., 2007).

Mitten crabs are capable of spawning up to two times from a single mating. Fecundity is about 1 million eggs for the first spawn. Second maturation of the ovary in captive crabs occurs about a month after the first spawn, which is much shorter than for the first maturation (3–4 months). A substantial reduction of broodstock survival, egg production and larval quality is however generally observed with subsequent spawns (Ji et al., 2006; Nan et al., 2006). Few hatcheries make use of re-matured broodstock due to the fact that egg supply is not a limiting factor in mitten crab larviculture.

3. Larvae rearing techniques

3.1. Indoor intensive larval rearing

In indoor intensive larval rearing system, the fertilized eggs can develop into zoeal larvae within 20 days at temperatures of 15–21 °C and a suitable salinity. According to the unpublished observations, a higher incubation temperature leads to a shorter incubation period but smaller-sized larvae. Time of egg hatch can be predicted by monitoring development of the embryo under a dissecting microscope. The grey color and transparency of the eggs, a heart beating rate of 170–200 min^{-1} and well developed eye spots with a purplish patch are all indications of imminent hatching. Before being transferred into the hatching tank, the berried crabs are usually disinfected in a 100–200 g L^{-1} formalin bath for 1–2 h to remove possible attached parasites and fungi. The berried crabs are normally placed in cages that are suspended inside the hatching tank, to which the initial diet for the larvae (usually *Chlorella* sp. and diatoms) had been added or bloomed in advance already. In order to maintain a better water quality, berried crabs are not fed 1–2 days before egg hatching. When larval density in the tank reaches a preset level, the cages are removed from the hatching tank and transferred into another hatching tank, where the females will continue to release larvae.

The eggs hatch as zoeal larvae at an optimal temperature of 18–21 °C. Larval development includes five zoeal stages and one megalopa stage. In order to shorten the production cycle and consequently reduce the production cost, in practice the temperature is gradually increased from 21 °C at zoea 1 (Z1) to 24–25 °C at Z5, and then decreases gently to the natural temperature range at megalopa stage (Su and Xu, 1996; Zhu and Li, 1998). In commercial hatcheries, larval development takes 2–4 days for each of the zoeal stages and 5–10 days for the megalopa stage depending on the rearing temperature, food availability and water salinity.

Although the initial breakthrough in the mitten crab larviculture was achieved in artificial seawater (Zhao, 1980), nowadays intensive larval rearing is mostly performed using natural seawater, ground-source brackish water (Zhu et al., 1997; Liu and Gao, 2004) and diluted brine with original salinity of 60–150. The optimal water salinity for egg incubation and larval rearing is 20–25. Several water exchange regimes are employed in intensive larval rearing systems. No water renewal is performed during the first 3 days of culture (Z1), afterwards daily water renewal is slowly increased from 10–20% (Z2–Z3) to 40–50% (Z4–Z5), and 100–200% at the end of the rearing cycle as biomass and feeding levels increase. In some hatcheries, the large tanks are partially filled with green water at stocking Z1 in order to concentrate the larvae, thus reducing the quantity of rotifers and algae required. Clean seawater is added daily, gradually filling up the tanks at Z2–Z3 stage. Towards the end of the culture period (Z4-megalopa), water is exchanged on a flow-through basis to dilute waste and reduce encounters between larvae and thus the risk of cannibalism. In order to maintain good water quality, the total water renewal is generally 5–8 times the rearing volume in an intensive rearing system.

In nature, mitten crab larvae migrate into estuaries as megalopa and move further upstream to rivers and lakes where they grow up to adults. Seawater is thus commonly diluted at the end of the zoeal stage or the beginning of the megalopa stage in the hatchery. After desalination, the megalopae are usually sold to a “coin-size crab culture farm” or are further reared in the hatchery until juveniles. Megalopa can tolerate long distance transportation (survival up to 90% during 24 h transportation), when placed on a surface of moist water grass or wet towels. The optimum transportation temperature is 14–18 °C.

Indoor intensive larval rearing technique is mostly described based on the farmer's experience. It is usually performed in concrete tanks with a typical size of 5 m × 5 m × 2 m l × w × d. Temperature and aeration are closely controlled in the rearing tanks and sufficient food (e.g. algae, egg yolk, live rotifers and *Artemia*, as well as frozen rotifers, copepods and *Artemia* biomass) is supplied. Z1 can be held at high densities (up to 1 million m^{-3}) for a limited period but then they need frequent feeding in order to maintain optimal rotifer density. Moreover, such high densities need to be reduced before the Z5 stage in order to prevent cannibalism. Yields are up to 0.15–0.5 kg megalopa m^{-3} at stocking densities of 0.2–0.5 million Z1 m^{-3}, and 0.5–0.9 kg megalopa m^{-3} at stocking densities of 0.5–1 million Z1 m^{-3}. Survival is usually about 10–15% from Z1 to megalopa.

3.2. Extensive larval rearing techniques

Extensive larval rearing systems are usually managed at low stocking densities in outdoor earthen ponds where the fluctuations of water temperature and salinity are similar to those occurring in the wild. Therefore the quality of the larvae is considered to be similar to wild larvae with better resistance to disease and extreme culture conditions, and thus they have a higher market price. Early larval stages (Z1–Z2) feed on natural food sources available in the ponds (e.g. microalgae, rotifers and other zooplankton), and later larval stages (Z3-megalopa) feed minced fish and *Artemia* biomass (Wu et al., 2006). The size of earthen ponds is between 0.06 and 1 ha, with a stocking density of 0.02–0.1 million Z1 m^{-3}. The yield of megalopa can be 2–10 kg/mu (1 mu = 667 m^2) depending on the food availability in the ponds. The less complicated management and the simpler facilities required have resulted in this technique widely adopted by local farmers since early 2000. However, the yield in these extensive rearing systems is usually low and unstable. Apart from the fluctuations of water quality parameters, other planktonic predators may exist in the water column and compete for space, food and dissolved oxygen with crab larvae, thus affecting the metamorphosis and larval survival (Wu et al., 2006).

Green water techniques are usually used in both outdoor earthen ponds and indoor concrete tanks and are thought to provide a “mature” culture medium. Except for experimental purposes, clear water systems are seldom applied in practice, and seem to result in small and black larvae. Mitten crab larvae eat primarily phytoplankton, rotifers, copepods and *Artemia*. Early larval stages (particularly Z1) ingest microalgae when swallowing water. The algal densities applied in the rearing medium range from 0.1 to 2 million cells mL^{-1} depending on their cell size. Although the presence of microalgae in the water improves the survival of Z1, they cannot molt to Z2 solely on it. *E. sinensis* larvae accept inert feed. Formulated diets modified from larval shrimp diets, dried *Artemia* flakes and decapsulated *Artemia* cysts (Yu et al., 1998) have been used in *E. sinensis* larviculture with some success. Meanwhile, egg yolk, soybean milk, frozen rotifers and copepods are also used as supplementary diets. Nevertheless, rotifers and *Artemia* are still the most widely used live foods in the hatcheries. In practice, Z1 and Z2 are usually fed rotifers, while *Artemia* nauplii are

usually introduced from Z3 onwards. Ongrown and adult *Artemia* provide a large sized prey item and are fed to Z5 and megalopa (Sui et al., 2008, 2009a).

4. Main obstacles in hatchery production and current scientific research achievements

A characteristic of mitten crab hatchery production is the highly variable survival, especially when molting from Z1 to Z2, and metamorphosis from Z5 to megalopa. Successful batches in a hatchery can reach an average survival of 10–15%, with maximum of 30–50% survival up to megalopa, but total mortality before Z2 or megalopa stage also frequently occurs. Although massive mortalities could be compensated for maintaining a large broodstock and stocking higher numbers of larvae, this would lead to a substantial waste of time and resources.

Mitten crab broodstock are usually fed natural foods during gonad maturation, which may cause nutritional unbalances or malnutrition of the broodstock, and consequently affect the egg and larval quality at hatch. Thus development of standardized optimal broodstock diets is crucial to improve and sustain hatchery production. Although a few studies have been done on the effects of total lipid (Wen et al., 2002), HUFA and phospholipids (Wu et al., 2007; Sui et al., 2009b) and vitamin sources (Ai et al., 2003) on the reproductive performance, egg quality and larval performance of *E. sinensis*, commercialization of the formulated dry diets for broodstock still has a long way to go.

Cannibalism often accounts for large mortalities at metamorphosis from Z5 to megalopa, which is mostly caused by asynchronous metamorphosis. The remaining Z5 is usually predated by newly-metamorphosed megalopa. In practice, although cannibalism can be reduced by increasing feeding ration and feeding larger prey such as juvenile or adult *Artemia* at the Z5 and megalopa stages, molting synchrony can only be achieved by providing the smaller larvae with more nutritious feeds and optimizing the feeding regime. Several studies reported that supplementation of microalgae with high HUFA content such as *Nannochloropsis oculata* and boosting the dietary n-3 HUFA in rotifers and *Artemia* using fish oil emulsion resulted in higher larval survival and/or better growth of *E. sinensis* zoea larvae (Shen and Huang, 1999; Chen et al., 2000; Jiang et al., 2000; Sui et al., 2007). Better resistance to osmotic stress was observed when larvae were fed *Artemia* nauplii containing higher level of n-3 HUFA and/or DHA/EPA ratio (Xin et al., 1999; Sui et al., 2007). Feeding regime is still empirical in the mitten crab hatchery, although several studies on rotifer and *Artemia* ingestion and feeding ration have been done in small scale (He and Gu, 1988; Sui et al., 2008; Sui et al., 2009a).

Bacterial interaction is considered to be a critical issue for the commercial development of mitten crab larvae culture. In intensive larval rearing systems, larvae are subject to infections by many opportunistic pathogens including *Pseudomonas putrefaciens*, *Vibrio* spp., filamentous bacteria, protozoa (e.g. *Zoothamnium* sp.), through attachment on the body surface and epithelia of the digestive organ or directly entering via wounds (Wu and Feng, 2004). Death may be caused by hypoxia or by interference with molting, locomotion or feeding. Under stressful conditions, such as poor water quality, unfavorable rearing conditions and feeding, and nutritional deficiency, the level of those pathogens rises rapidly and become deleterious to mitten crab larvae, particularly to the early larval stages. Although a certain survival rate can sometimes be recovered by reducing larval density and increasing water renewal, the mortality is usually high once the larvae get infected. The probiotic products have also been used in indoor intensive larviculture with limitation due to its high cost.

The shortage of broodstock and juveniles of good quality and the high market price of mitten crab have triggered the frequent exchange of resources among the different geographical populations (mainly transport from the Liao and Ou River areas to the Yangtze River basin) in the 1990s, which consequently has reduced the genetic diversity of this species due to the high level of gene flow (Wang and Li, 2002). Although these large scale exchanges are nowadays seldom carried anymore, maintenance of genetic diversity is crucial for conservation of this resource (Sui et al., 2009c).

As for many other intensive aquaculture operations, the important challenge for mitten crab larviculture is the development of safe and sustainable megalopa production. Future improvement of mitten crab hatchery techniques should be directed towards: development of high quality pellet broodstock diets and microcapsule larval feeds in order to improve reproductive performance and larval quality of mitten crab and to reduce the hygienic risk caused by using live food and frozen food; genetic selection for improved growth, adaptability to extreme culture conditions and disease resistance.

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