MEASURES AND DETERMINANTS OF EGG QUALITY IN TIGER PRAWN, *PENAEUS MONODON*

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PENGUKURAN DAN PENENTUAN KUALITI TELUR UDANG HARIMAU, *PENAEUS MONODON*

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DECLARATION

I hereby declare that this thesis contains my original research work. Sources of findings reviewed herein have been duly acknowledged.

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ABSTRACT

This thesis deals with the egg quality of tiger prawn, *Penaeus monodon*. The focus is on measures of egg quality and the factors that determine the quality. Egg quality was measured by examining the rates of fertilization, hatching, larval survival and the proportion of larvae successfully metamorphosing to the zoea stage. The data suggested that quality of egg was influenced by a number of intrinsic factors that included the dynamics of turnover of many biochemical constituents and the physiological mechanisms required to support those metabolic activities. The physiological status of the broodstock, particularly the nutritional condition was critically important in the development of the ovary as well as that of the oocytes inside it. A broodstock of tiger prawn was subjected to different dietary treatments and effects on robustness of the test specimens and the chemical quality of the oocytes were investigated. The condition of the broodstock was judged by several parameters such as specific growth rate, body specific growth, food conversion efficiency and food conversion ratio. The composition of the oocytes was determined by the nutrient reserves stored during the process of maturation and the chemicals that were transferred to the ovary to support intracellular synthesis. Experimental verification of egg quality was carried out. Bioencapsulation of live prey in the form of bloodworm was more effective in improving the broodstock condition and enhancing the ovarian development that manifested in egg quality improvement. This dietary treatment significantly:

- increased the efficiency with which the broodstock assimilated the ration (higher food conversion efficiency and lower food conversion ratio), gained biomass (higher absolute growth), and achieved more efficient growth (higher body specific growth and specific growth rate), and stimulated the maturation of gonads (earlier onset of maturity and higher proportion of specimens attaining maturity).
- improved the egg quality by way of increasing the capacity of the broodstock to ensure transfer of adequate quantities of nutrients to the intra-ovarian oocytes which were responsible for improving quality. The broodstock treatment accelerated RNA and protein biosynthesis, deposition of cholesterol and presumably also of highly unsaturated fatty acids for which there was no *de novo* synthesis in tiger prawn. The DNA remained unaffected by the accelerated metabolic activity in the egg cytoplasm, which ensured the integrity of the hereditary material necessary to control the processes of embryogenesis that unfolded following fertilization.

Quantitative data the above aspects and interpretation of the results are contained in this thesis. The information is of considerable interest to tiger prawn aquaculture, especially for the scientific management of the broodstock in the hatchery and for the production of healthy seeds.

PENGUKURAN DAN PENENTUAN KUALITI TELUR UDANG HARIMAU, *PENAEUS MONODON*

ABSTRAK

Kajian ini adalah berkenaan dengan penentuan kualiti telur udang harimau, *Penaeus monodon*. Fokus kajian ini tertumpu kepada pengukuran kualiti telur dan faktor-faktor yang mempengaruhinya. Kualiti telur ditentukan oleh peratusan persenyawaan, penetasan, kemandirian larva dan kadar kejayaan metamorfosis larva ke peringkat zoea. Kajian ini menunjukkan bahawa kualiti telur dipengaruhi oleh beberapa faktor intrinsik termasuk kadar pengantian dinamik bahan biokimia dan mekanisme fisiologi yang diperlukan untuk menyokong aktiviti-aktiviti metabolisme. Keadaan fisiologi induk udang terutamanya bekalan nutrisi amat penting dalam perkembangan ovari dan oosit yang berada didalamnya. Induk udang harimau diberikan diet yang berlainan dan kesan diet-diet tersebut dilihat pada kecergasan udang serta kualiti bahan kimia dalam oosit yang dihasilkan. Keadaan induk turut dinilai melalui beberapa parameter seperti kadar pertumbuhan spesifik, pertumbuhan badan spesifik, kecekapan penukaran makanan dan kadar penukaran makanan. Komposisi oosit pula ditentukan oleh simpanan makanan yang dikumpulkan semasa proses kematangan dan bahan kimia yang disalurkan ke ovari untuk menyokong aktiviti sintesis intrasel. Analisis kualiti telur melalui ujian makmal turut dijalankan. Keputusan eksperimen menunjukkan bahawa bio-pengkapsulan cacing laut terbukti lebih berkesan dalam memperbaiki keadaan induk udang sekaligus menampung perkembangan ovari khususnya dalam peningkatan kualiti telur. Jenis diet ini berjaya:

-mempertingkatkan kecekapan penyerapan makanan dalam udang (kecekapan penukaran makanan yang tinggi dan kadar penukaran makanan yang rendah), menambahkan biomas (pertumbuhan muktamad yang tinggi) dan pertumbuhan yang lebih berkesan (pertumbuhan badan spesifik dan kadar pertumbuhan spesifik yang tinggi) serta merangsangkan kematangan gonad (mencapai tahap kematangan lebih awal dan lebih banyak udang yang mencapai tahap kematangan)

-memperbaiki kualiti telur dengan meningkatkan keupayaan induk udang dalam memindahkan nutrient yang mencukupi kepada oosit dalam ovari yang penting untuk peningkatan kualiti telur. Diet induk udang ini mempercepatkan bio-sintesis RNA dan protein serta pemendapan kolesterol dan mungkin juga asid lemak tak tepu yang tidak dapat disintesis secara *de novo* dalam udang. DNA adalah stabil dan tidak dipengaruhi oleh peningkatan aktiviti metabolik dalam sitoplasma telur dan ini memastikan kesempurnaan bahan keturunan yang diperlukan untuk mengawal proses-proses embriogenesis yang diikuti oleh persenyawaan.

Data kuantitatif ke atas aspek-aspek ini dan taksiran daripada keputusan-keputusan eksperimen dibentangkan dalam penulisan tesis ini. Hasil kajian ini amat penting dalam pengurusan induk udang dalam pusat penetasan untuk tujuan pengeluaran benih anak udang harimau yang lebih sihat.

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ABBREVIATIONS

%	percentage
µg	microgram
‰	salinity
cm	centimeter
g	gram
h	hour
m	meter
mg	milligram
mg/L	milligram per liter
ml	milliliter
nm	nanometer
°C	degree Celsius
rpm	round per minute

CHAPTER I

GENERAL INTRODUCTION

1.1 Importance of prawn aquaculture

Prawn aquaculture has aroused a great deal of interest worldwide. In international trade, production of farm prawn has been a major force behind increased prawn trading and market price structure during the past decade. Fifteen years ago most of the prawn supply to the market depended on capture from the oceans. According to the Food and Agriculture Organization (FAO), production of farmed prawn has grown at the rate of 20–30% per year in the last decade (Primavera, 1994). In 2000, yields from prawn aquaculture represented more than 26% of the total prawn production. In 2002 prawn culture represented about 3.2% of the total aquaculture yield or approximately 1.109 million metric-tone valued at more than US$ 6.8 billion (FAO, 2002) when compared to its production in 1991 which amounted to 0.83 million metric-tone and a value of some US$ 5.2 billion.

Accurate statistics on even the basic aspects of world prawn farming do not exist. No one knows for sure how much farm-raised prawn is produced globally. Production estimates are, however, available from countries across the world.

The global aquaculture production of prawn is dominated by developing countries of the tropics. Six Asian and one Latin American countries contributed about 86% of the total farmed prawn yield in 1995. Regionally, Asia shares nearly four-fifth of the world's prawn production with an output of some 558,000 tones (Hagler, 1997). In 2001, Asia produced an estimated 90% of the global production. Since 1998, Thailand has maintained its status as the biggest exporter of farmed prawns in the world (Stickney, 2000), followed by Ecuador, Indonesia, India, Mexico, Bangladesh and Vietnam (FAO, 2002). Major markets of cultured prawns are the United States, Japan and the European Union. About one-third of the total prawn production is traded internationally and that makes prawn the single most valuable seafood product in world trade today.

Of the 300 species of penaeid prawns known worldwide, only a few dozen are commercially important in capture fisheries and a much smaller number in culture fisheries. Among the penaeid prawns, the giant tiger prawn (*Penaeus monodon*) is the most popular and valuable of the cultured species in Asia. Almost 60% of the farmed production in Asia comes from this species. Fleshly prawn (*P. chinensis*) forms 25%, and the reminder is mainly contributed by the banana prawn (*P. merguiensis*) and four or five other species (Barraclough and Finger-Stich, 1996). Globally, *P. monodon*, *P. vannamei*, and *P. chinensis* are the main cultured species that account for approximately 82% of total production. The market value of tiger prawn has increased steadily over the years. In 1999, *P. monodon* ranked first by value, fetching US$ 3.6 billion. Over the duration of five years its value increased at a 5.6% rate since 1994 (Rana *et al.*, 1996; FAO, 2002) and appears to have been driven by high market demand and consumer preference.

The scientific approach to prawn farming was introduced in the 1930's when the first larval rearing technique of kuruma prawn (*P. japonicus*) started in Japan. However, prawn farming as an industry is relatively new, having been started in the early 1970's when attention was given to organized culture outside the traditional growing practices in Southeast Asia (Yap, 1999). That period also witnessed interest in China and Taiwan (Asia) and France (Europe) in investigating the potential of prawn farming following decline in commercial capture fisheries. Dozens of penaeid species have been tested worldwide for their farming potential. The progress of prawn farming is facilitated by evolving technologies, researches, investment and increasing availability of farm area. Hatchery production of prawn larvae and formulated feed have been the focus of attention in prawn aquaculture technology.

The rapid expansion of prawn farming has had significant impacts on the economic, socio-economic and natural environments. Economically, the expansion has encouraged a number of associated businesses such as feed and chemical manufacturing, hatchery, storage, processing, export industry networks, and contract farming involving large private enterprises in association with a large number of small-scale shrimp farmers. While these businesses have generated employment both in coastal areas and in industrial and commercial centers, conversion of rice fields, salt farms, orchards, coconut plantations and other coastal land uses to shrimp farms has had detrimental socio-economic impacts on coastal rural populations in many developing countries. Similarly, the rapid growth of the prawn farming industry has had adverse environmental impacts, including the destruction of mangrove forests and other coastal wetlands, pollution of coastal waters and land from farm effluent discharge and sediment disposal, saltwater intrusion, land subsidence, land dereliction from abandoned farms, increased incidence of algal blooms in coastal waters, and possible changes in species composition in adjacent wetlands (Briggs, 1994; Dierberg and Woraphan, 1996). In addition to the off-site ecological impacts, large-scale and intensive prawn farming has caused many environmental feedback effects (for example through water exchange), which threaten the sustainability of the industry itself (Macintosh and Phillips, 1992).

In many places, intensive prawn farming has been characterized by a boom-and-bust cycle of rapid expansion followed by a crash (Csavas, 1992). Disease outbreak and crop losses were blamed for closure of many once-thriving farms. The disease outbreaks which destroyed the prawn crops were attributed to the stress created by the poor water quality. Apart from farm self-pollution, the coastal areas where the farms are normally situated also receive urban, industrial and agricultural waste water. Poor mixing of water due to characteristic topography of certain areas exacerbate the problem. Realization of the fact that overstocking and environmental stress are the major causes of disease outbreaks and are the most critical factors determining the economics of prawn farming, has prompted interest in tackling the environmental problems.

Prawn farming is carried out mainly in ponds and raceways. In a traditional culture system, where natural stocking is achieved through intake of tidal water carrying large numbers of prawn larvae, pond designs are simple and meant to serve largely as trap ponds. Many farmers release larvae directly into the rearing or production ponds. In more organized culture systems, nursery ponds are used for growing larvae to an advanced juvenile stage, before transfer to production ponds. With the adoption of techniques of controlled breeding, most modern prawn farms include hatchery units, together with nursery facilities, and even grow-out ponds or raceways. The basic techniques to spawn the giant tiger prawn, *Penaeus monodon*, were developed some twenty-five years ago (AQUACOP, 1977, 1983; Beard and

Wickens, 1980; Primavera, 1985). Low light levels, a day length of 12 – 16h and high quality natural feeds were observed to develop the animals into the spawning condition. The production of large numbers of larvae on a precise time schedule also required eyestalk ablation. This surgical procedure removes the source of hormones that inhibit development of eggs in the ovary. Eyestalk ablation has not proven to be effective for pond-reared spawners, whose productivity, and larval quality has been poor relative to wild-caught spawners (Menasveta *et al.*, 1993, 1994). Environmental factors, such as poor water quality, lack of key nutritional elements (Primavera, 1985) and genetic effects have been thought to be responsible for the poor performance of pond-reared animals (Sbordoni *et al.*, 1987). However, little progress appears to have been made in understanding the nature and degree of their effects on egg and larval quality (McVey, 1993). The recent years have witnessed a surge of interest in these areas of research and despite risks, prawn farming is increasing around the globe.

1.2 Prawn aquaculture in Malaysia

In Malaysia, prawn culture started on a small scale in the 1930's using the traditional trapping of wild post larvae and stocking them in grow-out ponds built in coastal areas. Major development occurred in the 1960's when the Department of Fisheries initiated research and development plans. In 1975, the first research hatchery was established in Penang for the mass propagation of tiger prawn and the giant Malaysian prawn. The brackishwater Aquaculture Research Centre was set up in 1978 for developing grow-out techniques. With the inception of the National Prawn Fry Production and Research Centre in 1987 research was intensified and training programs were regularly conducted especially in fry production technology and broodstock maturation.

Currently, prawn culture is the fastest growing sector of the aquaculture industry in Malaysia. The culture of tiger prawn in particular has received more interest than any other species. This is because of consumer demand, higher margin of profit, biological attributes of the prawn and feasibility of culture in the country. Prawn aquaculture production in the country increased at a rate of 21% during 1991–2000. In the year 2000, the production amounted to 16,000 metric-tone which was 14.3% of the prawn yield from capture and culture fisheries. The east Malaysian State of Sabah has the largest prawn farming area (3018 ha) and it has supported 13% of the total prawn production in 2000 (Subramaniam *et al.*, 2002). Sabah has the potential to become the largest prawn producer in the country. With more input of scientific skills and research, earnings from prawn farming can easily multiply. The availability of broodstock throughout the year is a great advantage for continued hatchery production of seed for farming.

Prawn culture technology includes three phases: hatchery, nursery and grow-out. The first phase of aquaculture comprises maturation and reproduction for production of seedstock (larvae), the second phase consists of the hatchery for production of post larvae, and the third phase includes the grow-out to adult stage in raceways or ponds. The seedstock produced in the maturation and reproduction phase supplies the hatchery phase, and the post larvae produced in the hatchery supply the grow-out phase. In the broodstock-hatchery phase, most hatcheries are totally dependent on the supply of wild gravid females for their operations. However, fluctuations in the availability of wild broodstocks in many regions, coupled with variable spawning performance result in the production of the seed often being not synchronized with the demands of the farms for grow-out production. Expansion of the prawn aquaculture industry needs hatchery-produced seed in sufficient quantity to support industrial level farming. Obviously, broodstock management, captive breeding and larval rearing are essential requirements. Good broodstock management should ensure production of high quality eggs which have better chances of survival (Carrillo *et al.*, 1995).

1.3 Application of scientific knowledge

Because egg quality is crucially important in prawn aquaculture, this topic is currently receiving much interest. Kjørsvik *et al.* (1990) have defined egg quality as the potential of eggs to produce viable larvae. However, little is known about the determinants of egg quality. A perusal of the recent literature suggests that egg quality is attributed to many factors. Generally, egg quality is determined by intrinsic properties of the egg and extrinsic factors that operate in the aquatic environment in which the eggs are spawned, fertilized and subsequently incubated. Intrinsic factors comprise the parental genes that are inherited and nutrient reserves that are stored inside the egg during oogenesis. Extrinsic factors such as salinity and temperature among others play important roles in determining quality and have been used to assess egg quality. However, these parameters do not always correlate with good survival and development in later embryonic stages in fish (Kjørsvik *et al.*, 1990) and the same appears to be the case in prawns.

The recent years have witnessed a surge of interest in examining the influence of broodstock diet on the quality of eggs and early larval stages in penaeid prawns (Bray *et al.*, 1990; Xu *et al.*, 1994; Marsden *et al.*, 1997). During maturation the broodstocks are believed to require more nutrient deposition in the storage organs and ovary. Obviously, nutrition is a critical factor in managing the broodstock condition from the onset of maturation to spawning. In penaeid prawn, successful reproduction is closely related to nutrient ingestion accompanying ovarian development (Bray and Lawrence, 1992). The quality and quantity of the nutrients transferred from the somatic organs to the ovary greatly depend on the broodstock diet, and these play vital roles in egg quality and fertility. Hence, understanding of the nutrition-reproduction interaction and of the qualitative and quantitative nutrient requirements for successful maturation and spawning is needed to improve seed quality. Researchers have shown that the diets given to the broodstock influence the nutrient accumulation in the ovary (Millamena *et al.,* 1986; Xu *et al.,* 1994). Malnutrition and poor food quality or reduced ration size have been reported to cause inhibition of gonad maturation in several fish species (Izquierdo *et al.,* 2001). Some of the marine invertebrates are well known as natural sources of critically important nutrients for prawns in captivity. Bloodworm is one such prey food item that contains hitherto unidentified ingredients that promote maturation in penaeid prawns. There are, however, some indications that the polyunsaturated fatty acids in the bloodworm might have a role in the maturation process. The biochemical composition of the bloodworm varies seasonally and this complicates the identification or elucidation of the efficacy of the active ingredients. Investigations on chemically active substances that trigger maturation whatever their sources might be, are therefore important. If they originate from live prey, the prey can be cultured on an industrial scale for adequate supply, or else substitute products could be found to increase egg fertility in the captive broodstock by way of nutritional manipulation.

1.4 Objectives and study approach

It is evident from above discussion that there is a dire need for investigations on egg quality in tiger prawn. Observations on the mechanisms involved in the quality of development of the intra-ovarian oocytes and practical measures that can be taken to improve the intrinsic control of egg quality are particularly important. This study was undertaken to fill the vacuum in knowledge on these aspects. The specific objectives were:

a. To examine the factors that determine egg quality

b. To develop feasible measures of improving egg quality

The eventual aim of this effort was to improve the survival of egg and larvae in the prawn hatchery and to economize aquaculture production.

The study necessitated analysis of the profiles of RNA, DNA, protein, lipid and cholesterol in the intra-ovarian oocytes for identification of determinants of egg quality. Nutritional manipulation of the broodstock was carried out essentially to influence these biochemical constituents and the mechanisms of their metabolic turnover.

The best size of the broodstock used in the hatchery for egg production is not clearly defined, but as a general rule the size of the prawn capable of breeding corresponds to the size at which onset of maturation occurs in the wild (Bray and Lawrence, 1992). Sexual maturity is defined as the minimum size of the penaeid prawn having spermatophores inside the terminal ampoule in the males and inside the thelycum in the females (Motoh, 1981). The optimum size is different for each species of penaeid prawn. For *P. monodon*, females of about 90 g and males of about 60 g are recommended with a minimum size of 60 g and 35 g, respectively. There is some correlation between body weight and fecundity in *P. monodon* (Motoh, 1981; Villegas *et al.*, 1986; Menastena, 1993). Larger-sized broodstocks produce more eggs. However, spawning, hatching and mating are lower in old broodstocks. Concordant views have been expressed by Villegas *et al.* (1986) who observed that larger and older spawners are not the most efficient in terms of number of eggs per unit body weight.

One of the major problems with captive breeding of penaeid prawn is the inability of the diet to supply the essential nutrients that are required for rapid ovarian development in the eyestalk-ablated broodstock. Broodstocks are usually fed natural seafood items such as squid, bloodworm, mussel, oyster, crab and clam together with formulated pellet diets for successful maturation. Studies have found that multiple component diets (combination of natural seafood and pellet feed) perform better for prawn maturation than commercial pellet feed only.

Studies on the maturation of penaeid prawn have demonstrated the role of bloodworm in enhancing the condition of the broodstock and oocyte in the hatchery. However, it is not clear which particular constituent in bloodworm stimulates gonad maturation in prawns. In Sabah, sourcing of bloodworm is easy. The worm burrows in the soft sediment of the intertidal and subtidal regions of the mud-flats. It is generally more abundant in the middle portion of tidal flats. The animal is distributed in many places in and around Kota Kinabalu. Earlier studies have found that the quality of bloodworm varies seasonally within the same locality. This problem could probably be overcome by using bioencapsulation. This technique increases consistency in the performance of diets and prevents fouling of the environment that is often associated with unused artificial feeds. Delivery of bioencapsulated food is easier. A problem with artificially formulated diets is the rapid loss of microbound substances, especially amino acids and water-soluble nutrients through diffusion across the binding material. Microencapsulated diets have the advantage that the capsule enclosing the ingredients has a low leakage rate of water-soluble nutrients, but their commercial production is generally expensive. Tricalcic Phosphate, ($Ca_3(PO_4)_2$), phosphate of lime is the bioencapsulating material was used in this study.

Tricalcic phosphate bioencapsulated bloodworm most probably enhanced the nutritional value of this prey organism for the prawn. Probably, the quality of eggs improves when bioencapsulated bloodworm favorably influences gametogenesis and promotes vitellogenesis.

Profiles of several biochemical constituents were established with the aim of seeking possible relationship between their quantitative turnover and broodstock condition with reference to egg quality. The constituents selected were protein, lipid, cholesterol fraction and the nucleic acids. The selection of these constituents was based on the roles that they play in oocytes maturation and development. The physiological functions of these substances had been briefly reviewed above. However as far as nucleic acid are concerned the technique of RNA/DNA ratio in determining egg quality has been applied for the first time. The RNA/DNA ratio had became one of the most promising biochemical tools for assessing growth rates and nutritional condition in a variety of marine fish larvae since the 1970's (Bergeron, 1997). However, use of this ratio as an egg quality assessment and measuring tool has not been established. DNA carries genetic information and occurs in a constant quantity in somatic cells so that its total quantity is an index of the number of cells (Buckley 1982, 1984). RNA concentration is dynamic and its changes indicate the rate of protein synthesis occurring in a cell (Buckley 1982, 1984). Thus, RNA/DNA ratio is an index of the efficiency of protein synthesis machinery in a cell (Buckley, 1984, Raae *et al.,* 1988). According to Millward *et al.* (1973) the theoretical basis of the RNA/DNA ratio is that 85% to 90% of RNA in a cell is ribosomal RNA and that the DNA content of a diploid cell is essentially constant. Tissues with high RNA/DNA ratio would contain large numbers of ribosomes per cell and should have greater capacity to synthesize protein. The rationale for measuring the quality of eggs by the RNA/DNA ratio is that the successful development of the egg and early larval stage up to the passive feeding period is dependent on the adequate maternal provision of egg constituents. Inadequacy of the essential substances or failure of the normal mechanisms of intracellular biosynthesis could impair the egg quality. Quantitative profiles of RNA and DNA could therefore be used as an index of the physiological status of the egg and its survival potential.

This thesis contains the results of investigations on the effect of various dietary treatments of tiger prawn broodstock on growth and fertility, and on concentrations of RNA, DNA, protein, lipid and cholesterol in the ovary. A special focus is on the efficacy of bioencapsulated live bloodworm on the quality improvement of eggs.

1.5 Tiger prawn – systematics and general features

The giant tiger prawn, *P. monodon* Fabricius (Figure 1.1) is one of the largest penaeid prawns in existence. It reaches 260 mm in body length or 250 g in weight (Motoh, 1981). This species was first described In 1798 by John Christ Fabricius. Several other scientific names for the same species also appeared in the literature (Motoh, 1981): *Penaeus carinatus*, Dana 1852; *Penaeus caeruleus,* Stebbings 1905;