

Comparison of Nucleic Acid Levels, Ratio and Ecophysiological Aspects among Three Populations of the Fleishy Prawn *Fenneropenaeus chinensis* in Korea

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Using biochemical methods, we determined the potential of local female shrimp populations as breeding stock to select the best adult prawns for improving larval production. As condition indexes, we selected total RNA, DNA, their ratio, and trypsin activity. The DNA content in the pleopods of each local population was similar, i.e., between 0.90 ± 0.06 and 1.02 ± 0.04 (SE) $\mu\text{g}/\text{mg}$. In comparison, the RNA contents differed markedly between 2.00 ± 0.09 and 0.96 ± 0.08 $\mu\text{g}/\text{mg}$. Therefore, the RNA/DNA (R/D) ratio in the pleopod could be used as a condition index because it represents a biochemical characteristic of the population. The mean pleopodal R/D ratio of the Goheung population was the highest at 2.52 ± 0.19 , which indicated the best condition. Trypsin activity was influenced little by shrimp condition and more by the amount of food ingested. The gonadosomatic index (GSI) and R/D ratio in the gonads provided offsetting information about the instantaneous gonad maturity. The Goheung population had the highest instantaneous GSI, despite some spawning. Based on the condition indexes and time of gonad maturation, the Goheung shrimp population is suitable for use as breeding stock.

Key words: *Fenneropenaeus chinensis*, RNA/DNA ratio, Trypsin activity, GSI

Introduction

The fleshy prawn *Fenneropenaeus chinensis* occurs in the northwest Pacific along Korea, Japan, and China and is locally abundant along the Korean coast. Two different geographical populations are found in the Yellow Sea: a large Chinese population and a smaller Korean population (Cha et al., 2002). The two move to coastal areas in March to spawn and offshore in November for wintering. In terms of use as breeding stock for aquaculture, the Korean population was divided into three smaller local populations according to where large numbers of shrimp are caught: Goheung (Narodo), Yeongkwang (Bupsungpo), and Taean (Chaesukpo). According to the National Fisheries Research and Development Institute (NFRDI, Korea), landings of the prawn decreased from 1997 to 2003, with some oscillation, from 1911 to 148 metric tons. For fleshy prawn aquaculture in Korea, it is important to monitor the

condition and state of gonad maturation of each prawn population for mass larval production because no techniques for domesticating adult shrimp have been developed, making aquaculture dependent on the selection of wild breeding stock. Unfortunately, few studies have been conducted on the local Korean populations of *F. chinensis* or on the environmental conditions favoring gamete production (Chen and Lin, 1992, 1995; Xu et al., 1994; Chen et al., 1996; Hur and Kim, 1996; Wang et al., 1998), distribution and migration (Kim, 1973), and reproduction and population dynamics (Cha et al., 2002) of the local wild populations. One morphometric analysis was conducted of commercially important penaeid shrimp in wild Chinese populations (Chu et al., 1995).

Several indexes have been proposed for monitoring the growth, feeding, reproduction, energy storage, and health of marine animal populations. In this study, a biochemical index, the RNA/DNA (R/D) ratio, and an enzyme factor, trypsin activity, were examined to determine the condition of wild shrimp. The tissue

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R/D ratio is a reliable indicator of the growth rate of experimental and natural populations of various marine organisms (Neidhardt and Magasanic, 1960; Bulow et al., 1981; Buckley, 1984; Clarke et al., 1989; Anger and Hirche, 1990; Chicharo and Chicharo, 1995). Moreover, many studies have shown that changes in the quantity and ratio of nucleic acids are a reliable, sensitive indicator of nutritional stress and condition (Wright and Hetzel, 1985; Clemmesen, 1994; Fukuda et al., 2001). Despite such positive findings, some studies on the quantitative relationship between nucleic acids and the growth of marine organisms have shown it to have limited predictive power. This is particularly true for crustaceans, as a result of the drastic internal morphological and physiological changes they undergo during molting (Anger and Hirche, 1990). Furthermore, tryptic enzyme activity in marine organisms, especially in fish larvae, is an appropriate indicator of digestive capacity and can be used to measure larval condition in response to a changing environment, such as the quality and quantity of ingested food (Nolting et al., 1999).

This study had two main goals. The first was to determine the characteristics of each local shrimp population using biochemical methods to select the best adult prawns for improving larval production. The second was to evaluate the use of the R/D ratio and trypsin activity as condition indexes for shrimp.

Materials and Methods

Sampling

As condition indexes, the total RNA and DNA contents in the pleopod, their ratio, and trypsin activity were examined. For this study, 30-50 mature female shrimp were collected from three locations in the middle of the spawning season. Mature females were sampled on 14 May at Goheung, 30 May at Yeongkwang, and 12 June at Taean, in 2005. All the shrimp sampled were measured morphometrically (body length, carapace length, body weight, and gonad weight); the digestive gland (hepatopancreas) and midgut were removed to determine the enzyme activity, and the 1st pleopods were examined to confirm shrimp condition. All the samples were deep frozen immediately at -80°C until analysis. The gonadosomatic index (GSI) was calculated as the percent of gonad weight to female body weight. The RNA and DNA contents in the gonad and their ratios were analyzed to determine gonad maturation.

RNA/DNA analysis

The nucleic acid content was determined fluoro-

metrically, as described by Clemmesen (1994) and Belchier et al. (2004), with some modifications. The tissues were freeze-dried (-50°C , 24 h), weighed (pleopod 5-6 mg; gonad 2-3 mg), and homogenized on ice with Tris-ethylenediaminetetraacetic acid (EDTA) (TE; 5 mM Tris-HCl, 0.5 mM EDTA, pH 7.5) buffer solution (400 μL) with a hand pestle (duration 15-30 s). After centrifugation (8 min, 6000 rpm), the nucleic acids were extracted and purified from the homogenate and the total content was determined fluorometrically using ethidium bromide (EB) dye. Then, the RNA was digested with RNase and the remaining DNA was determined using EB. Detailed instructions can be found in Kim et al. (2005).

Tryptic activity analysis

Tryptic enzyme activity was measured in the digestive organs (digestive gland, foregut, and midgut) of the shrimp with 0.2 mM Na-benzoyl-L-arginin-4-methylcoumarinyl-7-amid (Bachem, Bubendorf, Switzerland) and 0.5% dimethylsulfoxide (Merck, Whitehouse Station, NJ, USA) using the method of Ueberschaer (1992). The relative fluorescence was recorded five times every 1 min at extinction and emission wavelengths of 380 and 440 nm, respectively, with a fluorescence microplate reader (Fluoroskan Ascent FL; Thermo, Filderstadt, Germany). The resulting tryptic enzyme activity was given as the amount of substrate hydrolyzed per time unit (nmol/mg/min).

Protein analysis

For protein analysis, a Bio-Rad protein assay kit II (Bio-Rad, Hercules, CA, USA) was used. From samples homogenized in TE buffer for RNA and DNA analysis, 100 μL homogenate + 900 μL distilled water + 250 μL Coomassie Brilliant Blue (G-250) were mixed in a microcentrifuge tube and measured at 595nm (UV-spectrophotometer, Genesis 5; Genesis Technology Group, Miami, FL, USA). A standard curve was prepared for a range of 1 to 32 $\mu\text{L}/\text{mL}$ BSA (Bovine serum albumin, 0.5 mL of a 2 mg/mL solution in water containing 2 mM sodium azide).

Results and Discussion

The morphometric characteristics of the local shrimp populations in Korea are illustrated in Fig. 1. The greatest mean body length was recorded for the Goheung population as 186.3 ± 6.2 mm. The Yeongkwang population had a body length of 174.8 ± 7.4 mm and that of the Taean population was 167.6 ± 7.0 mm. The greatest mean body weight of the shrimp

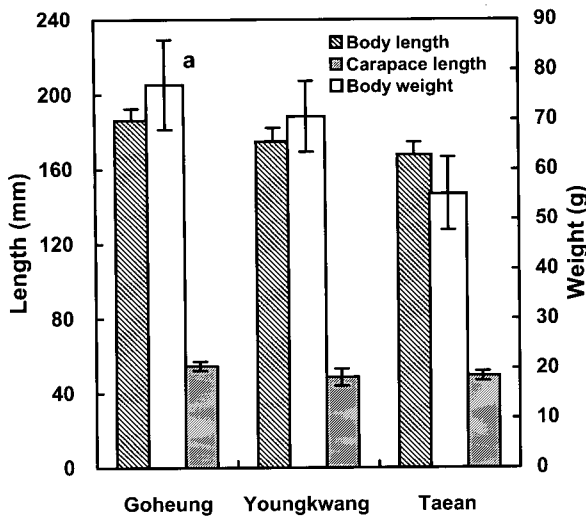


Fig. 1. Body length, body weight and carapace length of local shrimp populations in Korea. Data are presented by means of \pm S.D. (standard deviation, total n=120). Alphabets = statistical analysis using t-test ($p < 0.01$).

was recorded for the Goheung population as 77.0 ± 9 g, despite being the earliest collected. The body weights of the Yeongkwang and Taeon populations were 70.6 ± 7 and 55.1 ± 7 g, respectively. The weights and lengths of all of the populations differed significantly ($p < 0.01$, t-test; Statistika; StatSoft Inc., Tulsa, OK, USA). The ratio of carapace length to body length was 0.29 ± 0.8 in Goheung, 0.30 ± 0.8 in Taeon, and 0.28 ± 2.5 in Yeongkwang. The estimated carapace length at sexual maturity was 39.2 mm for mature females in a west coast shrimp population (Cha et al., 2002). In this study, all the sampled females had carapace lengths over 45.0 mm (54.7 ± 2.6 mm for Goheung, 48.6 ± 4.6 mm for Yeongkwang, and 49.4 ± 2.7 mm for Taeon).

The GSI was highest in the Goheung population (12.81 ± 1.22) and lowest in the Yeongkwang population (7.12 ± 0.65) in May (Fig. 2). The gonad weight of the Goheung population (9.98 ± 0.96 mg) was about twice the value of the other populations (5.26 ± 0.48 mg Taeon, 5.10 ± 0.48 mg Yeongkwang). Based on the low GSI for the Yeongkwang population, we could not determine whether some spawning of mature eggs had already occurred or the gonads were the middle of the maturation process (more information is shown in Fig. 4). The weight of ingested food in the midgut of each population was much higher in the Yeongkwang population (111.4 ± 7.60 mg; Fig. 2). Generally, the amount of ingested food can affect enzyme secretion and enhance nutrient

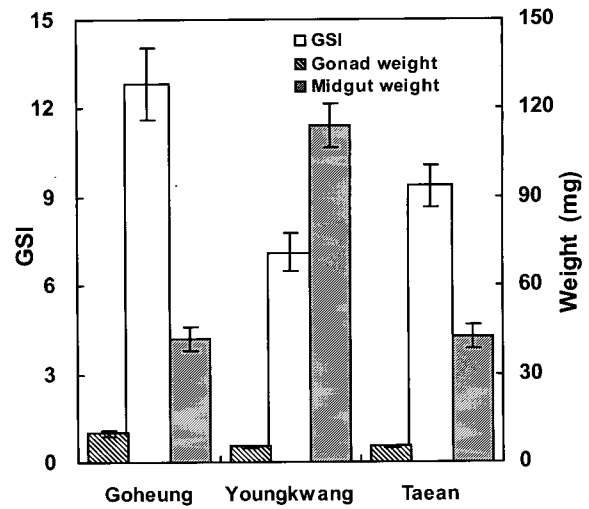


Fig. 2. The values of gonad, midgut weights and gonadosomatic index (GSI) of each local shrimp population. Data are presented by means of \pm S.E. (standard error, total n=120).

absorption. Therefore, food consumption by marine organisms can also be used to determine their health. In this study, the enzyme activity (trypsin) in the digestive gland, where enzyme synthesis and storage occur, was also influenced by the amount of ingested food (Fig. 3).

Despite having the highest trypsin activity in the digestive gland ($1,420.7 \pm 94.79$ nmol/mg/min), the enzyme activity in the midgut of the Yeongkwang population was very low at 104.4 ± 14.9 nmol/mg/

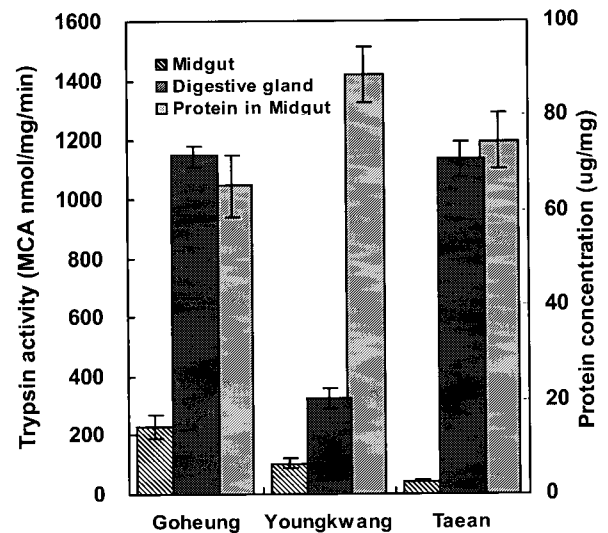


Fig. 3. Trypsin activity in midgut, digestive gland and protein concentration in midgut of three local shrimp populations. Data are presented by means of \pm S.E. (standard error, total n=120).

min (Fig. 3) in comparison to 229.3 ± 40.6 ng/mg/min in the midgut of shrimp from Goheung ($1,043.5 \pm 104.8$ nmol/mg/min in the digestive gland). Low or moderate enzyme activity indicates inadequate feeding or starvation (Nolting et al., 1999). In the Yeongkwang shrimp, the low activity might be related to a large amount of ingested food or inadequate feeding. In this study, the trypsin activity was not measured for the total midgut, but calculated per milligram dry weight of gut for the part of the midgut containing digested food. Therefore, a large amount of ingested food could have diluted the practical trypsin activity. Although the Goheung and Taean populations ingested the same amount of food, the mean trypsin activity in the midguts of Taean shrimp was lower (42.7 ± 4.0 nmol/mg/min). Therefore, it is difficult to estimate the effectiveness of digestion and food absorption using enzyme measures, when a large amount of food has been ingested. The use of trypsin activity in digestive organs as a condition index in wild shrimp remains questionable, as it is influenced more by the amount of food ingested, and not by health. We propose that trypsin activity only be used as a condition index for wild shrimp under continuous monitoring conditions. The protein content of the ingested food was very low in the Yeongkwang population (20.4 ± 2.1 μ g/mg) compared to the other populations (71.6 ± 2.3 μ g/mg, 70.7 ± 3.8 μ g/mg). Perhaps most of the ingested food consisted of nonanimal organisms. The ingested food in the Goheung and Taean shrimp had similar protein contents.

The RNA and DNA contents in the gonad were highest in the Yeongkwang population (Fig. 4). High DNA concentrations generally reflect small cell size and a large number of cells per unit weight of tissue (Bulow, 1970; Mustafa, 1977; Kim et al., 2005). In this study, the difference in the gonadal R/D ratio for each population reflects the different state of gonad maturation (Fig. 4). In the Goheung population, we postulate that the eggs in the gonads were still in the growing stage based on the low R/D ratio, although some mature eggs had been released based on the low GSI (12.81 ± 1.22) compared to the GSI determined on 18 April 2005 (17.4 ± 0.9 ; Kim, unpublished data). The eggs in the gonads of the Yeongkwang and Taean populations were in the mature stage based on the high R/D ratios of 9.82 ± 0.58 and 7.37 ± 0.47 , despite the low GSI (Fig. 3). We postulate that egg maturation does not occur all at once, but in batches. Lee (2005) determined that the main spawning season of the Boryoung shrimp population, located between Yeongkwang and Goheung geographically,

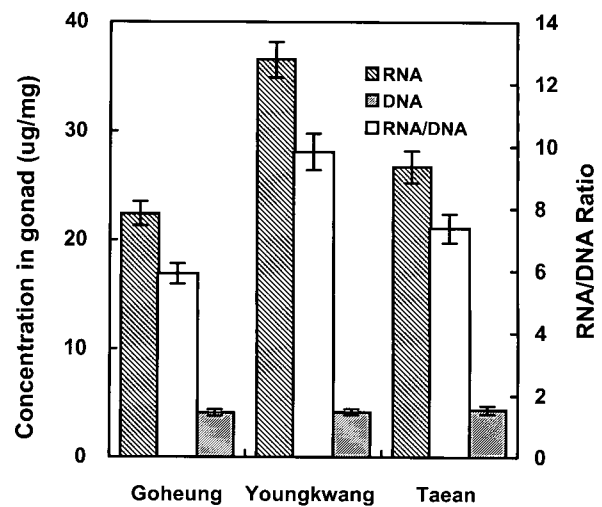


Fig. 4. The concentrations of nucleic acids (RNA, DNA) and its ratios in gonads of each local shrimp population. Data are presented by means of \pm S.E. (standard error, total n=120).

was in June. Therefore, it appears that the Yeongkwang population had still not reached the peak of gonad maturation or the spawning season. The Taean shrimp showed the same tendency as the Yeongkwang shrimp. The R/D ratio in the gonad can be used to identify the instantaneous state of maturity. If we monitored the nucleic acid changes in the gonads of local shrimp populations regularly, we should obtain more information on the gonad maturation process.

The distribution patterns of nucleic acids and their ratio in the gonads and pleopods of each shrimp population are illustrated in Fig. 5. The use of the tissue R/D ratio as a condition index is derived from the role of nucleic acids (DNA and RNA) in protein synthesis and hence in growth. Variation in the number of cells in an organism due to cell division may be estimated from the DNA content, based on the assumption that the DNA content per nucleus is constant in somatic cells. Theoretically, the amount of RNA carrying genetic information from DNA to the sites of protein synthesis, the ribosomes, varies within the cell in proportion to the rate of protein synthesis (Brachet, 1961). Therefore, the RNA concentration and the R/D ratio provide a measure of the average synthetic activity in growth-linked cells of an organism. In this study, the extracted tissue RNA and the R/D ratio in the pleopods of each population varied markedly, reflecting large differences in health. As the GSI increased, the nucleic acids in gonads decreased, reflecting the large size and small number of cells. The DNA concentration in pleopods was

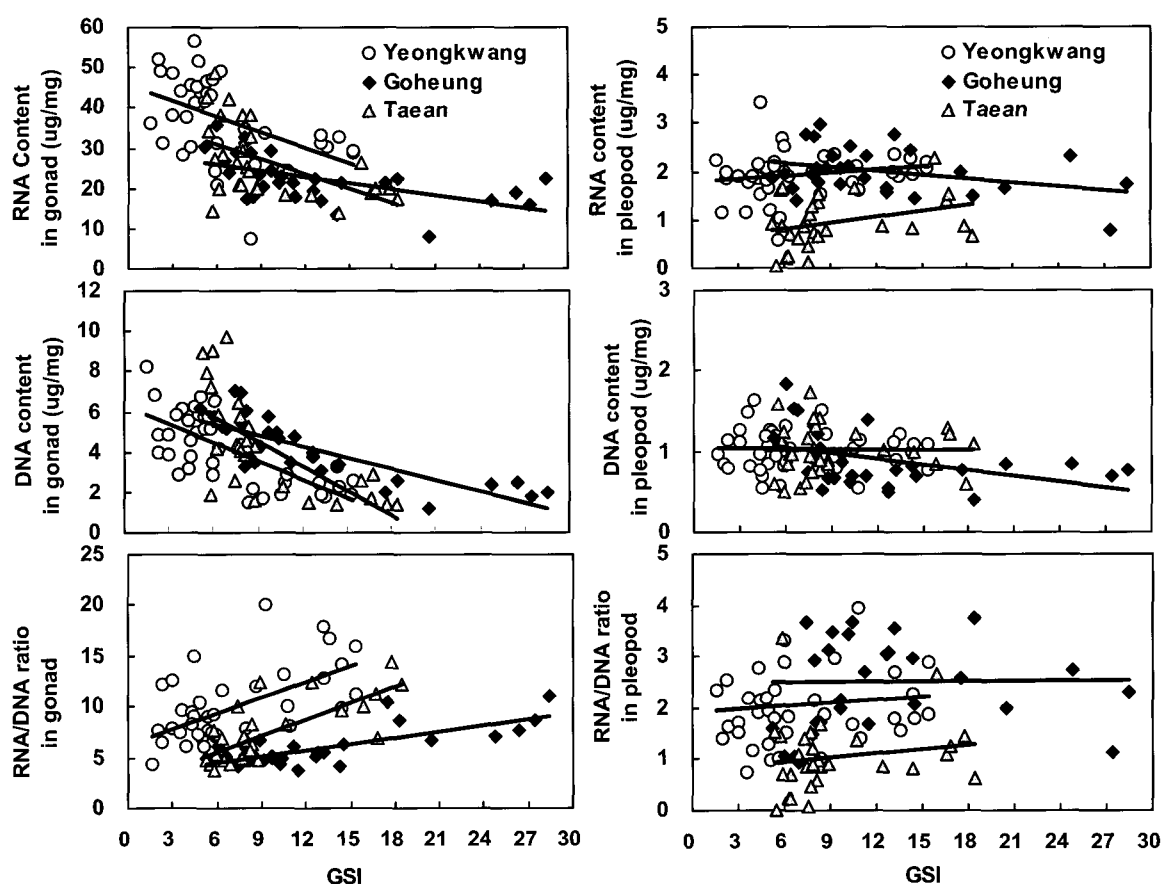


Fig. 5. The relationship between GSI and RNA, DNA concentration and their ratios in gonads and pleopods of three local shrimp populations (Goheung, Yeongkwang and Taean) in 2005, Korea.

relatively similar per milligram dry weight. Moreover, the regressions curves of pleopod DNA for Taean and Yeongkwang matched. The RNA and DNA levels and their ratio in the gonads and pleopods lead to two important conclusions. First, each of the three local shrimp populations are distinct populations in different environments based on the differences in gonad maturation and the regressions curves, which are strongly influenced by hydrological and climatological features, such as changes in temperature, current velocity, wind speed, primary production, and precipitation (Garcia, 1985). Second, based on the R/D ratios in pleopods, the condition of the shrimp is not strongly correlated with the state of gonad maturation, at least in this study. The gonadal R/D ratios varied drastically in individual shrimp that had the same or similar GSI.

The tissue DNA concentrations in the pleopods of each population had very similar mean values of between 0.90 ± 0.06 and $1.02 \pm 0.04 \mu\text{g}/\text{mg}$ (Fig. 6). In contrast, the RNA concentration ranged between 2.00 ± 0.09 and $0.96 \pm 0.08 \mu\text{g}/\text{mg}$. The shrimp in the

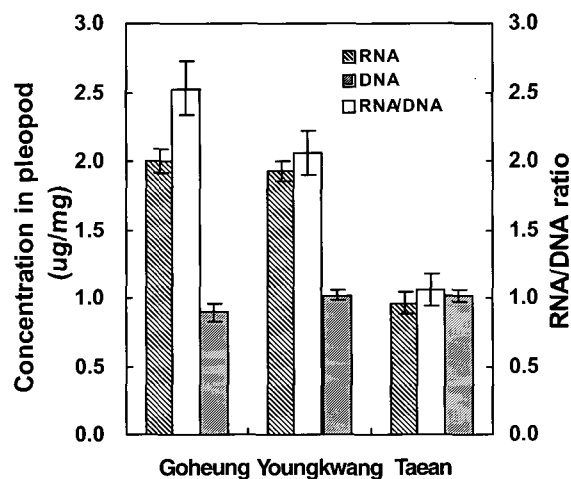


Fig. 6. The nucleic acid concentrations in 1st Pleopod of shrimps, *Fenneropenaeus chinensis*, and its ratios as condition indexes. Data are presented by means of \pm S.E. (standard error, total n=120).

Goheung population were in the best condition based on the R/D ratios in the pleopods as a condition index

(2.52 ± 0.19). The Taean population had a noticeably low RNA content and R/D ratio (1.06 ± 0.11). This may be attributable to the relative low body weight and small body size of shrimp in this region.

In summary, the Goheung shrimp population had the best condition index (pleopod R/D ratio), which can be interpreted as better growth due to increased synthetic activity in the cells, and the earliest gonad maturation, which are preconditions for the first step in brood production. Following the seasonal variation in the nucleic acids in each body part and the enzyme activities in the digestive organs should provide more detailed information on the factors affecting health. Furthermore, it can be used to determine the recruitment potential of each local population of wild shrimp in Korea.

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