STUDY ON FRY PERFORMANCE OF BLACK TIGER SHRIMP *Penaeus monodon* WITH SPECIAL REFERENCE TO ITS MORPHOLOGY AND RNA/DNA RATIO ANALYSIS

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ABSTRACT

Standard method to asses the performance of black tiger shrimp (*Penaeus monodon*) fry was needed for successful shrimp culture. The main purpose of this study was to determine standard method fry performance of *P. monodon* assesment based on its morphology and molecular RNA/DNA ratio analysis. Samples of *P. monodon* fry were collected from hatcheries in Bali, six hatcheries in East Java, three hatcheries in Central Java and six hatcheries in South Sulawesi. Each hatchery gave 25 appropriate sizes of fry samples taken from same tank culture. RNA/DNA ratio value was obtained from gene-quant measurement. Result of this study shown that morphology performance of shrimp fry correlated with RNA/DNA ratio. RNA/DNA ratio of shrimp fry from Bali hatcheries were obtained of 0.7121. Shrimp fry from hatcheries in East Java showed RNA/DNA value ranged between 0.2823-1.2132, while shrimp fry from hatcheries in Central Java and South Sulawesi ranged between 1.1810-17478 and 0.1798-0.5116 respectively.

KEYWORDS: morphological value, RNA/DNA ratio, *P. monodon*

INTRODUCTION

Shrimp fry production is important activities and has closed linkages with culture activities in pond. Fry supplied with good quality and sufficient quantity will determine a successful of production in shrimp farm. Recently, shrimp production in Indonesia always hampered by diseases out breaks, especially virus infection and deteriorated of environment pond culture. The other constrain also caused by low quality of fry which was produce by hatcheries, without good biosecurity.

Fry production process which was influenced by environment factors such as temperature, salinity, variation of food and nutrition, as well as by different genetic factor from the spawners, that will produce different shrimp fry quality in genetically. Some scientist stated that fish genetic diversity should be maintained in seed production process since genetic reduction occurred will cause some fish traits or genetic characters lost to their progeny (Sugama *et al.*, 1998; Gondie *et al.*, 1995; Benzie & Williams, 1996). The morphological performance traits and RNA/DNA ratio value is the reliable index to express the growth and fry quality of shrimp. The highest of morphological value and RNA/DNA ratio indicates that fry in better growth rate and better quality (Caldaron & Buckley, 1997; Jung & Clemmesen, 1997; Chicharo *et al.*, 1998; Segnini & Chung, 1997). The degradation of genetic variation of shrimp cultured in pond using shrimp fry from hatcheries will affect the performance of fry, observed from their lower growth and survival rate, feed conversion ability, survivability environment changes, and even from the abnormality shape. (Leary *et al.*, 1995; Sugama *et al.*, 1992; Ferguson & Drahushchak, 1990).

The correlation between morphological character and RNA/DNA ratio value by RNA and DNA extraction is expected to provide some information on standard quality of shrimp fry. Dahle (1991) showed that RNA/DNA ratio of godfish *Gadus morhua* L. is very low (≈ 1/3000), while Sato *et al.* (1995) succeed to analysis of RNA/DNA ratio of japanese sarden (*Sardinops melanostictus*) larvae and declared that value of 1.2 as index point of no return for first-feeding japanese sarden larvae.

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Therefore, the RNA/DNA ratio each individual of shrimp fry from different hatcheries were observed to determining its correlation with fry quality as well as expression of quantitative traits such more specifically the growth. The information obtained from this study could be used as an indicator of physiological, genetic, and morphological traits for basic development of standardized policy for shrimp, *P. monodon* fry quality.

MATERIALS AND METHOD

Morphology Assessment

P. monodon sample were collected from one hatchery in Bali, six hatcheries in East Jawa, three hatcheries in Central Jawa and six hatcheries in South Sulawesi. Same ages of samples were taken based on the appropriate size of fry for stocking into pond culture (post larvae stage 10—14). Each sample had 25 fry taken from every larval rearing tank. Life sample of shrimp fry and then assessed its morphological performance based on perfectly of organ. The parameters used for assessing their morphological performance including their value are presented in Table 1. The score of each sample was determined by the total value of all parameters. All sample were kept in low temperature at -20°C and some muscle were taken for RNA and DNA analysis.

DNA and RNA Genomic Extraction

DNA genomic was extracted from 100 mg of muscle tissue of *P. monodon* fry. The muscle tissue was blended in 500 µL of 10% Chelex-100 in eppendorf tube, added with 5 µL proteinase kinase (10 mg/mL) and incubated in water bath at 55°C for 4 h. Further, the incubation temperature was increased up to 89°C for 8 minutes and then cooled down to room temperature before the addition of 55 µL TE (Tris-EDTA) buffer at pH 8.0. Genomic DNA was collected after centrifugation at 13,000 rpm for 5 min. The clear solution at the upper layer was concentrated DNA genomic which is ready for DNA analysis using gene quant.

RNA genomic could be obtained from 100 mg of blended muscle tissue and extracted in 1,000 µL of Trizol. The solution was incubated for 5 min and centrifuged at 12,000 rpm for 10 min at 4°C. The supernatant was collected and added with 200 µL of chloroform and then vortexes for 20 second and incubated at room temperature for 10 min. The sample was then centrifuged for 10 min at 4°C and 670 µL isopropanol was added to the supernatant obtained. RNA pellet was collected by centrifuging the supernatant for 10 min. The pellet was washed with 500 µL of 70% ethanol and allowed for 30 min. Pellet was then dried and dissolved in 50 µL sterile double distilled H_2O.

Measurement of DNA and RNA Concentrations

DNA and RNA genomic were diluted 70 times and the concentrations then determined by Gene Quant (Gene Quant *pro*). For Gene Quant calibration, the blank sample consist of TE (Tris EDTA) was used and measured at wave length of 230, 260, 280, 320 nm. Each sample was transferred into a cuvette and the DNA

Table 1. Morphological parameters used in assessing *P. monodon* fry

Parameters	Percentage (%)
Antenulla	5
Hepatopancreas	20
ntestine	10
Midgut	15
Uropoda	5
Tail muscle	10
Chromatophora	5
Parasite	15
Stress	15

and RNA concentrations then were recorded. The ratio RNA to DNA was calculated from each sample.

RESULT AND DISCUSSION

The observation on total length and weight of *P. monodon* fry were showed in appropriate range. Total length of *P. monodon* fry from several hatcheries ranges between 8,052—10,812 mm, except from South Sulawesi relatively longer (Table 2).

However, the total length of *P. monodon* fry from varied hatcheries was not different. This might be related with similar length of rearing period of shrimp fry, where *P. monodon* fry were commonly harvested at the stage of PL 12—PL 14. Similar result showed in body weight of *P. monodon* fry.

Table 3 showed that two hatcheries in East Jawa and 6 hatcheries in South Sulawesi had morphological value relatively lower (46,8—65.0). While, RNA/DNA ratio from all of hatcheries sampled were showed variation of ratio. The highest of RNA/DNA ratio of *P. monodon* fry were obtained from Central Jawa hatcheries (1.1810—1.7478). Performance and relationship between morphological and RNA/DNA value of shrimp fry were showed on Figure 1, while correlation between body length, body weight and RNA/DNA value were showed on Figure 2 and 3.

This fact might be related with culture method of shrimp larvae and different environmental condition each hatchery. Beside that, shrimp spawners genetically would affect fry quality. Genetic variation of *P. monodon* showed high heterozigosity on fry, due to wild spawners used for fry production. Therefore, result of RNA/DNA ratio analysis of fry varied and DNA concentration was affected by genetic performance of shrimp fry.

Table 2. Total length and body length of *P. monodon* from different hatchery in Bali, East Jawa, Central Jawa, and South Sulawesi

Hatchery	Species	Total length (mm)	Body weight (g)
East Jawa			
G (Situbondo)	*P. monodon*	10.812	0.0036
SW (Situbondo)	*P. monodon*	8.976	0.0006
BA (Situbondo)	*P. monodon*	8.584	0.0016
S (Situbondo)	*P. monodon*	7.404	0.0014
SG (Besuki)	*P. monodon*	8.052	0.0018
JB (Situbondo)	*P. monodon*	7.996	0.0027
Central Jawa			
UB (Jepara)	*P. monodon*	9.780	0.0022
B (Jepara)	*P. monodon*	8.892	0.0015
BD (Jepara)	*P. monodon*	8.656	0.0013
Bali			
BB (Buleleng)	*P. monodon*	9.0	0.0010
South Sulawesi			
CH (Barru)	*P. monodon*	12.636	0.0042
MS (Barru)	*P. monodon*	12.320	0.0038
DW (Barru)	*P. monodon*	9.884	0.0004
PK (Bonne)	*P. monodon*	10.624	0.0020
MB (Takalar)	*P. monodon*	13.516	0.0045
WBM (Takalar)	*P.monodon*	11.352	0.0017

Table 3. Morphological value and RNA/DNA ratio of *P. monodon* fry from differences of hatcheries in Bali, East Jawa, Central Jawa and South Sulawesi

Hatcheries	Species	Morphological value	RNA/DNA ratio
East Jawa			
G (Situbondo)	*P. monodon*	71.2	0.8939
SW (Situbondo)	*P. monodon*	74.8	0.8862
BA (Situbondo)	*P. monodon*	78.0	1.2132
S (Situbondo)	*P. monodon*	71.2	0.6701
SG (Besuki)	*P. monodon*	54.6	0.8169
JB (Situbondo)	*P. monodon*	46.8	0.2823
Central Jawa			
UB (Jepara)	*P. monodon*	71.8	1.2889
B (Jepara)	*P. monodon*	74.4	1.7478
BD (Jepara)	*P.monodon*	81.4	1.1810
Bali			
BB (Buleleng)	*P. monodon*	76.8	0.7121
South Sulawesi			
CH (Barru)	*P. monodon*	59.8	0.1798
MS (Barru)	*P. monodon*	59.2	0.2287
DW (Barru)	*P. monodon*	56.6	0.1812
PK (Bonne)	*P. monodon*	63.4	0.5116
MB (Takalar)	*P. monodon*	40.2	0.4076
WBM (Takalar)	*P.monodon*	65.0	0.4111

RNA/DNA ratios of shrimp fry varied among hatcheries. Differences of this value might be related with *endogenous rhythm* in RNA production, and then will affect RNA concentration changes. The highest of RNA/DNA ratio also correlated with various food and nutrition of rearing condition, and those ratios could be used as indicator to physiology of rearing system (Chicharo *et al.*, 1998; Segnini & Chung, 1997).

In other words, RNA/DNA ratio concentration was affected to qualitative as well as quantitative traits of shrimp fry. Furthermore, the information obtained from this study could be used as an indicator for physiological, genetic, and morphological traits as a basis to development particularly for standardization policy for shrimp fry quality, *P. monodon*.

CONCLUSSION

- Performance morphology of *P. monodon* fry has correlation with RNA/DNA ratio
- RNA/DNA of *P. monodon* fry varied between 0.7121 for hatchery in Bali, while fry from hatcheries in East Jawa is 0.2823—1.2132 and fry from hatcheries in Central Jawa and South Sulawesi was 1.1810—1.7478 and 0.1798—0.5116 respectively.

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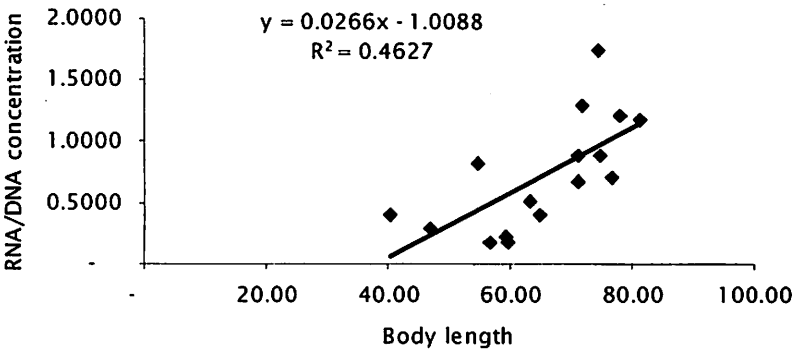

Figure 1. The relationship between morphological value and the RNA/DNA ratio of black tiger shrimp, *P. monodon* fry from Bali, East Jawa, Central Jawa and South Sulawesi hatcheries

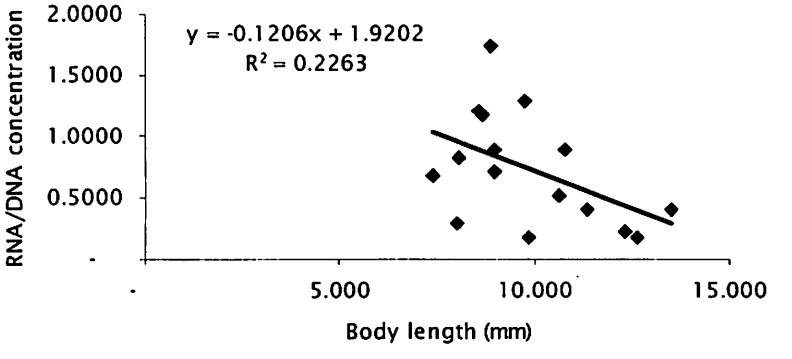

Figure 2. The relationship between total length (mm) and the RNA/DNA ratio of black tiger shrimp, *P. monodon* fry from Bali, East Jawa, Central Jawa and South Sulawesi hatcheries

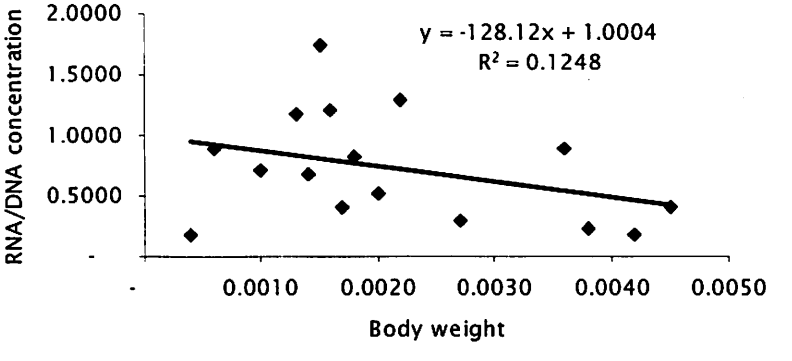

Figure 3. The relationship between body weight (g) and the RNA/DNA ratio of black tiger shrimp, *P. monodon* fry from Bali, East Jawa, Central Jawa, and South Sulawesi hatcheries

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