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Evaluation of induced triploid shrimp *Penaeus* (*Fenneropenaeus*) *chinensis* cultured under laboratory conditions

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Abstract

Triploid *Penaeus* (*Fenneropenaeus*) *chinensis* was successfully produced by heat shock. Their metamorphosis, the relationship between body weight and length and difference in appearance between triploids and their diploid siblings under laboratory culture were studied. Hematological studies showed a smaller number of haemocytes, but larger cell volume, in triploids than in diploids. Triploid shrimp did not show higher growth during the immature stage, but exhibited superior growth during the maturation stage. Characteristics of reproductive organs indicated that triploid shrimp may be sterile and sex ratio can be changed through triploidization of shrimp. This paper summarizes the progress made in triploid shrimp research which would be helpful in understanding more about triploids of crustaceans.

Keywords: Triploid shrimp; *Penaeus* (*Fenneropenaeus*) *chinensis*; Metamorphosis; Growth; Hematology; Gonad development

1. Introduction

Triploidy induction is an alternative way to improve cultured animals and has been commercialized in the production of oyster since 1985. In 1999/2000, triploid Pacific oysters constituted 30% of all the farmed Pacific oysters on the west coast of North America (Nell, 2002). Triploids have been induced and evaluated in Pacific oyster *Crassostrea gigas*, eastern oyster *C. virginica* and Sydney rock oyster *Saccostrea glomerata* (Nell, 2002). Benfey (1999) reviewed the physiology and behavior of triploid fishes. Although several species of crustaceans are of economic importance, research on triploid induction in crustaceans is far behind that of molluscs and fish because of their special reproductive characteristics. In recent years, some progress has been made in triploid production in shrimps (Xiang et al., 1991, 1998; Dumas and Ramos, 1999; Li et al., 1999, 2003a,b). These reports mainly focus on triploid induction and reproductive characteristics. During the last 10 years, our group has mainly concentrated on triploid shrimp research. This paper summarizes the progress in triploid research in our laboratory and provides an evaluation on triploid *Penaeus* (*Fenneropenaeus*) *chinensis* reared under laboratory conditions.

2. Materials and methods

Gravid shrimp were collected either from the wild population in the Yellow Sea or from an over-wintered population of a hatchery near Qingdao. Fertilized eggs

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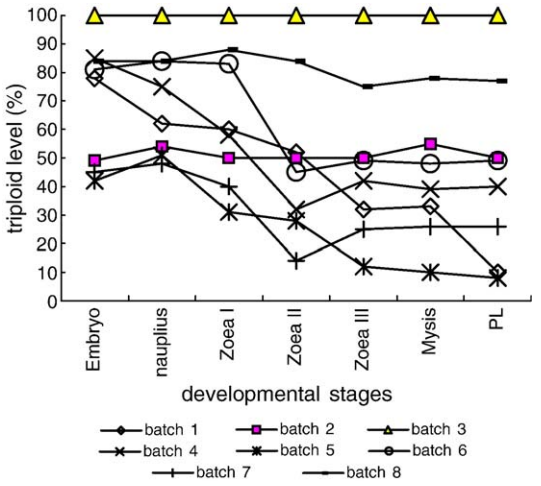

Fig. 1. Variation of triploid level at different larval development stage for the fertilized eggs treated with heat shock.

were treated by heat shock to inhibit the release of polar bodies to induce triploids. Induction was performed following the optimized conditions reported by Li et al. (2003a). Flow cytometry was used to detect the ploidy of shrimp at different larval/grow-out stages (Zhou et al., 1999). For ploidy detection at embryo stage, 100–200 embryos were put together and triturated in 0.2 ml dispersion fluid consisting of 2% citric acid and 0.5% Tween 20 in distilled water according to the method reported (Li et al., 2003a). For nauplius and zoea stage, 30–40 larvae from each batch were triturated in dispersion fluid and one replicate was analyzed. For mysis stage, 10 larvae from each batch were put together to triturate, and 4 replicates were measured each time. In post-larvae, ploidy can be detected individually, and more than 30 post-larvae were analyzed. For grow-out shrimp, one appendage of each animal was cut off and placed in dispersion fluid. The appendage was cut into small pieces and somatic cells in it were crushed out. Twenty to 30 shrimp were sampled each time. Tissue debris was removed, and 0.7 ml 2 mg/l DAPI solution was added to stain the nuclei. At the same time, embryos or larvae without any treatment were used as control following the above procedure. By comparing the peaks, the percentage of triploids in whole sample can be determined.

In order to know whether triploid larvae have the same survival rate as that of diploid larvae, triploid levels were analyzed at every larval stage and the relative survival rate of triploids during metamorphosis was inferred. When larvae were undergoing metamorphosis, ploidy level were determined separately for those that had just finished metamorphosis, and for those that yet to undergo metamorphosis in order to infer the time course for the development of diploid and triploid larvae.

Triploid or diploid shrimp used for the growth experiments at immature and mature stages were full siblings from one triploid population. For growth study, triploids and diploids were separated through flow cytometry detection using one appendage of the shrimp. Thirty individuals of triploids or diploids were selected from one triploid population through flow cytometry detection and placed in 300 l fibre glass container filled with natural seawater. Each ploidy group was replicated 3 times. Clam meat or shrimp food pellet was used as feed. The culture parameters of these tanks including temperature, salinity and light cycle were controlled and maintained at similar levels. The body length and weight of shrimp were measured and recorded every 10 days, measuring all individuals from each group. Growth experiments were performed at two different periods (19 Jun.–26 Aug.; 1 Oct.–4 Nov.).

In order to learn the relationship between body weight and length and difference in appearance between triploids and their diploid siblings, 3 triploid populations were analyzed at grow-out stage. Body length and weight were measured periodically for triploids and diploids of each triploid population. For each time, more than 50 triploids or diploids from each population were used.

Hemolymph of triploids and diploid siblings was sampled from shrimp of body length 8–9 cm. A haemocyte count was performed immediately for each of 20 triploids and diploids in a haemocytometer using a microscope. Total hyaline, semi-granular and granular cell numbers were recorded separately. Haemocytes were also fixed in 2.5% glutaraldehyde buffered with phosphate solution (pH 7.6) for 2 h, for later comparison of morphology using transmission electron microscopy.

The gonado-somatic index was calculated by the following equation: GSI (Gonado-Somatic Index)=weight of gonad/body weight×100. For testis somatic index, the gonad weight includes testis and vas deferens since the testis was difficult to separate from vas deferens. Pieces of ovaries and parts of vas deferens including some sperm, from triploids and diploids, were fixed in glutaraldehyde for later ultrastructure observation.

Table 1
Ploidy analysis when two kinds of larvae coexisted at one time

Date	May 21, 2001			May 23, 2001		
	Stage	No. of individuals	Triploidy rate (%)	Stage	No. of individuals	Triploidy rate %
Sample	Z_{II}	7	100	Z_{III}	6	100
	Z_{III}	7	10	Mysis	6	23