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The Effect of Artemia Sources on Growth, Survival and Salinity Stress of Crab (*Eriocheir sinensis*) Larvae

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Abstract

This experiment was conducted to investigate the n-3 HUFA requirements of the larvae of freshwater crab *Eriocheir sinensis*. Three strains of *Artemia* cysts which contain different levels of eicosapentaenoic acid (EPA) were selected. The zoea-3 stage crab larvae were divided into 3 groups and fed with one of the three strains of *Artemia*. After nine days of culture, the crab larvae had reached the megalopa stage. A significant higher sur-vival rate and dry weight ($P<0.05$) were found in the group fed with *Artemia* nauplii with a high level of EPA.

Crab larvae fed with a high-EPA *Artemia* nauplii displayed the highest tolerance in a salinity *stress* test.

Introduction

Crab larviculture has been successfully developed in PR China since 1970s (Luo et al. 1997). With the crash in shrimp culture in 1992, crab culture expanded quickly. In the Bohai Bay most of the shrimp hatcheries switched to crab hatcheries. Although the feasibility of crab larviculture was already demonstrated 20 years ago, the outputs are far from optimal which might be due to poor nutritional conditions. Although it is well known that highly unsaturated fatty acids (n-3 HUFA), especially docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) can significantly improve the growth and survival of marine fish and shrimp larvae (Sorgeloos and Leger 1992), the effect of n-3 HUFA has not been studied yet on Chinese crab species. The *Artemia* nauplii are an important live food for crab larvae. Since the HUFA

composition of *Artemia* can greatly vary from source to source (Léger et al. 1986), the aim of this study was to evaluate the effect of n-3 HUFA content of *Artemia* nauplii on survival and dry weight of crab larvae. Three strains of *Artemia* cysts which have different EPA contents were selected in this experiment: Karabogazgol lake (Turkmenistan), Tanggu saltworks (Tianjin PR China), and an undisclosed saltlake in Tibet.

Materials and Methods

Zoea-1 larvae of the freshwater crab *Eriocheir sinensis* were obtained from the Jingtang crab hatchery in Tanggu and transferred to a 400 liter culture tank with aerated seawater of 20ppt salinity. The crab larvae were fed with the rotifer *Brachionus plicatilis* during the first three days. When the crab larvae had developed to zoea-3, they were transferred into nine rectangular tanks with a volume of 20 liters each at a density of 150 larvae liter^{-1}. Twelve tanks were randomly divided into three groups. The crab larvae in the different groups were fed with rotifers for the first two days and followed by *Artemia* nauplii or decapsulated cysts from the 4th to the 8th day. During the first three days, one third of the seawater in the tanks was changed daily and half during the last five days.

Karabogazgol and Tibet cysts were hatched in 2 liter cones filled with 20 ppt seawater at 30 °C for 24 hours. The nauplii were separated from the shell and unhatched cysts and kept in the cones at 4 °C until feeding. Tanggu cysts were decapsulated according to the method of Sorgeloos et al. (1986). After decapsulation, the hatching percentage remained at 30%. The crab larvae were fed twice per day at 9:00 and 17:00. Three subsamples of 5ml were taken from each tank and the remaining nauplii were counted before feeding. The density of the nauplii in the tanks were adjusted twice per day by feeding new nauplii according to the food density rate given in Table 1. The decapsulated cysts were fed to the crab larvae according to Table 1. Small quantities of frozen *Artemia* adults were supplied to the larvae during the last two days when they had molted from the zoea-5 to the megalopa stage.

The number of megalopa stage larvae were counted at the end of the experiment. Ten larvae from each tank were selected at random and dried at 60 °C for 24 hours to determine the dry body weight.

The fatty acid composition of *Artemia* nauplii, decapsulated cysts and crab larvae were analyzed according to the method of Sui and Xin (1994).

In the salinity stress test ten larvae from each tank were transferred to beakers containing 60ppt seawater and the cumulative numbers of dead larvae were counted during different time intervals.

Results

The survival and dry weight of crab larvae after eight days of culture are given in Table 2. A significant difference ($p<0.05$) on survival was found

Table 1. Live food and food density (ind. ml^{-1}) was supplied to the crab larvae during the culture.

Day	1	2	3	4	5	6	7	8
Larval stage	Z3	Z4	Z4	Z5	Z5	Z5	Z5	Z5
Live food	Rotifer	Rotifer	*Artemia*	*Artemia*	*Artemia*	*Artemia*	*Artemia*	*Artemia*
Tibet	30	30	5	7.5	10	10	5	5
Karabogazgol	30	30	5	7.5	10	10	5	5
Tanggu	30	30	5	7.5	15	15	10	10

Table 2. The survival and dry weight of crab larvae after 9 days of the culture.

Groups	Survival (%)	Dry weight (mg/larvae) Mean (SD)
Tibet nauplii	$16.43^{a}(1.11)$	$1.63^{a}(0.05)$
Karabogazgol nauplii	$11.55^{b}(1.48)$	$1.28^{b}(0.11)$
Tanggu decapsulated cysts	$14.92^{a}(2.96)$	$1.10^{c}(0.12)$

Means within the same column and followed by a same letter are not significantly different at $p>0.05$ (Duncan's Multiple range test)

between the groups fed with Tibet nauplii and Karabogazgol nauplii, but no significant difference in survival was found between the groups fed with Tibet nauplii and Tanggu decapsulated cysts. The significant differences on dry weight were found in all three groups.

Table 3 showed the cumulative numbers of dead crab larvae in 60ppt seawater during different time intervals. It is clear that the crab larvae fed with Tibet nauplii and Tanggu decapsulated cysts were more tolerant to high salinity seawater than those fed with Karabogazgol nauplii. Within 120 minutes, 10 larvae in the Tibet and Tanggu groups were dead, while it took only 60 minutes in the Karabogazgol group.

The fatty acid composition and protein content of crab larvae fed with different nauplii and decapsulated cysts is shown in Table 4 while the biometrics and fatty acid composition of Tibet nauplii, Karabogazgol nauplii and Tanggu decapsulated cysts are given in Table 5. The EPA content in the crab larvae was strongly related to the EPA content in their food. The highest EPA content was found in the group fed with Tibet nauplii. When crab larvae developed from zoea-1 to megalopa stage, their protein content was gradually reduced from 62% to about 39%. The megalopa crab larvae of all three groups have similar protein content.

Discussion

The results clearly showed that the survival and the dry weight of crab larvae was significantly improved by increased EPA levels in their diet. The highest survival and dry weight was obtained in the group fed with Tibet *Artemia* nauplii that have the highest EPA content. It is clear that the crab larvae require n-3 HUFA during their developing stage like most other marine

Table 3. The cumulative numbers of dead crab larvae at different time intervals in 60ppt seawater (salinity stress test). The test was performed for each replicate of different groups.

Time (minutes)	30	40	50	55	60	80	100	110	120	125
Tibet1	0	0	0	0	0	1	2	5	5	10
Tibet2	0	0	0	0	0	1	4	6	10	
Tibrt3	0	0	0	0	0	0	0	7	7	10
Tibet4	0	0	0	0	0	0	0	2	4	10
Karabogazgol 1	2	4	10							
Karabogazgol 2	0	2	4	9	10					
Karabogazgol 3	0	1	4	6	10					
Karabogazgol 4	1	1	7	8	10					
Tanggu1	0	0	0	0	0	2	7	7	10	
Tanggu2	0	0	0	0	0	3	7	7	10	
Tanggu3	0	0	0	0	0	0	4	4	8	10
Tanggu4	0	0	0	0	0	0	4	4	10	

Table 4. Fatty acid composition and protein content of crab larvae fed on different *Artemia* sources.

Fatty acid (mg/g DW)	Zoea-1	Zoea-2	megalopa larvae		
			Tibet	Karabogazgol	Tanggu
16:0	8.27	11.48	17.40	7.03	12.87
16:1w7	5.96		20.08	5.45	12.14
18:0	3.93	6.97	6.43	2.61	4.63
18:1w9	12.35	8.07	12.40	9.46	18.78
18:1w7	4.39	4.48	16.19	5.98	12.61
18:2w6	1.32	1.17	4.80	2.30	4.64
18:3w6			0.84	0.20	0.53
18:3w3	0.45		2.94	1.33	2.84
20:4w6	1.76	1.26	2.53	1.68	3.37
20:5w3	5.00	5.27	24.62	7.80	14.83
22:6w3	1.29		1.99	1.01	1.42
Protein (%)	62.31	46.71	40.03	39.44	38.03

Table 5. Fatty acid composition, protein content and size of the live food from different *Artemia* sources.

Fatty acid (mg/g DW)	Tibet *nauplii*	Karabogazgol nauplii	Tanggu decapsulated cysts
16:0	18.36	11.64	5.76
16:1w7	25.91	4.02	18.18
18:0	6.52	5.63	4.00
18:1w9	22.97	15.04	21.93
18:1w7	17.60	9.78	12.60
18:2w6	5.27	6.16	6.47
18:3w6	1.38	1.08	4.93
18:3w3	6.47	33.22	3.85
20:4w6	2.00	2.32	
20:5w3	40.28	0.60	13.59
22:6w3	-	-	-
Total lipids		242.19	247.58
Protein (%)	58.54	59.62	58
Size (μm)	602.61 (40.31)	528.78 (30.30)	223.05 (15.37)

fish larvae and shellfish larvae (Sorgeloos and Léger 1992). The lowest dry weight in the group fed with Tanggu decapsulated cysts was probably caused by their limited availability in water column and poor digestibility. The EPA content in larval body were strongly related to the EPA content in their live food. The crab larvae fed with Tibet nauplii have the highest EPA content. Based on the stress results, the quality of crab larvae were also improved by high n-3 HUFA nauplii. It means that the crab larvae fed with high n-3 HUFA nauplii are healthier and stronger, and can resist the environmental changes during transportation and disease.

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