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Status of biological studies and aquaculture development of the mud crab, *Scylla serrata*, in China: an experimental ecological studies

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Abstract. This is an initial paper in a series of overviews of biological research and aquaculture development of the mud crab, *Scylla serrata*, in China. Results of experimental ecological studies on mud crabs are reported here. As a result of these experimental studies, results that are important for mud crab culture were also discovered and these include, methods to condition and manage broodstock, determination of ecological conditions that are suitable for embryonic development, and the influence of temperature, salinity, diet and larval density on development and survival of larvae. Results of this work will be useful in establishing a good method for artificial mass culture of larvae.

Introduction

The mud crab, *Scylla serrata*, is an important aquaculture species in Asia. In China, development of mud crab culture can be divided into three stages. Prior to 1980s, females with ovaries that were not fully developed were captured in the wild and held in ponds until the ovaries were mature and plump. In the next stage from 1980 to 1990s, young crabs of both sexes (about 100–150 g) were collected and raised in ponds for one or two months until they reached commercial size (about 200–250 g). In the late 1980s the mud crab culture industry developed rapidly resulting in shortages of young crabs (Wu 1992). The third stage began about 1990s, and the most important development in this stage was the successful commercial mass culture of larvae in hatcheries. Beginning in 1990s polyculture of mud crabs with shrimp, fish and algae became widely practised (Wei 1990; Wang et al. 1995a, b) and successful commercial mass larviculture technology was successfully developed by our research group (Wang et al. 1994, 1998) and transferred to the industry, first in southern China and then to northern China. Diseases devastated the shrimp culture industry in

China in the early and mid 1990s and many shrimp ponds were left idle. Many farmers turned to the culture of other species, including mud crabs, fish and algae and the polyculture of these species. Since, the development of hatchery technology has to produce larvae, mud crab culture has become an important industry in China.

Broodstock management

Female crabs used for breeding must be healthy and the ovaries that are fully developed. Fresh substrate (the mixture of mud and sand) is required in the broodstock pond to reduce microbial infections and enhance spawning and hatching. Berried females may be dipped in antiseptic baths (formalin, potassium permanganate, malachite green or methylene blue) for 15 min, every 2–3 days to eliminate microbial infection of the embryos (Lin et al. 1994).

There is a period of about one month between mating and spawning and this is different to the situation in the mitten crab, *Eriocheir sinensis*. Eyestalk ablation to stimulate gonadal maturation is sometimes practised but is not always successful. We found there are three types of neurosecretory cells (A, B and C) in *S. serrata*. Each cell type can be further divided into several subtypes (e.g. C cells can be divided into C_1–C_5) based on cell size, morphology and distribution (Shangguan and Li 1995). In the neurosecretory cells in the ganglion and X-organ, only secretions of two subtypes of C cells (C_3 and C_4) change with ovaries development. Secretion of C_3 cells in the X-organ decrease but secretions of C_4 in the thoracic ganglion increase as the ovary develops. It is likely that gonadal inhibitory hormones and gonadal stimulating hormones are secreted by C_3 and C_4 cells respectively (Shangguan et al. 1995).

Nutrition also plays a critical role in the development of the ovary of *S. serrata*. Total lipids along with classes of lipids and fatty acid composition of the muscle, gonad and hepatopancreas in female *S. serrata* change during the development of the ovary (Lin et al. 1994). Total lipid content in the gonad increased from 6.97% to 25.2% as ovaries developed from II to V stage and then decreased to 11.5% in the VI stage (the stage after spawning)(Figure 1). At the same time, total lipid in the hepatopancreas decreased from 17.1% to 13.8% as ovaries developed from I to V stage and then increased slightly (Figure 1). Total lipids in muscle tissue remain stable during ovarian developments (Figure 1). This suggests that as the ovary develops, lipids may migrate from the hepatopancreas to the gonads. Hence sufficient lipids should be provided in the broodstock diet to permit successful development of the ovaries (Lin et al. 1994). Sufficient amounts of the fatty acids (20:5ω_3, 22:6ω_3, 18:2ω_6 and 18:3ω_3) and the correct proportion of ω_3/ω_6 in the diet will also enhance gonadal development, hatching rate and larval metamorphosis (Li et al. 1994a, b; Cheng et al. 2000) Li et al. 1994 has been changed to Li et al. 1994a, b. Pl. check and approve.

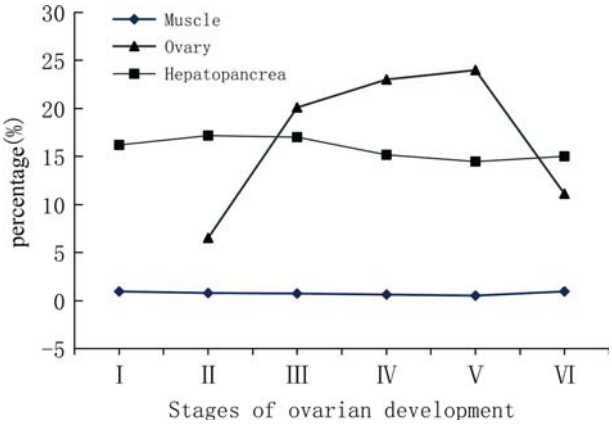

Figure 1. Total lipid content of the muscle, ovary, and hepatopancreas of *Scylla serrata* during ovarian development.

Embryonic development

Embryonic development of *S. serrata* was divided into 10 stages (Table 1) (Zeng et al. 1991). Several factors affect the embryonic development. As mentioned above, pond substrate for the broodstock and eyestalk ablation can affect embryonic development. If eyestalk ablation is performed it should be done after vitellus synthesis. Spawning may be induced if the operation is performed before vitellus synthesis but the hatching rate is low ($< 5\%$) (Li et al. 1994a, b; Wang et al. 1994). The reason for this result may be that insufficient nutrition has occurred in the vitellus if induced spawning is performed too early. Salinity and temperature also affect embryonic development. Suitable salinities and temperatures are 23–30 ppt and 20–30 °C, respectively (Figure 2) (Zeng et al. 1991).

Amounts of four hydrolytic enzymes (protease, α-amylase, cellulase and lipase) also change during developmental stages (Li et al. 1995). Three of the enzymes (protease, α-amylase and lipase) showed activities throughout embryonic development, especially during the latter stages, but cellulase activity only occurred in the eighth and ninth stages after the appearance of a beating heart (Figure 3). If the activities of the four enzymes do not increase at the eighth stage, the embryos will not hatch successfully or if they hatch the zoea larvae cannot metamorphose even though the larvae may appear active (Li et al. 1995). It is possible that increased activity of the enzymes during the eighth stage indicate that larvae are preparing for external feeding. Reasons for failures of increased enzymes activity in the eighth stage and why some embryos can hatch successfully but are not able to metamorphose are unknown.

Table 1. Morphology of embryonic stages of the mud crab *Scylla serrata.*

Stage	Morphology of egg
0	Newly extruded eggs completely filled with yellow-orange coloured yolk; no cellular cleavage
1	Cleavage starts; eggs still completely filled with yolk
2	Blastula; countless cells arrange on the surface of the eggs; embryo not visible
3	Late gastrula; two small, transparent, yolk-free areas become visible on one side of the eggs; first evidence of embryo
4	The two colour-less yolk-free areas expand and joint to form a new-moon shape transparent zone; buds of appendages in round shape recognizable within the transparent zone; yolk occupies approximately 4/5 of total egg volume
5	Transparent yolk-free zone further expands and buds of appendages elongated; yolk occupies about 3/4 of total egg volume; yolk colour close to embryo zone become lighter and more transparent; divisions of yolk lumps become visible
6	Yolk occupies approximate 3/5 of total egg volume; the colour of whole yolk zone becomes lighter and more transparent; division of yolk lumps clearly visible
7	Yolk reduced to about 1/2 of the total egg volume; red-brown eye pigmentation appears in eye-brown shape, gradually increases in size and turns dark
8	Yolk restricted to two butterfly shaped connected patches; heartbeat visible; eye black and enlarged in oval shape; two long strips of black chromatophore appear along the abdomen of embryos
9	Strong and fast heartbeat; yolk further reduced in size and colour; eyes differentiated and well formed; chromatophores appear on carapace and other parts of the embryos; larvae move inside egg membranes
10	Larvae hatching

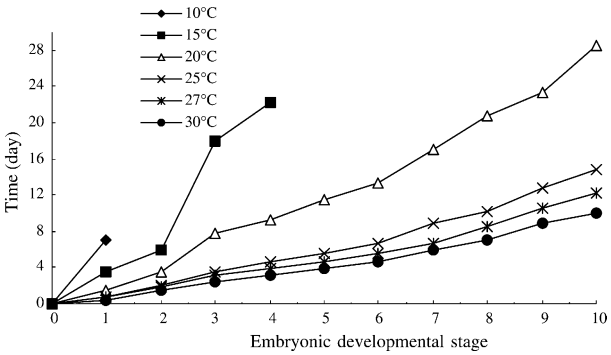

Figure 2. Time required for embryonic development of *Scylla serrata* at different temperatures.

Larviculture

Larvae develop through 5 or 6 zoeal stages before metamorphosing into megalops post-larvae. Temperature, salinity, and diet are the major factors affecting larval development. Suitable temperatures for larval culture are 25–30 °C (Zeng and Li 1992a) (Table 2). Larvae can survive in salinities of

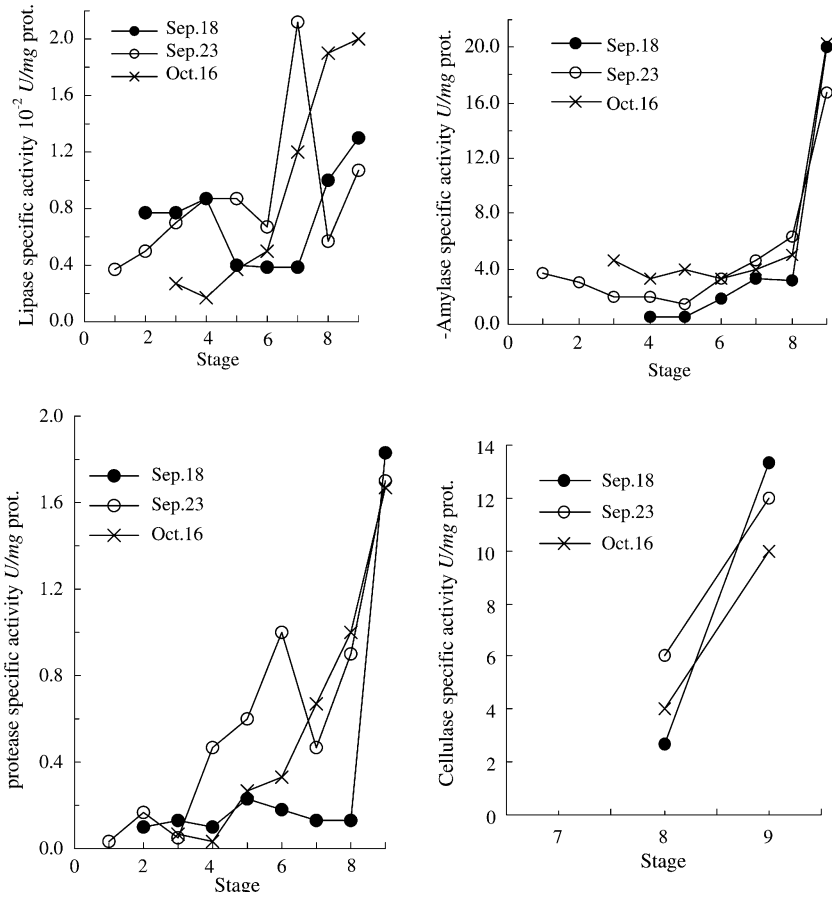

Figure 3. Specific activity of four hydrolytic enzymes during the embryonic development of *Scylla serrata*. Note: Spawning occured on 18 and 23 September, and 16 October.

23–35 ppt but optimal salinities for all larval stages are 27–31 ppt (Figure 4) (Wang et al. 1998).

Some of the first stage of zoea may survive 3 or 4 days without feeding but will die at the time of metamorphosis (Zeng and Li 1992b) (Figure 5). As discussed above, embryos can hatch successfully even when enzyme activities did not increase during the eighth embryonic stage. The larvae may have hatched from embryos with low enzymes activity and although they appear active they do not feed and will die after 3 or 4 days.

High rate of larval survival can be achieved when they are fed with proper proportions of rotifers and *Artemia* nauplii (Zeng and Li 1992b) (Figure 5). The density of rotifers is important in culturing zoea. Development of zoea is faster and megalopa appear earlier when high densities of rotifers are supplied (Figure 5). Mud crab zoeal larvae should be fed with rotifers, then a mixture of

Table 2. Cumulative percentage survival of zoeal instar stages of *Scylla serrata* larvae reared at different temperatures.

Groups:	A								B					
T (°C)	5	10	15	20	25	28	30	35	18	20	25	27	30	35
Z_1	0	0	0	50.0	95.5	80.0	80.0	0	16.0	22.6	73.3	58.5	32.0	1.3
Z_2				0	90.0	72.5	76.5		0	2.7	45.3	41.3	29.3	0
Z_3					54.3	57.5	72.5			0	22.7	–	24.0	
Z_4					40.0	46.6	66.7				22.0		24.0	
Z_5–M					22.5	34.0	27.5				13.4		10.7	
Z_5–Z_6					2.5	–	–				4.0		1.3	
Z_{total}					22.5	34.0	27.5				14.7		12.0	

Larvae in group B at 27 °C died accidentally on the 13th day after the experiment begin when water temperatures rise to 50 °C.
Z_1, Z_2, Z_3, Z_4, Z_5, and Z_6 represent the first to sixth zoeal stages, respectively.
M, represents megalopa.
Z_{total}, represents the cumulative percentage survival of all zoeal stages.

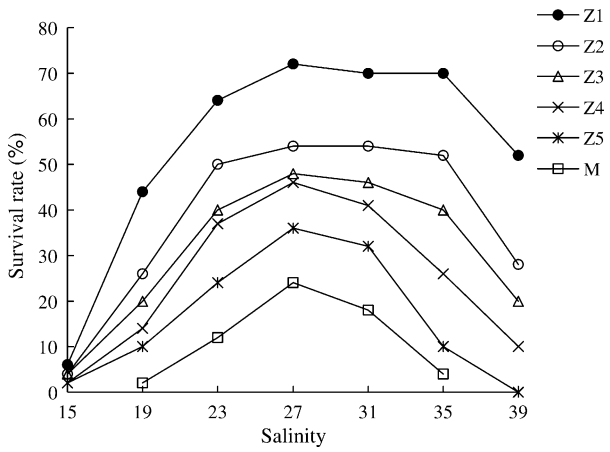

Figure 4. Survival rate of different developmental stages of *Scylla serrata* larvae at different salinities.

rotifers and *Artemia* nauplii as they develop (Figure 5). Megalopa post-larvae should be fed with *Artemia* nauplii. Crab larvae fed with different mixtures, proportions and densities of rotifers and *Artemia* nauplii will have different survival rates (Figure 5) and different organic composition (Wang et al. 1995b)(Figure 6). Further, if the rotifers and *Artemia* nauplii are not provided in proper proportional densities, crab larval development will be delayed and one extra stage (zoea 6) occurs (Wang et al. 1995a) (Table 3). When proper ratios of diets are provided (groups D, E_1 and E_2) zoea survival rate was high and there were only five zoea stages. In other group where the mixture of diets

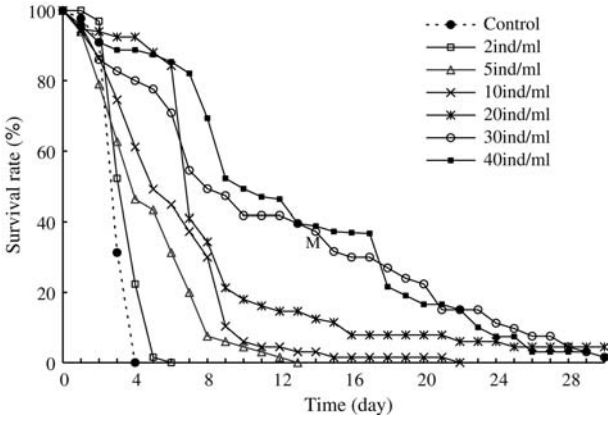

Figure 5. Daily survival rate of Zoea when fed rotifers at different densities. Note: M represents the time at which megalopa appeared. Animals in the control were not fed.

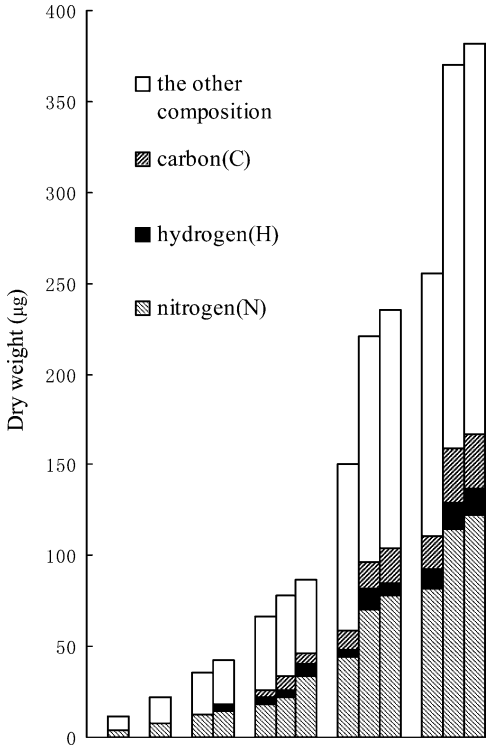

Figure 6. Total dry weight and C.H.N. content of newly moulted different developmental stages of *Scylla serrata* larvae reared under three different feeding regimes (A, B, and C, represent three experimental groups raised under different feeding conditions).

Table 3. Effect of diet composition on zoeal survival and frequency of the occurrence of the sixth zoeal stage.

Group	Appearance frequency of Z_6 (%)	Total survival rate in zoeal stage (%)	Percentage of Z_5 converted into Z_6 (%)
A	0	0	0
B	3.1	6.5	37.8
C	0	1.3	0
D	0	33.0	0
E_1	0	27.7	0
E_2	0	32.3	0
E_3	1.6	8.6	15.7
E_4	1.2	8.5	14.1
F	3.9	10.9	31.0
G	7.0	10.4	59.8

Groups A and B: Larvae were fed rotifers at a density of 40 ind./ml in group A and 60 ind./ml in group B throughout the zoeal stage.
Group C: Larvae were fed *Artemia* nauplius at a density of 10 ind./ml throughout the zoeal stage.
Group D: The Z_1 larvae were fed rotifers at 60 ind./ml; from Z_2 onwards the larvae were fed *Artemia* nauplius at 10 ind./ml.
Group E_1: The Z_{1-2} larvae were fed with rotifers at 60 ind./ml; from Z_3 onwards the larvae were fed *Artemia* nauplius at 10 ind./ml.
Group F: The Z_{1-3} larvae were fed rotifers at 60 ind./ml; from Z_4 onwards the larvae were fed *Artemia* nauplius at 10 ind./ml.
Group G: The Z_{1-4} larvae were fed rotifers at 60 ind./ml; from Z_5 onwards the larvae were fed *Artemia* nauplius at 10 ind./ml.
Group E_2: The Z_{1-2} larvae were fed rotifers at 60 ind./ml; from Z_3 onwards the larvae were fed a mixture of rotifers at 40 ind./ml and *Artemia* nauplius at 5 ind./ml.
Group E_3: The Z_{1-2} larvae were fed rotifers at 60 ind./ml; from Z_3 onwards the larvae were fed rotifers at 100 ind./ml.
Group E_4: The Z_{1-2} larvae were fed rotifers at 60 ind./ml; from Z_3 onwards the larvae were fed rotifers at 200 ind./ml.

was not correct, zoea survival rate was low and there were six zoeal stages (Wang et al. 1995a).

Besides rotifers and *Artemia* nauplii, unicellular algae (e.g. diatoms) and artificial diets (see Table 4 for the composition) were also tested. If all of these items are used in proper proportions, high larval survival can also be achieved (Wang et al. 1994).

Growout

The rate of metamorphosis is very low for megalopa in a hatchery, i.e., $< 5\%$ (Wang et al. 1994). Megalopa may be placed directly from a hatchery to earthen ponds for growout (Lin et al. 2000). We have proposed a model for *S. serrata* culture that is divided into four stages (Li and Wang 2001).

First step: zoea are reared in a hatchery using aeration (ventilating equipment) until they reach the megalopa stage.

Table 4. Proximate composition of formulated diets for mud crab *Scylla serrata* larvae.

Nutrient composition	Content (%)
Crude protein	47.6
Crude lipid	7.8
Crude fiber	3.2
ash	14.8
Lecithin	2.8
Cholesterol	0.8
Calcium (Ca)	3.0
Total phosphorus (P)	1.6

Second step: megalopa are placed in an elaborately designed earthen pond and reared to the first or second juvenile crab stage.

Third step: first or second stage juvenile crabs are placed in large earthen ponds and reared to the seventh or eighth juveniles stage, which is actually the young crab stage.

Fourth step: young crabs are reared to market size in large earthen ponds at very low densities.

The growth rate of *S. serrata* is relatively rapid, it requires 4 months from the first juvenile stage (carapace width about 3 mm) to attain market size (200–250 g) and 6 months to attain sexual maturity (250–500 g) (Wang et al. 1994).

Juvenile crabs can survive in lower salinities (upto 5 ppt) than larvae. In nature, larvae of *S. serrata* live in high salinity open waters and migrate to estuaries as they mature and become competent to settle (Li et al. 1994b).

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