REPRODUCTIVE MECHANISMS IN THE GIANT FRESHWATER PRAWN, *MACROBRACHIUM ROSENBERGII* AND COOPERATIVE RESEARCH TO IMPROVE SEED PRODUCTION TECHNOLOGY IN THE MEKONG DELTA REGION OF VIETNAM

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ABSTRACT

The giant freshwater prawn, *Macrobrachium rosenbergii*, is a commercially important species of crustacean cultured extensively throughout Southeast Asia. In Vietnam, where Japan International Research Center for Agricultural Sciences (JIRCAS) is currently implementing a comprehensive project entitled "Evaluation and improvement of farming systems combining agriculture, animal husbandry and fisheries in the Mekong Delta," *M. rosenbergii* is considered to be an important target species by the Vietnamese Government, and its aquaculture is being actively promoted. Farmers have traditionally depended on wild sources to obtain seed for aquaculture but are now faced with dwindling resources and a shortage of natural spawners. Development of improved means of artificial seed production for *M. rosenbergii* in the Mekong Delta is thus essential. JIRCAS is currently implementing basic studies on the reproductive endocrinology of *M. rosenbergii* as part of the Mekong Delta project. Biochemical and molecular biological research is being conducted both at the project site, Cantho University's College of Agriculture, in Cantho Province, Vietnam, in collaboration with Vietnam counterparts, and on JIRCAS Tsukuba premises. As part of our on-site studies in Vietnam, we are conducting an assessment of typical feeds utilized in freshwater prawn culture in Vietnam, and evaluating the effects of this feed on reproduction. This research is expected to be relevant in controlling female reproduction in captivity and improving seed production technology.

STATUS OF FRESHWATER PRAWN CULTURE IN VIETNAM

The Mekong Delta of Vietnam possesses more than four million hectares of natural land area, of which water bodies excluding rivers comprise 954,350 ha. Freshwater bodies total 641,350 ha or 67.2% of total water surface, and brackishwater areas comprise 313,000 ha (Tien 1993). The flat lowland, moderate climate, and rich natural aquatic resources of the Mekong Delta provide favorable conditions for agricultural and aquacultural development in the region (NEDECO 1991). Aquacultural activity is often integrated with other farming enterprises such as rice cultivation, or is implemented on a mono-culture basis, in ponds or in garden canals. Several species of fish, including common carp, tilapia, and silver barb are cultured in the Mekong Delta. Among crustacean species, the giant freshwater prawn, *Macrobrachium rosenbergii*, is widely targeted in freshwater areas.

In Vietnam, annual production of *M. rosenbergii* varies from 5,000 to 8,000 t (Lin and Lee 1992). However, these production statistics are based on total harvest from both aquaculture and capture fisheries; actual production due to aquaculture is estimated to be as low as 2,000-3,000 t (Hien et al. 1998). Compared to an annual production of 50,000 t of saltwater *Penaeus*

monodon or black tiger prawn (Aqua Farm News 1996; Nien and Lin 1996), *M. rosenbergii* culture appears to be a much less developed industry. The Vietnamese Government aims to promote the culture of *M. rosenbergii* because of its high export value and potential to contribute to increased income levels among farmers in the Mekong Delta.

Freshwater prawn culture in the Mekong Delta is greatly dependent on the use of prawn juveniles collected from natural bodies of water. Most farmers engaged in *M. rosenbergii* culture stock prawns at low density; thus, productivity levels are typically low, being about 250-300 kg/ha in monoculture, and 150-180 kg/ha in rice-prawn combined farming systems in which prawn culture is integrated with rice cultivation (Dung 1991; Tuan and Phuong 1994). Much of the freshwater prawn culture is practiced in the Phung Hiep District of Cantho Province (Sanh et al. 1993), and several districts in Angiang, Tiengiang, Vinhlong and Travinh Provinces (Lin and Lee 1992). This may be related to the natural distribution of *M. rosenbergii*. In the wild, *M. rosenbergii* is distributed from Nhatrang in the central part of the country to the South, but is found mostly in Cantho, Vinhlong, Travinh, and Soctrang Provinces (Thang 1993) (Fig. 1).

At a current market price of approximately US $10/kg, *M. rosenbergii* is a high-priced species and incentive among farmers to engage in its culture is significant (Hien et al. 1998). Income levels per capita in the Mekong Delta vary with province, but a value of US $130-200/yr may be fairly typical (Danida Report 1996). In contrast, many prawn farmers earn up to US $1,000/yr in terms of net income, especially those conducting rice-prawn farming (Hung 1992; Tuyen 1993).

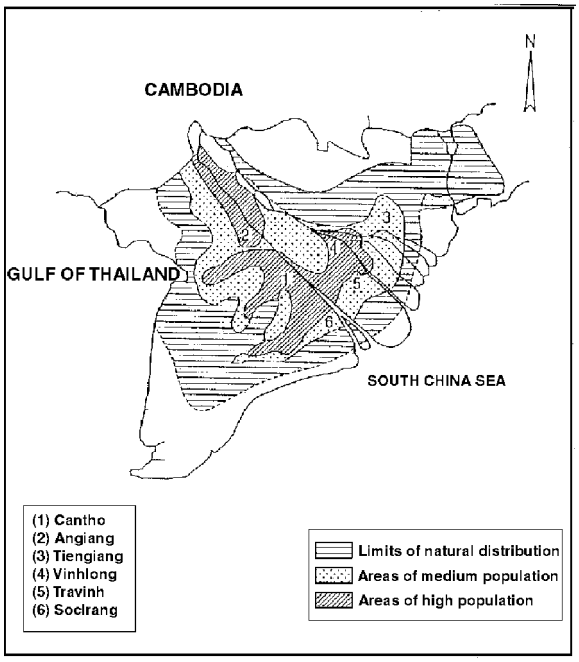

Figure 1. Distribution of *Macrobrachium rosenbergii* populations in the Mekong Delta (redrawn from Thang, 1993). *M. rosenbergii* is found in most freshwater areas of the Mekong Delta, but populations are especially concentrated in central regions of the Delta. Horizontally-shaded areas show limits of natural distribution, while dotted areas and diagonally-shaded areas show areas of medium and high populations, respectively. Numbers indicate approximate locations of provinces where *M. rosenbergii* is found.

Rice-prawn combined farming systems, in which freshwater prawns juveniles are released into rice fields and are allowed to forage, has become a familiar site in the Mekong River Delta. A schematic diagram of the rice-prawn system is shown in Fig. 2. This type of culture is extensive. Canal area, which is used for prawn culture, covers about 15-20% of the total area of the rice field itself. Water levels are controlled to be 1-1.2 m in depth in the canal and 0.2-0.3 m depth on the rice field. Juveniles are stocked often at a

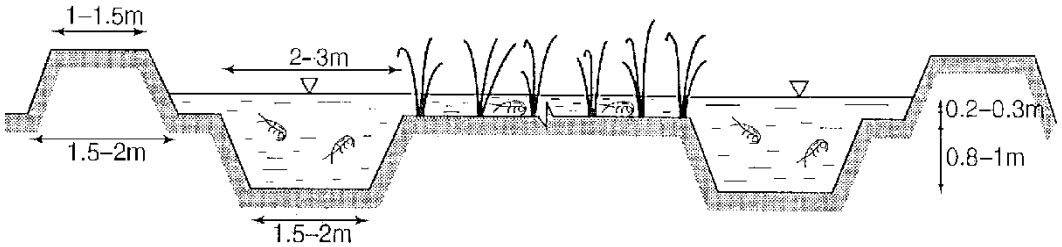

Figure 2. Transect drawing of a rice-prawn field. Canal area comprises 15-20% of total area as described in the text. Prawns are initially stocked in the canals, but can also be found foraging within the rice fields as well.

size of 5-10 g with a stocking density of 0.5-2 individuals/m^2. Stocking activities are usually conducted from December to February. Many farmers do not provide feed, allowing prawns to forage only on natural feed sources occurring in the rice fields (Tuyen 1990). Some farmers, however, utilize agro-byproducts such as rice bran, broken rice, cassava, and coconut pulp as a means of feed.

The culture period in rice-prawn systems varies from 4 to 8 mo depending on farming practices and available capital of the farming households. Yield differs from place to place within a specific region. For instance, in the Thotnot District of Cantho Province, average production reaches 268 kg/ha. In Phunghiep District also in Cantho Province, production is about 100-200 kg/ha (Hien et al. 1998). The major obstacle to further development of rice-prawn farming is not the system itself; it is rather due to the lack of a stable supply of seed and the inability of national hatcheries to meet farmers' demands, as described in the following section.

Natural Sources of Seed; Status of National Hatcheries

As described above, much of the commercial culture of *M. rosenbergii* is dependent on the use of juvenile prawns collected from natural sources. Fishing gear such as brushwood, stow nets, straw nets, or shelter traps are commonly used to obtain juvenile prawns (Hien et al. 1998). Brushwood gear (Fig. 3a) consists of a bundle of tree branches placed in the river, allowing juvenile prawns to be entrapped. Using stow net gear, a net is placed across the river in a zig-zag fashion (Fig. 3b), while straw net gear consists of an enclosed net which is pulled by a boat (Fig. 3c). Shelter traps, square-shaped structures consisting of bamboo frames enclosed with netting (Fig. 3d), are placed along the edges of rivers, and periodically checked for prawn juveniles trapped inside (Lin and Lee 1992). Persons engaging in the above activity are usually fisher folk who sell their catch to middlemen, through which juvenile prawn seed is made available to farmers. Although there are five national hatcheries in Vietnam for the production of *M. rosenbergii*, many farmers prefer to use prawn juveniles obtained in the above manner, as hatchery output is insufficient to meet demands in both quality and quantity.

The Vungtau hatchery, located southeast of Ho Chi Minh City, was the first national hatchery to be established for *M. rosenbergii* seed production, and was built by the Mekong River

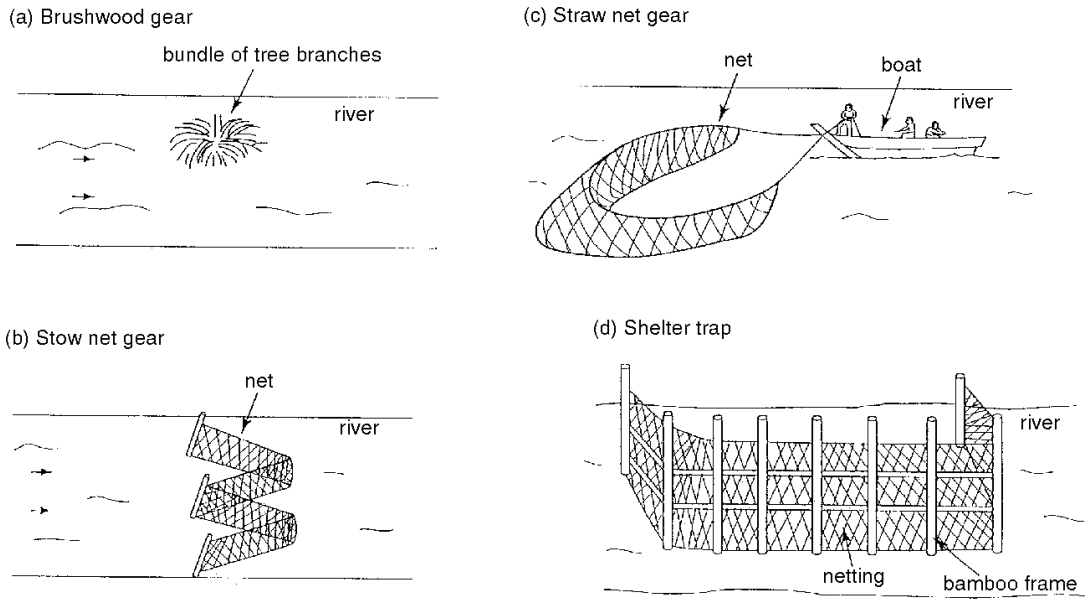

Figure 3. Schematic diagrams of fishing gears used for obtaining juvenile *M. rosenbergii*. (a) Brushwood gear; (b) stow net gear; (c) straw net gear; (d) shelter traps. More detailed explanations are given in the text.

Commission and the Vietnamese Government. It has been in operation since 1987 (Hien et al. 1998). There are four other national hatcheries for *M. rosenbergii* in Vietnam: Nhabe outside of Ho Chi Minh City, Gocong in Tiengang Province, Long My in Cantho Province, and Travinh in Travinh Province. The Long My hatchery is the newest of the five and is still under construction. Annual production capacities for these national hatcheries range from 4,000,000 to 5,000,000 post-larvae/yr at the Vungtau and Nhabe hatcheries, and 2,000,000 to 3,000,000 post-larvae/yr at the other hatcheries; however, most hatcheries are not operating at full capacity due to low survival and technical constraints, which include securing good-quality broodstock and controlling the outbreak of disease (Hien et al. 1998). There is, however, a significant demand for high quality artificially-produced seed, thus there is an urgent need to improve seed quality and decrease price in order to decrease the country's dependence on the use of wild juveniles.

According to personal communications from personnel at the National University, Faculty of Fisheries (Ho Chi Minh City); Research Institute for Aquaculture No. 2 (RIA2), Ministry of Fisheries; and research counterparts at the College of Agriculture, Cantho University, the control of female maturation under captive conditions is the most significant obstacle in establishing a stable means of broodstock cultivation in Vietnam. In the wild, female prawns first mature after reaching a size of 20-40 g; eggs obtained from these females are of good quality and their larvae show high percent survival after hatching. However, females of hatchery origin which are cultured as broodstock often mature while only 7-10 g BW. Use of such precociously mature females results in eggs and larvae of poor quality; offspring of these females may mature even more precociously. For the above reasons, broodstock collected from wild sources, 20-50 g individuals, are presently employed in most hatchery operations. However, this is causing resources of spawners to decline due to over-exploitation, and the dependence of such wild broodstock in hatchery operations is limiting the ability of hatcheries to meet the needs of the aquaculture industry (Hien et al. 1998).

Outline of Cooperative Research

In research between JIRCAS and Cantho University, we are examining how rearing conditions in the hatchery may contribute to precocious reproductive development in female *M. rosenbergii*. Preliminary work has focused on the effects on reproduction and growth of typical feeding regimens practiced in the Mekong Delta. We have conducted an analysis of nutritional content of locally-available feed resources, and are considering how manipulation of diet may be used to control growth and reproductive development (Hien et al. in preparation). In a second phase of these joint studies, we are investigating the effects of water temperature and salinity on reproductive development by simulating typical conditions of culture sites found in the Mekong Delta. Basic studies at JIRCAS are being conducted to elucidate the physiological mechanisms of reproduction in *M. rosenbergii* in parallel to on-site studies in Vietnam. In particular, research at JIRCAS is focused on the hormonal control of vitellogenin (yolk-protein) synthesis and uptake in relation to ovarian development (Wilder et al. 1994) and mechanisms of osmoregulation in relation to salinity adaptation (Wilder et al. 1998). It is expected that a more fundamental understanding of reproductive function at the molecular level will enable better interpretation of how environmental and nutritional conditions influence maturation, and will be applicable in the on-site control of maturation. The research at both JIRCAS and Cantho University is described below.

Basic Research on Reproductive Mechanisms

In *M. rosenbergii* and most other species of decapod Crustacea, reproduction is thought to be under the control of various hormones, including vitellogenesis inhibiting hormone (VIH) and vitellogenesis stimulating hormone (VSH). The presence of VIH in the eyestalk has been well-established, but less is known about VSH which is thought to originate in the brain and thoracic ganglia. Thus, many aspects of reproductive function in Crustacea, including regulatory mechanisms of vitellogenesis, remain unclear. This is due in great part to the fact that the

biochemical nature of vitellogenin (yolk protein) is not fully known. The chemical characterization of vitellogenin and the elucidation of regulatory hormones responsible for mediating reproduction in crustaceans are highly urgent.

In most crustacean species, vitellogenin exists as the precursor of yolk protein and has a molecular mass of more than 200 kDa. In *M. rosenbergii*, vitellogenin is thought to be first synthesized in the hepatopancreas and thereafter secreted into the hemolymph. Subsequently, during vitellogenesis, vitellogenin is taken into the ovary and processed into several subunits to serve as an important source of nutrients during the processes of ovarian and embryonic development. These subunits are known as vitellin.

In research at JIRCAS, we are currently examining the primary structure of vitellin and vitellogenin in *M. rosenbergii*, and are attempting to elucidate the site of vitellogenin synthesis. In order to do so, we first extracted vitellin from a mature ovary and filtered the extract with microconcentrators to cut off low molecular weight proteins. The filtrate was then subjected to reversed-phase high performance liquid chromatography (HPLC), and four major proteins (fractions A, B, C and D) were separated using a linear gradient of acetonitrile/trifluoroacetic acid (TFA) (Fig. 4). The results of Western blotting suggested that the four fractions were vitellins. Using TOF (time-off-flight) mass spectrometry, it was observed that the four fractions recovered from HPLC exhibited protonated molecular ion peaks at m/z 89560.7, 88721.1, 88963.6 and

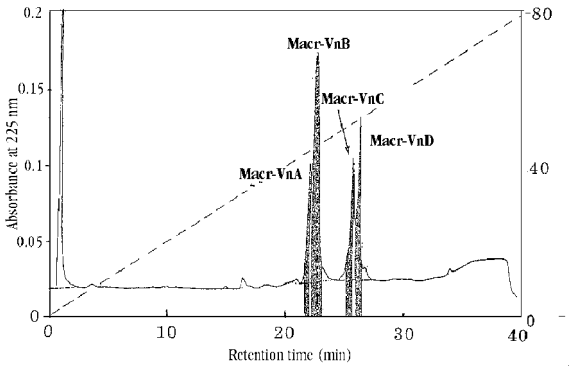

Figure 4. Reversed-phase HPLC elution profiles of M. rosenbergii ovarian extract, showing 4 peaks (Macr-VnA, VnB, VnC and VnD) of purified vitellin.

88900.9, respectively, indicating molecular weights of approximately 90 kDa for all vitellins.

The four fractions were initially subjected to N-terminal amino acid sequence analysis, and we were able to identify more than 30 amino acid residues. To obtain more information about the amino acid sequences, the four fractions were digested with lysyl endopeptidase and the digested fragments were separated by reversed-phase HPLC on an ODP-50 column with a linear gradient of acetonitrile/TFA. A total of 48, 53, 57 and 46 fragments (for fractions A, B, C and D, respectively) were thus obtained and amino acid sequences for several of these fragments were determined.

In order to clone the four cDNA-encoding fractions (fractions A, B, C and D), total RNA isolated from the ovary and hepatopancreas were subjected to reverse transcription (RT) reaction in order to synthesize cDNA. The resultant cDNAs were then subjected to polymerase chain reaction (PCR) using degenerate oligonucleotide primers. The PCR products were subcloned into a plasmid vector and analyzed to determine the DNA sequences. The complete DNA sequences of the four vitellin cDNAs were determined and the conceptually translated amino acid sequences were identical to those of the N-terminal and lysyl endopeptidase fragment sequences. In subsequent research, this will enable us to determine the full DNA sequences of the four vitellins, and thus their complete amino acid sequences. This will provide important structural information whereby it will be possible to more fully understand the process of vitellogenesis in *M. rosenbergii*.

In addition, to identify the synthetic site of vitellogenin, we are analyzing the specific expression of mRNA using Northern hybridization. The site of expression of mRNA for fractions C and D has already been identified as the hepatopancreas. Furthermore, in order to obtain a complete picture of the dynamics of vitellogenin synthesis, expression of mRNA in various tissues (hepatopancreas, ovary, hemocytes, subepidermal adipose tissue and muscle) are being analyzed at different stages of reproduction.

The above research will serve as a basis for developing a bioassay system which will

enable us to identify VIH and VSH. Using such a bioassay system, the role of VIH and VSH, as well as of other hormonal factors in regulating vitellogenesis, may be examined. In turn, we expect that this will result in an understanding of how various environmental and nutritional factors affect the onset of maturation. Details of the above research are reported in Yang et al. (In press).

On-site Studies in Vietnam

Initial research was conducted to address how rearing conditions practiced in the Mekong Delta affect reproductive development in *M. rosenbergii*. We selected three representative feeding regimens, consisting of commercial pellets, hand-prepared pellets made from rice bran, fish meal, and vitamin supplements, and chopped trash fish. Treatments were analyzed for proximate composition (Table 1), as well as amino acid and fatty acid composition (data not shown). Results differed among treatments, although commercial and hand-prepared pellets showed similar profiles. A large-scale experiment, in which 30 male and female prawns for each treatment were reared together in 1.3-t tanks, was designed to look at the effects of differing feeds at saturated and unsaturated levels. A small-scale experiment, in which females were reared individually in 60-L tanks, was designed to examine the effects of feed and compare reproductive development between prawns of wild and hatchery origin.

In both the large- and small-scale experiments, prawns were examined for ovarian maturation by observing the size of ovaries through the carapace. In the small-scale experiment, prawns were initially blood-sampled, and extent of maturation was assessed by measuring hemolymph vitellogenin levels via enzyme immunoassay (EIA).

In both experiments, prawns were an initial size of 5 g, and were reared for up to 20 wk. In the large-scale experiment, prawns fed trash fish matured for the first time at a larger size, more than 15 g BW, while prawns fed with commercial and hand-prepared pellets matured at a size of approximately 12 g, indicating that trash fish were effective in obtaining mature prawns of larger size. In the small-scale experiment, prawns of hatchery origin showed elevated hemolymph vitellogenin levels and matured in 4-7 wk after the start of experimentation while those of wild origin required 16-20 wk. This revealed that the phenomenon of precocious maturation in hatchery-reared prawns is related to an earlier onset of vitellogenin synthesis.

Total essential amino acid (EAA) contents were higher in trash fish than in other feeds, including lysine, an amino acid known to strongly stimulate growth. On the other hand, fatty acids of the n-6 (Σn-6) family and linoleic contents in trash fish were very low compared to commercial and hand-prepared pellets, while fatty acids of the n-3 (Σn-3) family and linolenic acid were high in trash fish and commercial pellets. It is suggested that the balance of amino acid and fatty acid composition present in diets employed by farmers in the Mekong Delta is a main factor affecting the dynamics of growth and reproduction in *M. rosenbergii*. Differing nutritional conditions to which wild and hatchery-reared prawns are exposed during the early life stages may be a factor in determining the onset of vitellogenin production, and thus ovarian maturation.

The connection between nutritional condition and reproductive function is unclear, but this research suggests a link between diet and hormonal makeup. However, a better understanding of the role of nutrition in reproduction can only be established after basic hormonal mechanisms in Crustacea are more fully elucidated.

In a second phase of study, we are currently evaluating the effects of salinity and water temperature on growth and reproductive

Table 1. Proximate composition of representative diets used in freshwater prawn culture in the Mekong Delta. Results are shown as percent (%) dry weight and moisture content (%).

Component	Commercial pellets	Hand-prepared pellets	Trash fish
Crude protein	37.7	26.0	60.0
Crude lipid	6.3	7.2	10.6
Ash	11.1	18.9	13.0
Carbohydrate	43.4	43.7	19.2
Fiber	11.6	4.2	-
Moisture	9.5	9.3	77.8

development using a similar experimental protocol. In addition, we are conducting studies on the development of larval feeds using locally-available resources. Based on the results of this research, we plan to conduct trial investigations in freshwater prawn seed production at a mini-hatchery established by Cantho University.

The above is a partial summary of collaborative research on freshwater prawn culture conducted as the fisheries component of an international comprehensive project between the Japan International Research Center for Agricultural Sciences (JIRCAS), Cantho University, and the Cuu Long Delta Rice Research Institute entitled "Evaluation and improvement of farming systems combining agriculture, animal husbandry, and fisheries in the Mekong Delta." The first phase of the project, initiated in 1994, was concluded in 1999. The authors continue to engage in collaborative research on this topic under the framework of the second phase project, "Development of new technologies and their practice for sustainable farming systems in the Mekong Delta" which is scheduled to run from 1999 to 2003.

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LITERATURE CITED

Aqua Farm News. 1996. Production trends, Vol. XIV (No. 4&5): 2-4.

Danida Report. 1996. Geographical, social and socio-economic assessment of the fishery industry in Vietnam (Danish Ministry of Foreign Affairs' Aid Agency): I1-I31.

Dung, L.C. 1991. Implementation of freshwater rice-shrimp systems at Dai Thanh Village, Phung Hiep District, Cantho Province, Vietnam. Cantho University In-house materials: 1-7. [In Vietnamese]

Hien, T.T.T., T.H. Minh, N.T. Phuong and M.N. Wilder. 1998. Current status of freshwater prawn culture in the Mekong River Delta of Vietnam. JIRCAS Journal 6: 89-100.

Hung, L.T. 1992. Integration of crustacean aquaculture with coastal rice farming in Vietnam. NAGA, the ICLARM Quarterly, April Issue: 27-29.

Lin, C.K. and C. Lee. 1992. Production of freshwater prawns in the Mekong Delta. NAGA, the ICLARM Quarterly, April Issue: 24-26.

NEDECO. 1991. Review and assessment of possibilities and potential of expanding shrimp and prawn culture. Mekong Delta Master Plan. Outline Inception Report; Review and Assessment Reports. 14 pp.

Nien, N.M. and C.K. Lin. 1996. *Penaeus monodon* seed production in central Vietnam. World Aquaculture, September Issue: 6-18.

Sanh, N.V., T.Q. Phu, F. Villanueva and J.P.T. Dalsgaard. 1993. Integrated rice prawn farming in the Mekong Delta, Vietnam: A route towards sustainable and profitable systems. NAGA, the ICLARM Quarterly, April-July Issue: 18-20.

Thang, N.V. 1993. Some biological characteristics and seed production of freshwater prawn (*M. rosenbergii* DeMan 1879) in the South of Vietnam. Ph.D Thesis, 148 pp. [In Vietnamese]

Tien, V. 1993. Fisheries development in the Mekong Delta, Vietnam. In: Fisheries Magazine, published by the Ministry of Fisheries: 22-30. [In Vietnamese]

Tuan, N.A. and N.T. Phuong. 1994. Aquaculture in the Mekong Delta: Present status and potential, Cantho University In-house materials. [In Vietnamese]

Tuyen, N.Q. 1990. Implementation of freshwater rice-shrimp systems at Mangcai Hamlet, Dai Thanh Village, Phung Hiep District, Cantho Province, Vietnam. Cantho University In-house materials: 21-40. [In Vietnamese]

Tuyen, N.Q. 1993. Rice-freshwater prawn (*Macrobrachium rosenbergii*) farms in the Mekong Delta, Vietnam. NAGA, the

ICLARM Quarterly, October Issue: 18-20.

Wilder, M.N., T. Okumura, Y. Suzuki, N. Fusetani and K. Aida. 1994. Vitellogenin production induced by eyestalk ablation in juvenile giant freshwater prawn *Macrobrachium rosenbergii* and trial methyl farnesoate administration. Zool. Sci. 11: 45-53.

Wilder, M.N., K. Ikuta, M. Atmomarsono, T. Hatta and K. Komuro. 1998. Changes in osmotic and ionic concentrations in the hemolymph of *Macrobrachium rosenbergii* exposed to varying salinities and correlation to ionic and crystalline composition of the cuticle. Comp. Biochem. Biophys. A. 119A: 941-950.

Yang, W-J., T. Ohira, T. Tsutsui, S. Subramoniam, D.T.T. Huong, Aida, K. and M.N. Wilder. In press. Determination of amino acid sequence and site of mRNA expression of four vitellins in the giant freshwater prawn, *Macrobrachium rosenbergii*. J. Exp. Zool.