Original Article

Timing for Male Reproductive Tract Gene Expression and Gonopore Complex Development of Giant Freshwater Prawn (*Macrobrachium rosenbergii*)

Prapruddee Piyaviriyakul[1*] Damri Darawiroj[2]

Abstract

Mono-sex culture of giant freshwater prawns (*Macrobrachium rosenbergii*) gives more benefits than mixed-sex culture because of high market demand for male prawns. Unfortunately, sex differentiation in crustaceans could not obviously be observed in their early larval stage. Therefore, sex segregation or sex reversal techniques need to be developed and timing for sex differentiation in *M. rosenbergii* during larval development needs to be examined. In this study, three genes related to male reproductive hormone (*IAG, MRR* and *MAL*) were isolated and amplified from terminal ampulae (TA) of adult male *M. rosenbergii*. The male specific transcripts demonstrated that they could be used to monitor sex differentiation time during larval development. Observation of male transcripts expression together with macro and microscopic pictures of phenotypic characters, the appendix masculina (AP) and the gonopore complex (GP), during larval and juvenile development demonstrated that the sex differentiation process of male freshwater prawns began when the larvae were 2 months old or after complete metamorphosis. Moreover, this is the first report on SEM depicting an ultra-valve like structure on gonopore complex surface during sex development of male prawn.

Keywords: androgenic gland, freshwater prawn, gonopore complex, sex differentiation timing

[1] *Unit of Biochemistry, Department of Physiology, Faculty of Veterinary Science, Chulalongkorn University, Bangkok 10330, Thailand*

[2] *Department of Anatomy, Faculty of Veterinary Science, Chulalongkorn University, Bangkok 10330, Thailand*

***Correspondence:** ppraprud@chula.ac.th*

Introduction

Male giant freshwater prawn (*Macrobrachium rosenbergii*) gains more growth rate and body weight than female. Male prawn also exhibits better taste and fetches a better price. Mono-sex culture is, therefore, the gold culture method for shrimp farmer to achieve the most economical advantages. However, specific problems of all male culture remain such as a proper timing and methods for sex segregation.

As we have known, crustaceans sexing system is not similar to that of higher vertebrates. Sex differentiation in crustaceans does not immediately occur at the time of fertilization, in addition, their gender could also be reversible from environmental factors (Zupo et al., 2014). Similarly, the gender of *M. rosenbergii* is identified by its morphology after pond rearing. For many decades, factors influencing sex differentiation of prawn had been unclear. However, the clue was unmasked, since androgenic gland (AG) and its function were reported in Atlantic blue crab (*Callinectes sapidus Rathbun*) (Cronin, 1947; Charniaux-Cotton, 1962). AG is widely accepted as a key organ for sex differentiation in male decapods crustacean (Charniaux-Cotton and Payen, 1985; Okumura and Hara, 2004).

One of the key factors that are responsible for male morphotypic differentiation and spermatogenesis is androgenic gland hormones (AGHs), which are produced from AG (Okumura and Hara, 2004; Nagaraju, 2011). Recently, an insulin-like androgenic hormone (*IAG*) which is the most important hormone has been isolated and characterized in many decapods species such as red-claw crayfish (*Cherax quadricarinatus*) (Manor et al., 2007), giant freshwater prawn (*M. rosenbergii*) (Ventura et al., 2009), kuruma prawn (*Marsupenaeus japonicus*) (Banzai et al., 2011), black tiger shrimp (*Penaeus monodon*) (Mareddy et al., 2011) and Chinese shrimp (*Fenneropenaeus chinensis*) (Li et al., 2012). The hormone was strongly expressed in AG in all species (Ventura et al., 2009; Mareddy et al., 2011; Li et al., 2012) and showed adverse effect on male phenotypic gender differences and spermatogenesis after introduced with RNAi (Ventura et al., 2009).

Male reproduction-related gene (*MRR*) was identified only in male reproductive tract of crustacean, e.g. *M. rosenbergii* (Cao et al., 2006) and blue swimming crabs (*Portunus pelagicus*) (Sroyraya et al., 2013). The *MRR* gene was subsequently characterized and was suggested to involve sperm capacitation and fertilization (Phoungpetchara et al., 2012; Sroyraya et al., 2013), while other male reproductive-related hormones such as *M. rosenbergii* androgenic gland hormone-like protein (*MAL*) (FJ595507) (unpublished data) were subsequently isolated from male reproductive tract. However, characterization of this gene has not yet been studied.

Since sexual identification by phenotypic observation from prawn larvae is very difficult, more specific timing for sex differentiation could be helpful to manipulate sexing techniques and to serve mono-sex culture in the future. The expression of these male reproductive tract genes (*IAG*, *MRR,* and *MAL*) might be used to investigate the relevance of gene expression and male phenotypic development during the larval stage.

Materials and Methods

Animals: Adult blue-claw males, juveniles and larvae of *M. rosenbergii* were obtained from a local culture farm in Chachoengsao province, Thailand. Reproductive tracts of the adult males were dissected. Vas deferens (VD) and terminal ampullae (TA) were collected and then preserved in RNA (Ambion, USA). The samples were kept at room temperature for 1 d and later at -20^{o}C until used. Whole bodies of 1-, 2-, 3- and 4-week-old larvae (20 each) were harvested. Cephalothorax area of the 5th periopod from 2- and 3-month-old prawns (5 each) was collected. All samples were kept similarly as the adult samples.

Total RNA extraction and androgenic specific gene expression: Total RNA from all samples was extracted using Trizol reagent (Sigma, USA). A 1st stranded cDNA was synthesized using RevertAid™ first Strand cDNA Synthesis Kit (Fermentas, Canada). Open reading frames (ORFs) of androgenic gland hormone genes were amplified by the primer sets described in Table 1. The efficiency of cDNA was assessed by housekeeping gene, beta actin primer (Table 1). PCR products were subjected to run in 1.0% agarose gel electrophoresis and to be visualized under UV using gel documentation (Biorad, USA).

Sequence analysis: The PCR products were separated in agarose gel, cut, purified and ligated into pGEM-T-easy vector (Promega, USA). Recombinant plasmids were transformed into competent cells, *E.coli* (JM109). Selected clones were subjected for plasmid extraction (Macherey-Nagel, Germany) and submitted for DNA sequencing (First base, Singapore). Nucleotide sequence analysis was carried out using Genetyx V 7.0 and Blast algorithm (http://www.ncbi.nlm.nih.gov/blast/blastx).

Table 1 Primer sets used in this study

ORF of AG specific genes	**Product length (bp)**	**Accession no.**
IAG ORF s 5′ ATGGGATACTGGAATGCCGA 3′	519	ACJ38227
IAG ORF as 5′ CCTGGAACTGCAGGTGTTAA 3′		
MRR ORF s 5′ ATGGCTTCCTTCTTCAAGTT 3′	330	ABQ41239
MRR ORF as 5′ ATCATAAAAACGTCCTGAAT 3′		
MAL ORF s 5′ ATGAAGTGGTTCTCTCTTTT 3′	261	ACM18117
MAL ORF as 5′ TTTCTCGCAGCATTCCAGGT 3′		
Housekeeping genes		
Mr β-act s 5' CCCAGAGCAAGCGAGGTA 3'	461	AAV71158
Mr β-act as 5' GTGGTCGTGAAGGTGTAGCAG 3'		

Table 2 Nucleotide homology of amplified fragment with other genes in Genbank data base

Gene	Accession no.	Match spp.	E-Value	Identity	Gaps
IAG					
insulin-like androgenic gland specific factor	ACJ38227	*M. rosenbergii*	5.00E-113	172/173 (99%)	0/173 (0%)
insulin-like androgenic gland factor	BAJ78349	*M.lar*	2.00E-75	128/183 (70%)	11/183 (6%)
insulin-like androgenic gland factor	BAJ84108	*P. paucidens*	5.00E-67	114/173 (66%)	2/173 (1%)
insulin-like androgenic gland factor	BAJ84109	*P. pacificus*	2.00E-40	83/174 (48%)	4/174 (2%)
insulin-like androgenic gland factor	ABH07705	*C. quadricarinatus*	6.00E-12	46/169 (27%)	17/169 (10%)
MRR					
male reproductive-related protein Mar-Mrr	ABQ41239	*M. rosenbergii*	3.00E-55	109/110 (99%)	0/110 (0%)
MAL					
androgenic gland hormone-like protein	ACM18117	*M. rosenbergii*	4.00E-49	87/87 (100%)	0/87 (0%)

SEM study in larva: Forty prawn larvae of 1-4 weeks old and 20 of 2- and 3-month-old prawns were collected from a hatchery for comparative anatomical study using stereomicroscopy and scanning electron microscopy (SEM). For stereomicroscopy, sizes and shapes of fresh samples of 1- to 4-week-old prawn larvae were compared, particularly the second pleopods (swimming legs). For SEM study, all specimens were preserved in 2.5% glutaraldehyde in 0.1 M phosphate buffer. Then, the whole bodies of prawn larvae and cephalothorax with the 4th and 5th walking legs of 2- and 3-month-old prawns were post-fixed in 2% OsO_4 and were subsequently dehydrated by ethanol and isoamylacetate, respectively. Then, the specimens were mounted on aluminum stubs and were coated with gold. After that, all prawns were visualized under a scanning electron microscope (JEOL, JSM 5410LV).

Results

Specific gene expression in reproductive tract of male prawn: To confirm the expression of androgenic hormone genes (*IAG, MRR* and *MAL*) from adult male prawn reproductive tract, TA and VD were used as a source of cDNA templates. The expected products were shown from TA but not from VD cDNA template (Fig 1).

The DNA fragments were subsequently cloned for DNA sequencing. DNA sequence analysis indicated that a full ORF amplified from the three genes was obtained and showed high homology with the reported genes (Fig 2). The ORF of obtained *IAG* gene showed 173 deduced amino acids which was 99% identity with insulin-like androgenic gland specific factor from *M. rosenbergii* accession no. ACJ38227. One nucleotide of the 2nd frame was changed (A^{395} to G^{395}) (data not shown), resulting in a change in one amino acid (Asp^{131} to Gly^{131}) (Fig 2a). The *MRR* gene revealed 99% identity with male reproductive-related protein *Mar-Mrr* from *M. rosenbergii* accession no. ABQ41239. The ORF contained 110 deduced amino acids with a change in one nucleotide of the 2nd frame (G^{161} to A^{161}) (data not shown), leading to an alteration in one deduced amino acid (Arg^{54} to Lys^{54}) (Fig 2b). The *MAL* gene (87 deduced amino acids) gave 100% identity with androgenic gland hormone-like protein from *M. rosenbergii* accession no. ACM18117 (Fig 2c). Searching homology using Blast algorithm was done and results are shown in Table 2.

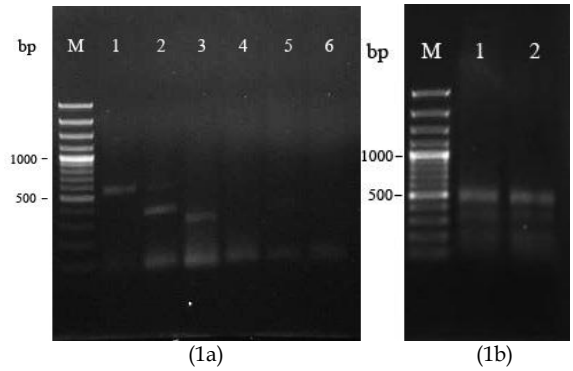

Figure 1 Amplification of specific genes using TA (1-3) and VD (4-6) as cDNA templates. *IAG* (2, 4), *MRR* (3, 5), *MAL* (4, 6), 100 bp DNA ladder (M) **(1a)**. *β-act* from cDNA of TA (1) and VD (2) as internal control **(1b).**

Expression of male reproductive genes in larval and juvenile prawn: The three genes were used as markers for male prawn development. RT-PCR was performed using whole body RNA extraction from 1- to 4-week-old prawns after hatching. No expected bands were amplified in all samples, albeit β-actin was observed (data not shown). The three genes can be detected from 2- and 3-month-old prawns after hatching (Fig 3)

```
2a
Query   1    MGYWNAEIKCVLFCSLVASLLPQPSSSYEIECLSVDFDCGDITNTLASVCLRHNNYINPG  180
             MGYWNAEIKCVLFCSLVASLLPQPSSSYEIECLSVDFDCGDITNTLASVCLRHNNYINPG
Sbjct   1    MGYWNAEIKCVLFCSLVASLLPQPSSSYEIECLSVDFDCGDITNTLASVCLRHNNYINPG  60

Query  181   PTYVSKERRSADIYTVPSTKSPSLAHPRATHLTMADEETQKVSKVEEEIQHMTLSREEAN  360
             PTYVSKERRSADIYTVPSTKSPSLAHPRATHLTMADEETQKVSKVEEEIQHMTLSREEAN
Sbjct   61   PTYVSKERRSADIYTVPSTKSPSLAHPRATHLTMADEETQKVSKVEEEIQHMTLSREEAN  120

Query  361   NMLHskrrfrrgsvrrsprEECCNNASFRRCNFEEVAEYCIELRPGVNTCSSR  519
             NMLHSKRRFRR SVRRSPREECCNNASFRRCNFEEVAEYCIELRPGVNTCSSR
Sbjct  121   NMLHSKRRFRRDSVRRSPREECCNNASFRRCNFEEVAEYCIELRPGVNTCSSR  173

2b
Query   1    MASFFKLISVMVMVAMGFILVSEAASDLQDAAMHDYPTVLEIIGSPRMKRSPHKAESGFY  180
             MASFFKLISVMVMVAMGFILVSEAASDLQDAAMHDYPTVLEIIGSPRMKRSPH+AESGFY
Sbjct   1    MASFFKLISVMVMVAMGFILVSEAASDLQDAAMHDYPTVLEIIGSPRMKRSPHRAESGFY  60

Query  181   GSNRGMEADFFgdsgtystgyglgglsrltgKFSGEGEFAGGAHSGRFYD  330
             GSNRGMEADFFGDSGTYSTGYGLGGLSRLTGKFSGEGEFAGGAHSGRFYD
Sbjct   61   GSNRGMEADFFGDSGTYSTGYGLGGLSRLTGKFSGEGEFAGGAHSGRFYD  110

2c
Query    1   MKWFSLFLLMALVYVSGQQSVYDKSEEEQIEILGpllrkllmprprpVYQIHQLLRVHVQ  180
             MKWFSLFLLMALVYVSGQQSVYDKSEEEQIEILGPLLRKLLMPRPRPVYQIHQLLRVHVQ
Sbjct    1   MKWFSLFLLMALVYVSGQQSVYDKSEEEQIEILGPLLRKLLMPRPRPVYQIHQLLRVHVQ  60

Query  181   KCCNSQPMMDCCTPALCCDMNLECCEK  261
             KCCNSQPMMDCCTPALCCDMNLECCEK
Sbjct   61   KCCNSQPMMDCCTPALCCDMNLECCEK  87
```

Figure 2 Deduced amino acid sequence comparison of amplified specimen (Query) to the reported gene (Sbjct) *IAG* (2a), *MRR* (2b), *MAL* (2c). Different amino acids are indicated by bold underline symbol.

Microscopic morphology changes in larval prawn: Stereomicroscopy and scanning electron microscopy (SEM) were employed to observe morphological changes in larval prawn (Fig 4). Microscopic study of forty larvae demonstrated that no significant male characteristic appeared during 1-4 weeks old. Pleopods were firstly observed in the 2-week-old larvae and grew rapidly in the 3- and 4-week-old larvae. However, an appendix masculinus could not be seen, while SEM showed very clear biramous appendages (endopods and exopods) in the 1- and 2-week-old larvae. However, exopods gradually reduced within 3 weeks (Fig 4b).

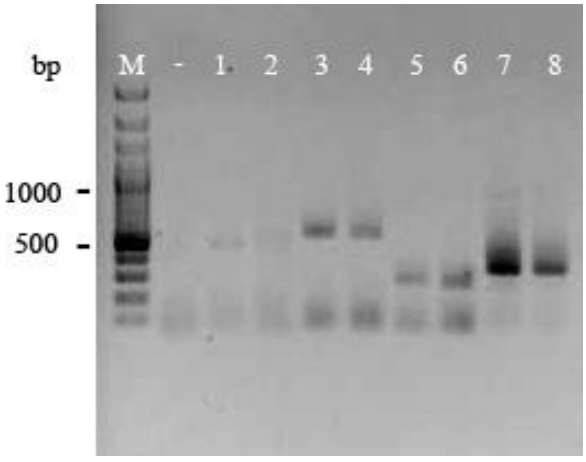

Figure 3 RT-PCR showing expression of 3 genes from 2-month-old prawn (1, 3, 5, 7) and 3-month-old prawn (2, 4, 6, 8). β-actin, 461 bp (1, 2); *IAG*, 519 bp (3, 4); *MAL*, 216 bp (5, 6); and *MRR*, 330 bp (7, 8); negative control (-)

Investigation into external reproductive organs: After rearing in pond for 1 and 2 months (2- and 3-month-old prawns), the prawns were harvested for sex determination. External characters of the male and female juvenile prawns were observed by naked eyes. The gonopore complex (GP) was initially observed in the 2-month-old juveniles. Within 3 months old, it was more clearly observed (Fig 5a). In addition, the appendix masculinus (AM) was obviously presented at the 2nd pleopod of the 3-month-old juveniles (Fig 5b).

Gonopore complex was examined in the 2- and 3-month-old juveniles under SEM. A pouch-liked structure was located at the base of the 5th periopod of the male prawns (Fig 6M). These prawns were previously examined for the existence of GP or AM. While, the GP did not exist in absent AM prawns (Fig 6F).

Applying with high magnification (x7,500), ultrastructure on a surface of the GP was observed. A valve-like structure approximately 8x18 um in dimension was shown. This structure was found on the GP surface from the 3-month-old prawns but not from the 2-month-old prawns (Fig 7).

Discussion

Since AG is a very small gland located only on male TA (Ventura et al., 2009), it is difficult for pure dissection. Total RNA extraction from TA, therefore, could provide transcripts from AG and parts of reproductive tract. The amplified sequences (*IAG, MRR, MAL*) showed high homology to reported genes of *M. rosenbergii*. Hence, the specific genes extracted from TA could be used as gene markers for time tracking during male larval development. From

previous reports of *M. rosenbergii*, *IAG* expression was observed only in AG (Ventura et al., 2009) and ejaculatory bulbs (EBs) or TA epithelial cells (Phoungpetchara et al., 2011). Similar to our study, *IAG* expression was detected only in TA. Interestingly, two forms of *IAGs* (*Fc IAG1 and Fc IAG2*) of *F. chinensis* can be expressed in a low level (40 PCR cycles) in hepatopancrease and nerve cord in both sexes. However, *Fc IAG2* was mainly expressed in AG (Li et al., 2012).

We used the specific genes as the male marker to demonstrate the timing for sex differentiation by using whole body larvae for RNA extraction. Similar to our result, *IAG* transcript was early observed at post larva 20 (PL20) from freshwater prawn larvae (Ventura et al., 2011), while *IAG2* from *F. chinensis* was detected from post larva 60 (PL60) (Li et al., 2012). These data together with our result could be concluded that *IAG* of *M. rosenbergii* was not produced within the 1st month after hatching. It was suggested that *MRR* might depend on androgen since its expression increased during AG maturation and localized in the epithelium of reproductive duct (VD and TA) (Cao et al., 2006; Phoungpetchara et al., 2012). However, our PCR product was not obtained from adult VD cDNA template while strongly clear band was depicted from the 2- and 3-month-old larval cDNAs. Similar result was observed in *MAL*. This is the first report on the earliest detection of *MAL* in larvae.

Besides gene expression, prawn morphology is routinely used for gender identification. Adult male morphotypic characters such as body weight/size or claw color/ size are obviously observed when they reach adult stage (Okumura and Hara, 2004). However, before adulthood, GC and AM are used as morphotypic criterion after earlier metamorphosis. From pictures of larval morphology, we suggested that

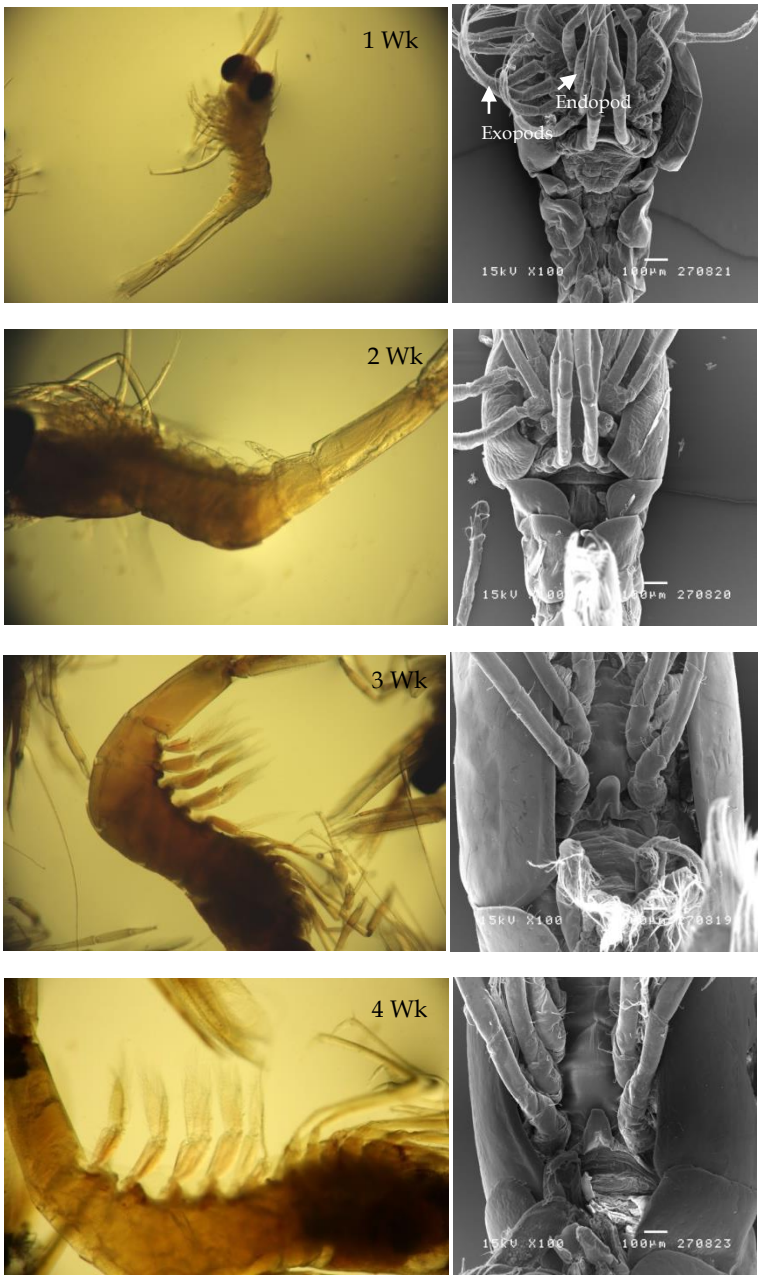

Figure 4 Morphological changes in 1- to 4-week-old larvae observed from stereomicroscope (left panel) (x30) and by SEM (right panel) (x100)

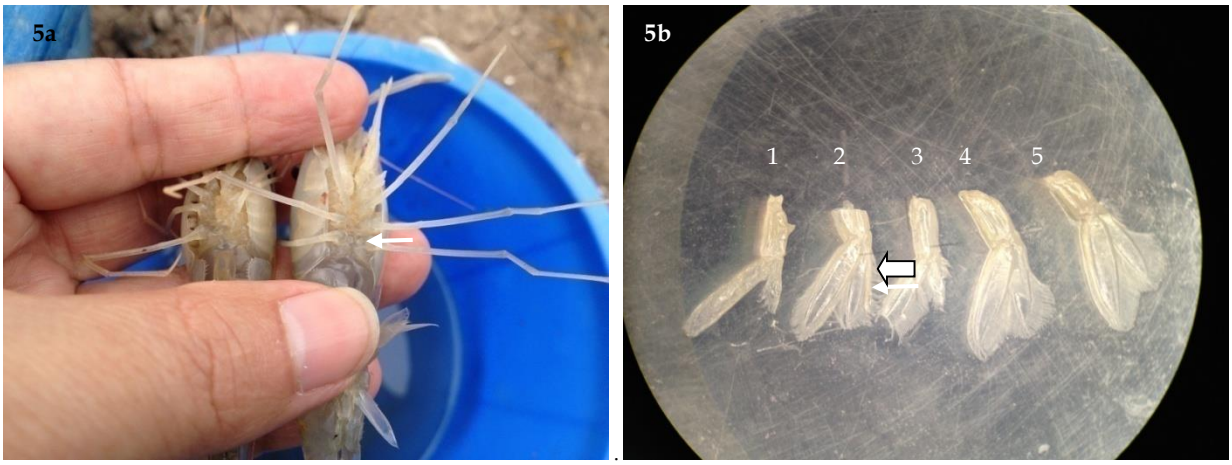

Figure 5 Male external characters of 3-month-old juvenile prawn. (5a) gonopore complex (white arrow), (5b) appendix masculinus (white arrow) located at the 2nd pleopod.

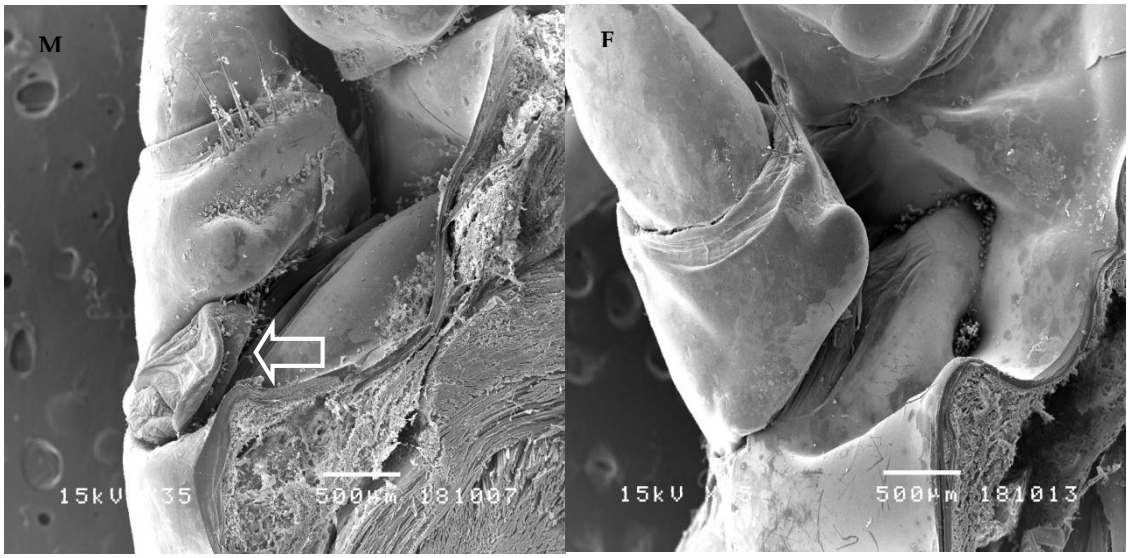

Figure 6 SEM illustrating a structural difference at the base of the 5th periopod of male (M) and female (F) prawns. The arrow indicates the gonopore complex of male prawn.

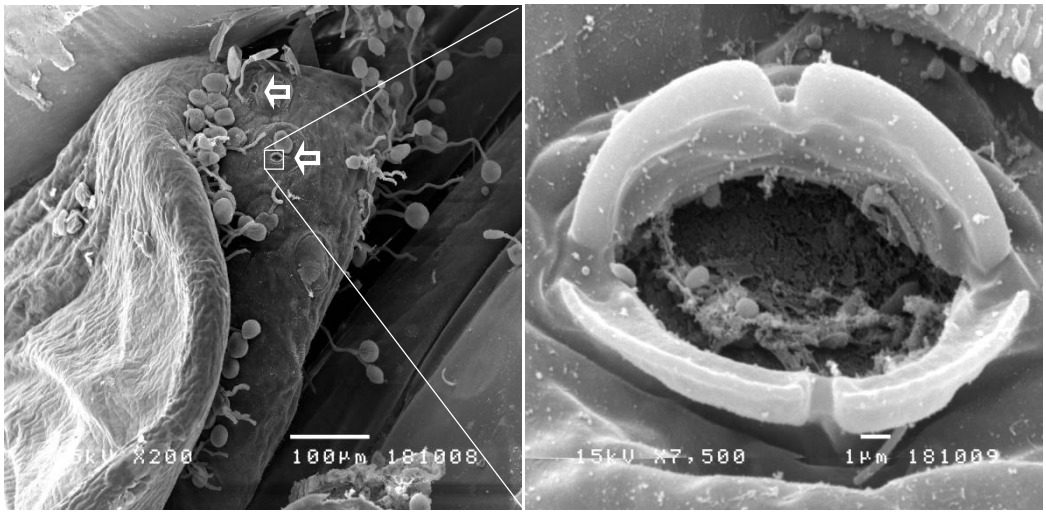

Figure 7 Ultrastructure on gonopore complex surface were depicted by SEM from 3-month-old male prawn (white arrows). With high magnification, a valve-like structure was observed.

no sex differentiation occurred during the first month. A major activity of the larvae seems to focus on metamorphosis. All our specimens underwent metamorphosis as shown by the degeneration of exopods within the first month. The degeneration occurs in crustaceans since they are classified as primitively biramous which exopods are absent during metamorphosis (Boxshall and Jaume, 2009). Within the metamorphosis period, GP and AM were not observed. These may be because there was no male specific gene expression during the first month. Thereafter, the male specific genes were detected. GP and AM were observed in the meantime. Our observing time was in accord with previous discoveries (Campos-Ramos et al., 2006; Ventura et al., 2011). From these data, we addressed that the sex differentiation process of male freshwater prawn began when the larvae were 2 months old or after complete metamorphosis.

This study is also the first to present the ultrastructure on juvenile male prawns. The valve-like structure should be further examined in adult prawn GP surface to confirm its function. However, we hypothesized that this structure might be an opening valve that control sperm fertilization during spermatophore implantation.

Phenotypic characters of male prawn were presented in agreement with the timing for transcript expression. The detection of male specific transcripts reveals the time point for sex differentiation in prawn. This could be used as a key to successful mono-sex culture manipulation. Therefore, further researches on timing for sex reversal should focus on the stage of post larva (PL) prawns.

Acknowledgements

This study was financially supported by Faculty of Veterinary Science, Chulalongkorn University (grant number: RG 12/2554).

References

Banzai K, Ishizaka N, Asahina K, Suitoh K, Izumi S and Ohira T 2011. Molecular cloning of a cDNA encoding insulin-like androgenic gland factor from the kuruma prawn Marsupenaeus japonicus and analysis of its expression. Fish Sci. 77: 329-335.

Boxshall GA and Jaume D 2009. Exopodites, Epipodites and Gills in Crustaceans. Arthropod Systematics and Phylogeny. 67(2): 229-254.

Campos-Ramos R, Garza-Torres R, Guerrero-Tortolero DA, Maeda-Martinez AM and Obregon-Barboza H 2006. Environmental sex determination, external sex differentiation and structure of the androgenic gland in the Pacific white shrimp Litopenaeus vannamei (Boone). Aqua Res. 37: 1583-1593.

Cao JX, Yin GL and Yang WJ 2006. Identification of a novel male reproduction-related gene and its regulated expression patterns in the prawn, Macrobrachium rosenbergii. Peptides. 27(4): 728-735.

Charniaux-Cotton H 1962. Androgenic gland of crustaceans. Gen Comp Endocrinol. Suppl 1: 241-247.

Charniaux-Cotton H and Payen G 1985. Sexual differentiation. Vol 9. In: The Biology of Crustacea Academic Press, New York. 217-219.

Cronin LE 1947. Anatomy and histology of the male reproductive system of Callinectes sapidus Rathbun. J Morphol. 81(2): 209-239.

Li S, Li F, Sun Z and Xiang J 2012. Two spliced variants of insulin-like androgenic gland hormone gene in the Chinese shrimp, Fenneropenaeus chinensis. Gen Comp Endocrinol. 177(2): 246-255.

Manor R, Weil S, Oren S, Glazer L, Aflalo ED, Ventura T, Chalifa-Caspi V, Lapidot M and Sagi A 2007. Insulin and gender: an insulin-like gene expressed exclusively in the androgenic gland of the male crayfish. Gen Comp Endocrinol. 150(2): 326-336.

Mareddy VR, Rosen O, Thaggard HB, Manor R, Kuballa AV, Aflalo ED, Sagi A, Paterson B and Elizur A 2011. Isolation and characterization of the complete cDNA sequence encoding a putative insulin-like peptide from the androgenic gland of Penaeus monodon. Aquaculture. 318: 364-370.

Nagaraju GP 2011. Reproductive regulators in decapod crustaceans: an overview. J Exp Biol. 214(Pt 1): 3-16.

Okumura T and Hara M 2004. Androgenic gland cell structure and spermatogenesis during the molt cycle and correlation to morphotypic differentiation in the giant freshwater prawn, Macrobrachium rosenbergii. Zoolog Sci. 21(6): 621-628.

Phoungpetchara I, Tinikul Y, Poljaroen J, Changklungmoa N, Siangcham T, Sroyraya M, Chotwiwatthanakun C, Vanichviriyakit R, Hanna PJ and Sobhon P 2012. Expression of the male reproduction-related gene (Mar-Mrr) in the spermatic duct of the giant freshwater prawn, Macrobrachium rosenbergii. Cell Tissue Res. 348(3): 609-623.

Phoungpetchara I, Tinikul Y, Poljaroen J, Chotwiwatthanakun C, Vanichviriyakit R, Sroyraya M, Hanna PJ and Sobhon P 2011. Cells producing insulin-like androgenic gland hormone of the giant freshwater prawn, Macrobrachium rosenbergii, proliferate following bilateral eyestalk-ablation. Tissue Cell. 43(3): 165-177.

Sroyraya M, Hanna PJ, Changklungmoa N, Senarai T, Siangcham T, Tinikul Y and Sobhon P 2013. Expression of the male reproduction-related gene in spermatic ducts of the blue swimming crab, Portunus pelagicus, and transfer of modified protein to the sperm acrosome. Microsc Res Tech. 76(1): 102-112.

Ventura T, Manor R, Aflalo ED, Weil S, Khalaila I, Rosen O and Sagi A 2011. Expression of an Androgenic Gland-Specific Insulin-Like Peptide during the Course of Prawn Sexual and Morphotypic Differentiation. ISRN Endocrinol. 2011: 476283.

Ventura T, Manor R, Aflalo ED, Weil S, Raviv S, Glazer L and Sagi A 2009. Temporal silencing of an androgenic gland-specific insulin-like gene affecting phenotypical gender differences and spermatogenesis. Endocrinology. 150(3): 1278-1286.

Zupo V, Juttner F, Maibam C, Butera E and Blom JF 2014. Apoptogenic metabolites in fractions of the Benthic diatom Cocconeis scutellum parva. Mar Drugs. 12(1): 547-567.

บทคัดย่อ

ระยะเวลาที่มีการแสดงออกของยีนในระบบสืบพันธุ์เพศผู้และพัฒนาการของ Gonopore Complex ในกุ้งก้ามกราม (*Macrobrachium rosenbergii*)

ประพฤติดี ปิยะวิริยะกุล[1*] ดำริ ดาราวิโรจน์[2]

ความต้องการกุ้งก้ามกรามเพศผู้ในตลาดมีมากกว่ากุ้งเพศเมีย การเพาะกุ้งก้ามกรามเพศผู้ล้วนจึงเป็นผลดีต่อเกษตรกร ดังนั้นการพัฒนาวิธีการการคัดแยกเพศและเทคนิคการแปลงเพศเพื่อให้ได้กุ้งเพศผู้ล้วนจึงเป็นสิ่งจำเป็น อย่างไรก็ตาม เนื่องจากการคัดแยกเพศในสัตว์กลุ่มคลัสตาเชียนทำได้ยากในช่วงอายุน้อย การหาระยะเวลาที่แน่นอนที่สามารถทราบเพศลูกกุ้งก้ามกรามจึงจำเป็นอย่างยิ่ง การศึกษานี้ได้เพิ่มจำนวนและแยกยีน 3 ยีนที่เกี่ยวข้องกับฮอร์โมนสืบพันธุ์เพศผู้ (*IAG, MRR* and *MAL*) จาก terminal ampulae (TA) จากกุ้งก้ามกรามเพศผู้ตัวโตเต็มวัย ยีนทั้งสามถูกใช้สำหรับติดตามการเปลี่ยนเพศในลูกกุ้งก้ามกราม การแสดงออกของยีนดังกล่าวร่วมกับข้อมูลเฉพาะของลักษณะเพศผู้ เช่น appendix masculina (AP) and gonopore complex (GP) ที่สังเกตจากภายนอก ทั้งจากการดูด้วยตาเปล่าและภายใต้กล้องจุลทรรศน์ ทำให้สรุปได้ว่า ลูกกุ้งก้ามกรามมีการแสดงออกของเพศผู้หลังจากเสร็จสิ้นกระบวนการเมตามอร์ฟอร์ซีส หรือเมื่ออายุ 2 เดือนขึ้นไปหลังจากฟัก นอกจากนี้กล้อง SEM ได้รายงานภาพของโครงสร้างที่เล็กมากลักษณะคล้ายทางออกของน้ำเชื้อบนผิวของ gonopore complex ระหว่างการเจริญของลูกกุ้งเพศผู้เป็นครั้งแรกด้วย

คำสำคัญ: ต่อมแอนโดรจีนิค กุ้งก้ามกราม โกโนพอร์คอมเพลกซ์ เวลาในการแยกเพศ

[1] หน่วยชีวเคมี ภาควิชาสรีรวิทยา คณะสัตวแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย ปทุมวัน กรุงเทพฯ 10330

[2] ภาควิชากายวิภาคศาสตร์ คณะสัตวแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย ปทุมวัน กรุงเทพฯ 10330

***ผู้รับผิดชอบบทความ E-mail:** ppraprud@chula.ac.th*