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OSMOTIC RESPONSE OF TIGER SHRIMP (*Penaeus monodon*) BROOD STOCK IN VARIOUS MOLTING STAGES

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ABSTRACT

The attempt to optimize shrimp culture production has faced with several problems related to its biotechnical management, which was suspectedly unable to meet the needs of the cultured species. This is primary because the ecophysiological characteristics of shrimp, particularly those related to the osmoregulation mechanism in connection with their growth, have not been sufficiently understood.

The research was aimed at studing the ecophysiological characteristics of tiger shrimp (Penaeus monodon) *involved in biotechnical management of shrimp hatchery with special emphasis on the osmotic response and isosmotic medium requirement for adult spawners in various molting stages.*

The result showed that osmotic responses were closely related to the salinity of water medium and molting phases. It was also found that the minimum osmotic works of tiger shrimp occurred in isosmotic medium, i.e. 34 - 35 ppt for premolt, 33 - 34 ppt for molt and 30 - 31 ppt for intermolt stages.

Keywords : Osmotic response, Tiger shrimp (*Penaeus monodon*), Broodstock, Molting.

I. INTRODUCTION

In principle, any failure occurring in shrimp culture could be attributed to the inappropriateness between biotechnical management used and the ecophysiological needs of the cultivated shrimp.

Some researcher (Nurdjana, 1986; Tim Satgas Tambak Nasional, 1995) stated that there were some indicators that could be used to determine the success of a shrimp culture operation, i.e. :

1. The ability of brood stock to survive and spawn in the right time.

2. Sufficiently high hatching rate with adequate number and high quality of larvae.
3. Large number of biomass production, characterized by high survival rate and rapid growth rate.
4. Efficiently used feed while maintaining the quality of water medium.

However, some profound problems still exist in the attempt to achieve the above success. Issues predominantly occurred in shrimp culture operation in Indonesia which contribute largely to the failure of shrimp culture are (Tim Satgas Tambak Nasional, 1995) :

1. Low hatching rate and high mortality of brood stocks due to molt death syndrome (MDS) and osmotic stress (OS), especially those which were spawned using ablation technique.
2. Poor quality and quantity of shrimp larvae.
3. Frequent failure in harvesting due to high mortality rate or retarded growth of shrimp being cultured.
4. Degradation of water quality owing to the accumulation and putrefaction of feed residue and fecal matters which in turn lowering the carrying capacity of pond environment.

In principal, it was considered that there are 2 major factors that contribute to the above condition, i.e. :

1. Internally, the ecophysiological characteristics of shrimp, particularly those related to the need of molting and osmoregulation processes which are not fully understood.
2. Externally, the optimum level of water quality and feeding suitable for molting and osmoregulation have not been determined.

It is, therefore, highly crucial to address the issues in order to improve shrimp production technique, and thus improving shrimp production at large.

The general objective of this experiment was to examine the eco-physiological aspects of *P. monodon* especially the relatonship of molting stages and osmoregulation pattern, as a basic information for the improvement of biotechnical management of shrimp culture. Specifically, this experiment was aimed at investigating more deeply the ecophysiological characteristics of *P. monodon* that relate to the biotechnical management of on-growing and spawning of shrimp brood stock, with special emphasis on the osmotic response and the need for isosmotic medium by brood stocks in various molting stages.

II. MATERIALS AND METHODS

The examination of osmoregulation was conducted on the brood stocks of *P. monodon*. In addition, osmotic response of brood stock's haemolymph was also observed during molting stage. Brood stocks were collected from the coastal waters of the North of Central Java region. They were kept in the acclimation tanks and treated according to Anggoro's method (1992). In order to prevent spawning during the transportation of adult shrimp, temperature of water medium was set below 25°C (using ice flakes scattered around the vessel). As for osmoregulation and molting examination, brood stocks were acclimatized in a medium similar to their natural environment (a salinity of 32 ppt) prior to the main treatment.

Osmotic response of *P. monodon* was examined during 3 molting stages, including pre-molt, molt/post-molt and intermolt phases. This experiment was arranged using Completely Randomized Design.

Three similar concrete tanks each with a capacity of 500 l were used as experimental units. The treatment was designed as follows :

a. Tank 1; containing brackishwater at a salinity of 25 ppt or equal to 726.2 mOsm/ l H_2O. This condition is close to postmolt isosmotic level.
b. Tank 2; containing brackishwater with a salinity of 30 ppt or 875.46 mOsm/l H_2O. This is close to intermolt isosmotic level.
c. Tank 3; containing brackishwater with a salinity of 35 ppt or 1026.31 m Osm/l H_2O. This is close to pre-molt and molt isosmotic level.

Each tank was partitioned into 3 similar compartments with nylon netting and covered with plastics on the top. Each compartment was then filled with 1 adult spawner with a body length ranged between 27.50 and 10.00 cm and weighing about 189.45 to 206.55 gr. Aerators were used in each tank to supply oxygen, and temperature regulator and biofilter recirculation unit were also placed in the tank to maintain water quality in the desired condition. The treatments was done in 29 ± 1° C.

Tested shrimps were fed with minced fresh squids weighing 17.3 g in average every afternoon and dawn.

Osmotic response (osmolarity) examination on adult spawner was conducted following the method previously applied by Anggoro and Nakamura (1996). Equipment used for this purpose was Automatic Microosmometer Roebling (for 0.10 ml sample).

The data were then analyzed using Analysis of Variance (ANOVA) with a statistical software (SPSS program). Water quality parameters data were analyzed using descriptive statistics.

III. RESULT AND DISCUSSION

Osmotic responses of brood stock shrimp were evaluated based on the osmolarity of their haemolymph and the osmotic work level in various molting stages. This was done in the three treatments with different salinity of water medium.

The results could be seen, in Fig. 1 and 2, and revealed that the osmolarity of haemolymph and the osmotic work level of brood stock shrimp were changing according to their molting stages. Such changes were necessary to occur from the viewpoint of their ecophysiological mechanism in accordance with the need of molting and osmoregulation processes (Ferraris *et al*, 1987).

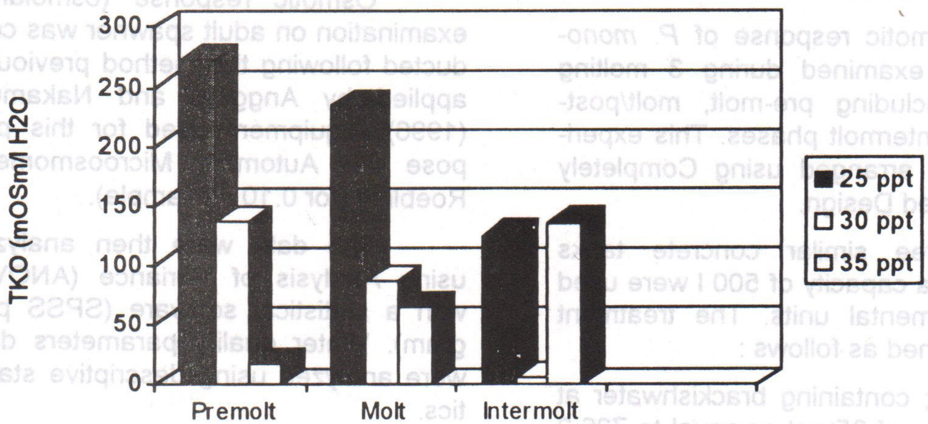

Fig.1. Osmotic work of adult spawners in various molting stages and in different osmotic media (molting cycle I)

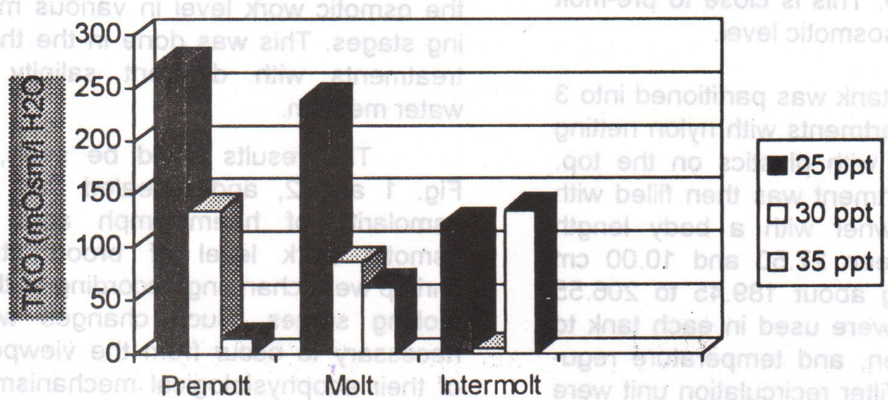

Fig. 2. Osmotic work of adult spawners in various molting stages and in different osmotic media (molting cycle II)

In the pre-molt phase, the osmolarity of haemolymph was considerably high, i.e. :

a. In 25 ppt medium : 999.08 - 1000.32 m Osm/l H_2O or equal to salinity of 34.4 - 34.5 ppt
b. In 30 ppt medium: 1010.55 - 1010.57 m Osm/l H_2O or equal to salinity of 34.6 - 34.7 ppt.
c. In 35 ppt medium: 1010.70 - 1010.72 mOSm/l H_2O or equal to a salinity of 34.8 ppt.

In this phase the minimum level of osmotic work of the shrimp was 15.59 - 15.60 m Osm/l H_2O, which was achieved in 35 ppt medium. Such a very high level of haemolymph osmolarity in pre-molt phase was also found in previous study by Anggoro and Nakamura (1996). There were two factors considered responsible for the above result. First, during pre-molt phase, mobility and accumulation of osmoefector substance reserve, especially calcium, phosphorus and organic nutrient, occur in haemolymph and hepatopancreas as an initial step of molting process. Secondly, the preparation of new integument formation is accompanied by the absorption of organic nutrient and calcium from the old integument to haemolymph (Yamaoka and Scheer, 1970; Mantel and Farmer, 1983; Ferraris et al, 1986). The regulation and transport of calcium into haemolymph are sustained by ion pumping system (Na-K-ATP-ase and Ca-Mg-ATP-ase) and calmoduline (Ferraris, 1986; Che Mat, 1987). In this case, calmodulin is responsible for modulating Ca concentration in the cells (CIS). The modulation is stimulated by Ca transfer from CIS (in the epidermis) into the haemolymph using the energy supplied from ATP. It is through this process that Ca is pumped out from epidermis cells (old integument) into haemolymph and finally accumulates in hepatopancreas (as a raw material for the formation of new integument). Consequently, the increase of osmotic concentration occurs in the extra cellular fluids, which results in a considerable increase in haemolymph osmolarity.

During molt or early post-molt phases haemolymph osmolarity level is slightly lower than that in pre-molt phase as described below :

a. In 25 ppt medium ; 960.25 - 965.45 m Osm/l H_2O or equals to a salinity of 33.0-33.2 ppt
b. In 30 ppt medium : 961.30 - 061.91 m Osm/l H_2O or equals to a salinity of 33.10-33.11 ppt.
c. In 35 ppt medium ; 962.86 - 962.94 m Osm/l H_2O or equals to a salinity of 33.2 ppt.

The decrease of haemolymph osmolarity in the early molt/post-molt stage was suspectedly caused by : (1) the increase in water absorption during molt phase (2) the increase in the utilization of osmoefector (organic and inorganic nutrient) in the haemolymph as raw materials of somatic tissue formation (Gilles, 1979; Dalla Via, 1986).

Intermolt stage is the longest period in the entire molting cycle of shrimp, that is around 70% of the whole cycle (Anggoro, 1992; Anggoro and Nakamura, 1996). In this phase, haemolymph osmolarity was relatively low and steady within the ranges described below :

a. In 25 ppt medium ; 847.66 - 850.44 m Osm/l H_2O or equals to salinity of 29.5 - 29.8 ppt.

b. In 30 ppt medium ; 880.25 - 880.76 m Osm/l H_2O or equals to salinity of 30.3 - 30.4 ppt.

c. In 35 ppt medium ; 889.44 - 891.04 m Osm/l H_2O or equals to salinity of 30.6 - 30.7 ppt.

The minimum value of osmotic work of the shrimp occurred at a salinity of 30 ppt, that was 4.80 - 5.31 m OSm/l H_2O. Osmolarity and osmotic work level in intermolt phase is considered ideal as a standard reference for shrimp culture (Cheng and Liao, 1986; Ferraris et al, 1987). The length of intermolt cycle is related to several contributing factors mentioned below :

a. The process of somatic cells and tissues growth and the hardening of new integument that stimulate X-organ to continuously produce molt inhibiting hormone (MIH).

b. The production of MIH will inhibit the activity of Y-organ in such a way that the secretion of molt alternating hormone is restrained.

c. The accumulation of materials and energy for the successive molting process is highly dependent upon the availability of foods and suitable environment, thus it needs a longer time to happen (Ferraris *et al*, 1987; Anggoro and Nakamura, 1996).

The results of data analysis indicated that there were estimated correlation between osmotic work level (Y) and the salinity of water medium (X) as shown in Table 1. The formulations and regression equations resulted are as follows :

Table 1. Correlation between osmotic work level (Y) and water medium salinity (X)

Phase	Equation
Premolt :	
□ Molting cycle I	$Y = 913.033 - 25.7280 X$ ($r^2 = 0.84$; $p < 0.01$)
□ Molting cycle II	$Y = 917.210 - 25.8532 X$ ($r^2 = 0.98$; $p < 0.01$)
Molt	
□ Molting cycle I	$Y = 693.6066 - 17.0610 X$ ($r^2 = 0.85$; $p < 0.01$)
□ Molting cycle II	$Y = 657.3400 - 17.5833 X$ ($r^2 = 0.84$; $p < 0.01$)
Intermolt	
□ Molting cycle I	$Y = 4460.1240 - 298.7640 X + 4.899 X^2$ ($r^2 = 0.85$) $X_{min} = 30.49$
□ Molting cycle II	$Y = 4328.7067 - 29.1333 X + 4.8073 X^2$ ($r^2 = 0.99$) $X_{min} = 30.48$

From the above discussion, it could be predicted that the salinity of water medium that ensures the minimum osmotic work load in compliance with the molting process in shrimp could be described as follows :

a. Pre-molt phase: 34-35 ppt or equals to osmolarity of 996.98 - 1026.30 m Osm/l H_2O.

b. Molt phase : 34 - 35 ppt or equals to osmolarity of 967.60 - 996.98 m Osm/l H_2O.

c. Intermolt phase : 30.5 ppt or equals to osmolarity of 890.04 m Osm/l H_2O.

IV. CONCLUSION

1. Osmotic response of adult shrimp was closely related to the osmolarity/salinity of water medium and molting stages of shrimp.
2. The minimum osmotic work of the shrimp occurred in isosmotic media, i.e. 34 - 35 ppt for premolting stage, 33 - 34 ppt for molting stage and 30 - 31 ppt for intermolting stage.
3. The range of isosmotic media for the spawning of tiger shrimp was : 30 - 35 ppt or equals to 875.46 - 1026.31 m Osm/l H_2O.

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