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Identification of genes involved in reproduction and lipid pathway metabolism in wild and domesticated shrimps

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ABSTRACT The aims of this study were to identify genes involved in reproduction and lipid pathway metabolism in *Penaeus monodon* and correlate their expression with reproductive performance. Samples of hepatopancreas and ovaries were obtained from a previous study of the reproductive performance of wild and domesticated *Penaeus monodon* broodstock. Total mRNA from the domesticated broodstock was used to create two next generation sequencing cDNA libraries enabling the identification of 11 orthologs of key genes in reproductive and nutritional metabolic pathways in *P. monodon*. These were identified from the library of *de* novo assembled contigs, including the description of 6 newly identified genes. Quantitative RT-PCR of these genes in the hepatopancreas prior to spawning showed that the domesticated mature females significantly showed higher expression of the *Pm Elovl4*, *Pm COX* and *Pm SUMO* genes. The ovaries of domesticated females had a significantly decreased expression of the *Pm Elovl4* genes. In the ovaries of newly spawned females, a significant correlation was observed between hepatosomatic index and the expression of *Pm FABP* and also between total lipid content and the expression of *Pm CYP4*. Although not significant, the highest levels of correlation were found between relative fecundity and *Pm CRP* and *Pm CYP4* expression, and between hatching rate and *Pm Nvd* and *Pm RXR* expression. This study reports the discovery of genes involved in lipid synthesis, steroid biosynthesis and reproduction in *Penaeus monodon*. These results indicate that genes encoding enzymes involved in lipid metabolism pathways might be potential biomarkers to assess reproductive performance.

Keywords: *Penaeus monodon*; Reproductive performance; Lipid metabolism; Gene expression

1. **Introduction**

Over the past decade, many shrimp farmers have switched from farming the Giant Tiger shrimp *Penaeus monodon* to the Pacific white shrimp *Litopenaeus vannamei* which now dominates global shrimp production. One of the key reasons that farmers prefer *L. vannamei* is the comparatively slow progress in the domestication and selective breeding of *P. monodon* (Briggs et al., 2005). Although some advances have been made in domesticating *P. monodon* (Preston et al., 2009, Preston et al., 2010), seedstock production is less efficient than wild broodstock (Arnold et al., 2013, Coman et al., 2013, Marsden et al., 2013) and most *P. monodon* hatcheries continue to rely on wild-caught broodstock (Klinbunga et al., 2010). A broad range of parameters have been used to evaluate reproductive performance in wild and cultured crustacean populations. These include biological (survival, growth-molting, fecundity, spawning and hatching rate), morphometrical (weight and carapace length), histological (mainly ovary and hepatopancreas function-anatomical changes) and biochemical parameters, such as elemental and proximal composition, lipid classes and fatty acids, and digestive enzymes (cf. Arnold et al., 2013, Glencross et al., 2013, Rotllant et al., 2013, Rotllant et al., 2014). The domestication of *P. monodon* has been constrained, in part, by the lack of basic information about ovarian development and maturation in Penaeid shrimp (Uawisetwathana et al., 2011, Talakhun et al., 2012, Hiransuchalert et al., 2013, Wimuttisuk et al., 2013, Phinyo et al., 2014, Treerattrakool et al., 2014).

In Penaeid shrimp, ovarian development is characterized by the accumulation of a major yolk protein vitellin and the formation of cortical rods in the oocytes. The precursor of vitellin, vitellogenin (Vtg), is synthesized in the ovary and hepatopancreas, transported to the oocytes, and accumulate in the ooplasm as vitellin for utilization as a

nutritional source during embryogenesis. After the completion of yolk accumulation, the cortical rods are formed radially around the periphery of the oocyte plasma membrane, and mature oocytes are spawned. Upon spawning, the content of the cortical rods is released around eggs and forms into a jelly-like layer which envelops the eggs. The process is under the control of a neuroendocrine organ in the eyestalk (the X-organ sinus gland complex) (Okumura et al., 2006). Vitellogenin receptor (VgR), vitellogenin inhibiting hormone (VIH) and cortical rod proteins (CRP) have been identified in *P. monodon* (Tiu et al., 2006, Tiu et al., 2008, Treerattrakool et al., 2008, Lehnert et al. unpublished-NCBI) and might be used as indicators for ovarian maturation.

Nutrition plays a substantial role in reproductive processes of Penaeid shrimp, particularly the levels of dietary total and specific lipids. Lipid content has been reported to decrease in the hepatopancreas and transferred to the ovary during maturation (for reviews see Wouters et al., 2001, Glencross, 2009). For *P. monodon* broodstock the dietary total lipid requirement is around 11% of total fed dry matter (Marsden et al., 1997) and the essential cholesterol to 1.7% in juveniles (Smith et al., 2001). Several studies have shown that arachidonic acid (ARA) plays a key role in egg development and spawning in *P. monodon* (Huang et al., 2008, Hoa et al., 2009). The prostaglandins (eicosanoids) are a suite of hormones known to influence a range of physiological processes including reproduction. Arachidonic acid is a precursor in prostaglandin synthesis and is therefore considered to have a further key functional role in reproduction. When prostaglandin biosynthesis was examined in different stages of *P. monodon* ovaries, Wimuttisuk et al. (2013) found that the amounts of prostaglandin F synthase gene transcripts and prostaglandin F2α decreased as the ovaries matured. Therefore, it has been proved that lipid metabolism is a key process in *P. monodon* ovarian maturation.

In contrast to peptide hormones related to reproduction, the study of lipid metabolism requires complex biosynthetic pathways for their synthesis. This in turn relies on the identification of genes encoding these biosynthetic steps, namely fatty acid, eicosanoid and cholesterol biosynthesis. The aim of the study was to discover genes involved in reproduction and lipid pathway metabolism, and to correlate their expression with reproductive performance parameters in wild and domesticated broodstock of *P. monodon*.

2. **Material and methods**

2.1. Shrimp broodstock origin and rearing conditions, tissue sampling and virus screening

A previous study (Arnold et al., 2013) used cultured wild broodstock (W) caught off the coast of Innisfail (17°53′S, 146°01′E, Queensland, Australia) and eighth generation domesticated (D) *P. monodon* stock originated from wild founder stocks collected from a population off the coast of Weipa (12°48′, 141°32′, Gulf of Carpentaria, Queensland, Australia) to determine reproductive performance in a series of reciprocal cross matings. Taking into account the previous study results for hatching rate, we classified the females into good spawners and poor spawners and we compared them with females just before spawning (I=initial). The characteristics of each group of broodstock are summarized in Table 1. For gene expression analyses we sampled ovaries from the initial group and hepatopancreas for all the groups. To rule out the effects of occasional pathologies in Australian farms, we analyzed hepatopancreas tissues for virus screening;

Mourilyan virus and gill associated virus by qPCR following the method of De la Vega et al. (2004) and Rajendran et al. (2006), respectively.

Table 1. Selected wild (W) and domesticated (D) female broodstock from Arnold et al. (2013) and classification according their hatching rate (I= initial, before spawning; GS=good spawner; BS=bad spawner).

	WI	DI	WGS	WBS	DGS	DBS
N	5	5	3	3	4	6
Ovarian Stage[1]	IV	IV	0	0	0	0
Female weight (mg)	140.98±22.19	161.80±26.02	141.57±23.17	151.63±6.80	164.80±33.13	159.40±10.00
Hepatosomatic index	2.96±0.32	2.86±0.25	2.71±0.43	2.74±0.34	2.58±0.21	2.60±0.60
Hatching rate[2] (%)			78.07±11.26	41.10±11.32	62.35±9.29	25.57±5.87
Relative fecundity			4.00±1.09	5.02±1.60	1.77±0.52	2.30±0.86

[1]Ovarian stage following Tan-Fermin & Pudadera (1989).[2]Hatching rate calculation only included the fertilized eggs.

2.2. RNA extraction, gene screening, P. monodon libraries generation and primer design

RNA was extracted from the hepatopancreas and ovary tissues from three to six individuals from each treatment. Total RNA was extracted using RNeasy RNA Extraction Columns and on-column DNase digestion (Qiagen, Germantown, MD, USA) according to the manufacturer's instructions. RNA quantity was assessed using a Nanodrop spectrophotometer (Thermo Fisher Scientific, USA), and subsequently each sample diluted to 100 ng μL^{-1}. The quality of all RNA samples was assessed using the Agilent RNA 6000 Nano Kit and the Agilent 2100 Bioanalyser (Agilent Technologies, Waldbronn, Germany). For the hepatopancreas and ovary next generation sequencing libraries, a pool of RNA extractions from 12 and 10 animals, respectively, to contain 300 μg of total RNA was used. Samples were sent for pyrosequencing using two separate regions of ¼ plate on a Roche 454 GS-FLX Titanum sequencer (Macrogen, Korea). Results were trimmed removing the low quality data (quality of phred score<30), 454 adaptors and 50 bp minimum read length, and a partial transcriptome for both ovary and hepatopancreas was *de novo* assembled using CLC Genomics

146 Workbench software (CLC Bio, Aarhus, Denmark). These libraries were investigated
147 for target genes in shrimp reproduction using the most closely related species in
148 scientific journals and NCBI data bases sequences (Ibarra et al., 2007) and/or lipid
149 nutritional pathways to the Kyoto Encyclopedia of Genes and Genome (KEGG) as fatty
150 acid, steroid and eicosanoid biosynthesis (http://www.genome.jp/kegg/). Putative gene
151 orthologs were identified by BLASTX sequence similarity searches using CLC Main
152 Workbench software (CLC Bio, Aarhus, Denmark). Primers for each specific gene
153 target were designed using PerlPrimer program (Marshall, 2004), using the following
154 conditions: temperature: 58-62 ^{0}C, distance between pair of primers: 100-300 pb and,
155 GC%: 45-55%. The list of pairs of primers for each gene is detailed in table 2.
156

Table 2. Genes, accession numbers and primer sequences used in quantitative real-time PCR analysis.

Gene Name	Gene Abbreviation	Genbank Accession Number	Primer name	Primer Sequence
Ovarian Development				
Vitellogenin	*Pm Vtg*	DQ288843	Pm Vgt F4	TTGTAATGAGTGAAGTGAGTGG
			Pm Vgt R4	CCTTGATTTCCTCCTTTGCC
Vitellogenin receptor	*Pm VgR*	EU024890	Pm VgR F1	GAATCCAGATATCAAACGCCT
			Pm VgR R1	CCCAAATCAAGAGACATCATCC
Cortical Rod Protein	*Pm CRP*	AF510332	Pm CRP F1	CTGCTACCCAAATGAACCTC
			Pm CRP R1	CATCGAAGACCATACCATCTG
Lipid Metabolism				
Fatty acid elongase 4	*Pm Elovl4*	JX128699	Pm Elovl4 F1	CAGTTTACCTCACCTTCGTC
			Pm Elovl4 R1	TTGACAGAACCAGCTATATCGG
Fatty Acid synthase	*Pm FAS*	JX128703	Pm FAS F1	AATGCTTCAGTTCTCACTTGTAGG
			Pm FAS R1	CTGGATGATAACACCCTGACAC
Fatty Acid Biding Protein	*Pm FABP*	ABE77154	Pm FABP F1	AGGAATTTGAAGAGACCACAG
			Pm FABP R1	CACTCCATTAGCATCTTGTC
Steroid Hormone Biosynthesis				
Cyclooxygenase	*Pm COX*	JX128701	Pm COX F2	GACCGACTACAAGAAGGGAC
			Pm COX R2	GGAAGAAGTGCGAAGAAAGG
Cytochrome P450 enzyme 4C	*Pm CYP4C*	JX128700	Pm CYP4 F1	CGGAGAACTGCATTAAAGAG
			Pm CYP4 R1	GGAACATAAGCATAAGGATGAC
Neverland	*Pm Nvd*	JX128704	Pm Nvd F1	GTACCATTCCATCCTGAAGAC
			Pm Nvd R1	TAGTACACGTAGAAGATGCTG
Transcriptional Regulation				
Retinoid X Receptor	*Pm RXR*	JX128702	Pm RXR F1	CCTCCAGTTACTCCAACACC
			Pm RXR R1	GCAACCTTCACAACTGTAGAC
Small ubiquitin like modifier	*Pm SUMO*	ACD13593	Pm SUMO F4	CAATGAGATCCACTTCCGAG
			Pm SUMO R4	ACTTCCTTAACCTAACGATGTC
Control Genes				
18 S	*Pm 18s*	AY074921	18S rRNA qPCR.For	GCCGGCACGTTTACTTT
			18S rRNA qPCR.Rev	ACCGAGGTCCTCTTCCATCA
Luciferase	*Luc*	na	Luc qPCR For	GGTGTTGGGCGCGTTATTTA
			Luc qPCR Rev	CGGTAGGCTGCGAAATGC

157
158
159 *2.3. Gene expression analysis and quantification*
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161 cDNA synthesis was performed on the 26 hepatopancreas and the 10 ovary samples
162 using the SuperScript III First-Strand Synthesis System for RT-PCR (Invitrogen,
163 Carlsbad, CA, USA, Cat. No: 18080-051). An equivalent of 1 μg of total RNA was
164 reverse transcribed following previously published protocols (Callaghan et al., 2010),
165 which incorporated 400 pg of non-endogenous Luciferase RNA (Promega L4561,
166 Venlo, The Netherlands) as an internal control.
167 Real-time PCR reactions were carried out using 1x SYBR Green PCR Master Mix
168 (Life Technologies Corporation, Carlsbad, CA, USA), 0.2 μmol^{-1} of each primer and
169 the equivalent of 7.5 ng of reverse-transcribed RNA. Amplification cycle conditions
170 were 2 min at 50°C, 10 min at 95°C, followed by 40 cycles of 15 s at 95°C and 40 s
171 at 60°C, and completed by a dissociation melt-curve analysis. Reactions were set up in
172 triplicate and run on an ABI ViiA7 Real-time PCR system (Applied Biosystems, Life
173 Technologies, Carlsbad, USA). Removal of gDNA contamination was verified by gene-
174 specific PCR amplification of an equivalent amount of DNase treated RNA sample that
175 had not been reverse transcribed. Optimal template concentration and PCR efficiencies
176 (95%) for each primer pair were calculated using a five-fold serial dilution of a mixture
177 of cDNA. Normalisation was performed against Luciferase and 18S internal control
178 genes. For comparison between treatments, relative quantification (RQ) expression of
179 targets genes is shown as relative fold change against wild initial group.
180 To confirm the amplification of each specific gene, products of the qPCR reactions
181 were sequenced by Sanger sequencing using BigDye V3.1 and an Applied Biosystems
182 ABI Hitachi 3130xl Prism Genetic Analyzer according to established methods.
183 Sequencing PCR reactions were cleaned with Agencourt CleanSEQ Sequencing
184 Reaction Clean-Up system utilizing Agencourt's patented SPRI® paramagnetic bead
185 technology (Beckman Coulter, Beverly, MA, USA).

2.4. Lipid analysis

Total lipid content (LP) was quantified gravimetrically after extraction in chloroform/methanol (2:1) and evaporation of the solvent under a stream of nitrogen followed by vacuum desiccation overnight (Folch et al., 1957).

2.5. Statistical analysis

Where comparison between individual measurements was required, statistical significance was assessed by single-factor analysis of variance (ANOVA), followed by Tukey's HSD test allowing 5 % error. Pearson's correlation was used to determine linear dependence between measurements, using a 5 % confidence interval and a Student's t test to attribute statistical significance. All statistical analyses were performed using StatPlus:Mac 2009 (AnalystSoft Inc, 2009).

3. **Results**

Next-generation sequencing returned 277,335 reads for the hepatopancreas library and 287,211 reads for the ovary library, of which more than 99 % were retained after quality trimming, resulting in an average length of 501 bp and 508 bp, respectively. Along with published ESTs, sequence data were assembled into 5,110 contigs for the hepatopancreas and 9,345 contigs for the ovary with an average contig length of 811 bp in the hepatopancreas and 899 bp in the ovary (Table 3).

Table 3. Next-generation sequencing data from the hepatopancreas and ovary of Australian East Coast wild *Penaeus monodon*.

		Hepatopancreas	Ovary	Combined data set
Raw Data	Read count	277,335	287,211	564,546
	Total bases (bp)	141,069,311	148,268,491	289,337,802
	Average read length (bp)	509	516	513
Trimmed Data	Read count	275,336	285,307	560,673
	Total bases (bp)	139,043,111	146,169,303	285,212,414
	Average read length (bp)	501	508	505
De novo assembly	Number of contigs	5110	9345	13,288
	N50 (bp)	885	986	995
	Total bases (bp)	4,141,895	8,397,326	11,840,729
	Average contig length (bp)	811	899	891
	Maximum contig length (bp)	8568	7912	8525
	Map reads	5,104	9,332	13,269
	Unmap reads	10,190	9,921	15,867

Within those, we identified and further analysed eleven key genes in the reproduction pathway and lipid metabolism of *P. monodon*. Three genes that belonged to the vitellogenesis synthesis pathway were previously described in *P. monodon* and identified in our combined library: (1) vitellogenin (Tui et al. 2006), (2) cortical rod proteins (also called shrimp ovarian peritrophin; Lehnert et al. unpublished), and (3) vitellogenin receptor (Tui et al. 2008). The first two genes are expressed in the hepatopancreas and ovary while the last gene is only expressed in the ovary. Three genes were identified as associated with the fatty acid metabolism. Two new genes were first described in *P. monodon*, fatty acid elongase 4 (*Pm Elovl4*) and fatty acid synthase (*Pm FAS*). The fatty acid binding protein (FABP) was already described in *P. monodon* by Soderhall et al. (2006). From the ecosanoid biosynthesis pathway we have newly identified for *P. monodon* the cyclooxygenase enzyme (*Pm COX*). Three new sequence from *P. monodon* were identified in our combined library from steroid metabolism: cytochrome P450 enzyme 4 (*Pm CYP4*), Neverland gene (*Pm Nvd*) and retinoid X receptor (*Pm RXR*). Finally, a gene encoding the small ubiquitin like modifier (SUMO) previously described in *P. monodon* by Leelatanawit et al. (2009) was also identified. Results of the BLASTX similarity searches of the newly described genes belonging to

lipid metabolism are detailed in Table 4, and raw sequence reads have been made available through the CSIRO Data Access Portal (https://data.csiro.au/dap/home).

Table 4. TBLASTX analyses of *Penaeus monodon* genes belonging to lipid metabolism.

Gene code	Pm accession #	AA	Matched accession # (Reference species)	Identity (%)	E-value
Pm Elovl4	JX128699	515	NP_956266 (*Danio rerio*)	55	4e-49
Pm FAS	JX128703	803	ADM88556 (*Litopenaeus vannamei*)	95	3e-149
Pm COX	JX128701	1,337	ADB65786 (*Gammarus* sp.)	65	1e-178
Pm CYP4	JX128700	846	ADD63783 (*Litopenaeus vannamei*)	90	1e-180
Pm Nvd	JX128704	741	XP_001656693 (*Aedes aegyptii*)	62	1e-88
Pm RXR	JX128702	977	BAF75376 (*Marsupenaeus japonicus*)	97	5e-101

In stage IV females sampled at the beginning of the experiment (initial), domesticated females presented lower lipid content in the hepatopancreas when compared with wild animals (17.8±5.3 vs 24.4±10.9 mg g-1; P=0.174), while lipid content in the ovary was similar for both groups (13.6±1.3 vs 14.2±4.9 mg g-1). The relative expression of genes involved in a range of metabolic pathways in initial mature females in the hepatopancreas and the ovary is represented in figures 1 and 2, respectively. In the hepatopancreas, the expression of three genes was significantly overexpressed in domesticated animals: *Pm Elovl4* belonging to the fatty acid biosynthesis pathway, *Pm COX* belonging to the eicosanoid biosynthesis pathway and *Pm SUMO*, a gene that encodes peptides that become conjugated to target proteins via a cascade of enzymatic reactions involved in organelle biogenesis. In the ovary, no genes showed significantly different expression levels between wild and domesticated females (Fig. 2).

Fig. 1 - Rotllant et al.

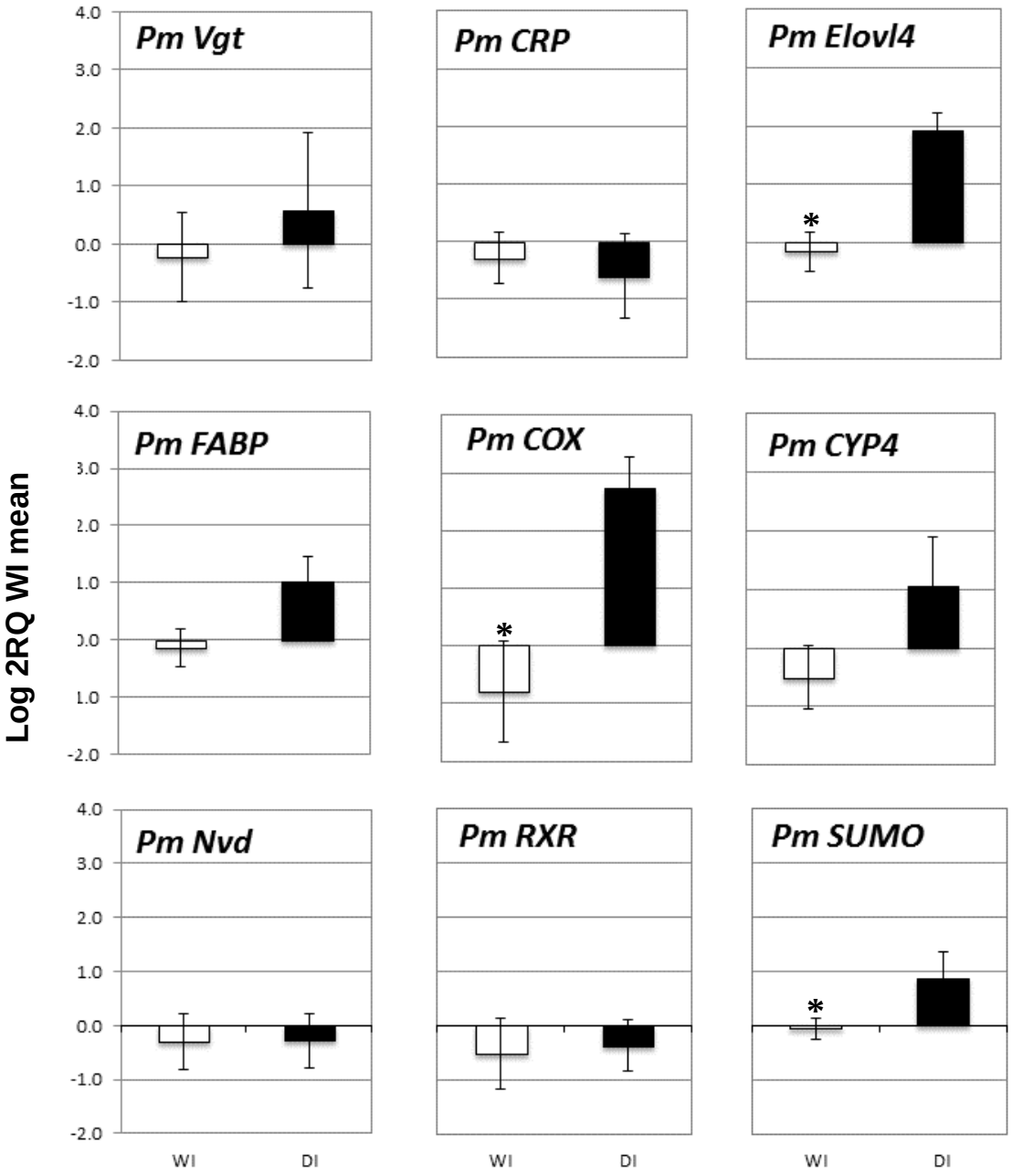

Figure 1. Comparative gene expression from mature *Penaeus monodon* females (stage IV) in wild (WI) and domesticated (DI) specimens in the hepatopancreas. * Indicate significant differences (P< 0.005). *Pm COX*= Cyclooxygenase; *Pm CRP*= Cortical Rod Protein; *Pm CYP4*= Cytochrome P450 enzyme 4; *Pm Elovl4*= Fatty acid elongase 4; *Pm FABP*= Fatty Acid Biding Protein; *Pm Nvd*= Neverdeland; *Pm RXR*= Retinoid X Receptor; *Pm SUMO*= Small ubiquitin like modifier; *Pm Vgt*= Vitellogenin.

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Fig. 2– Rotllant et al.

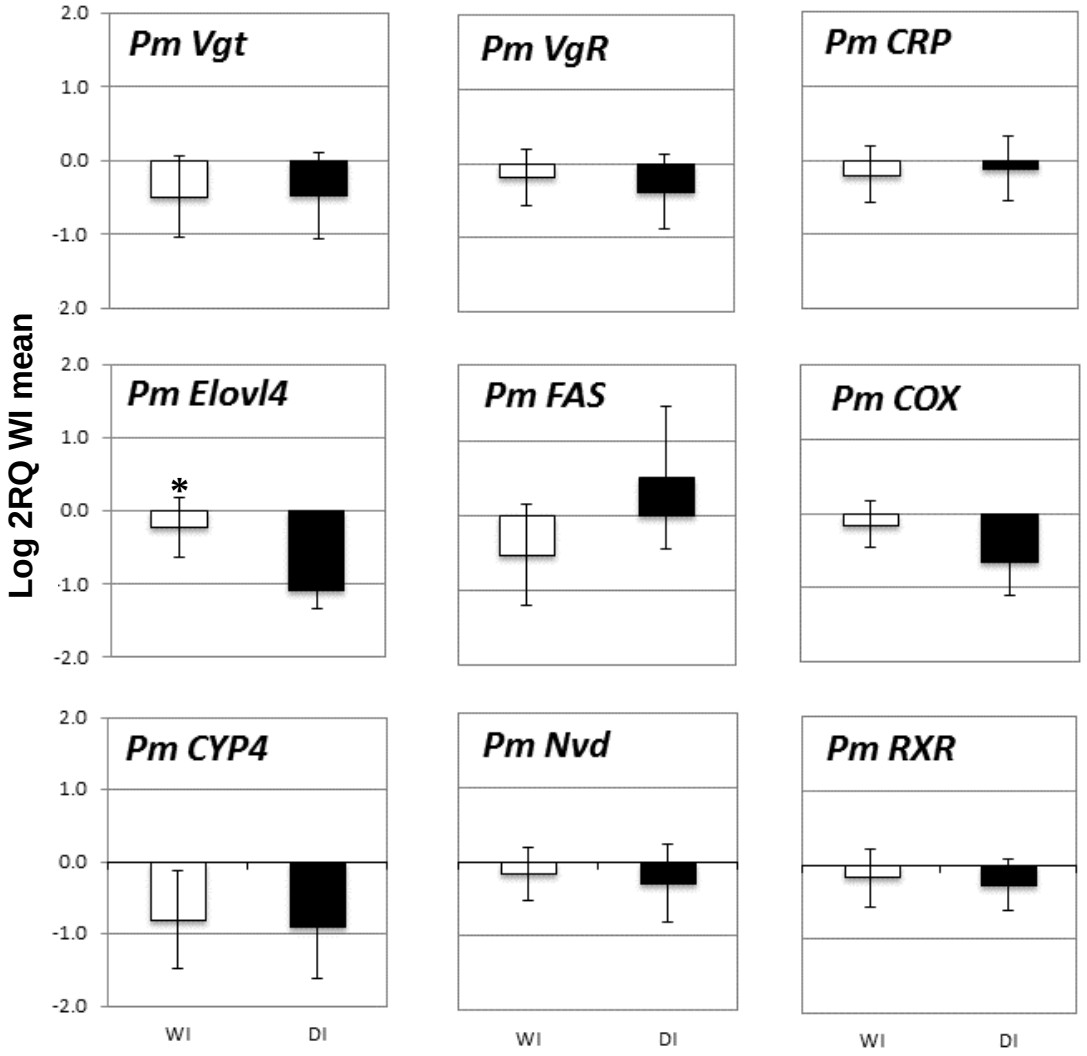

Figure 2. Comparative gene expression from mature *Penaeus monodon* females (stage IV) in wild (WI) and domesticated (DI) specimens in the ovary. * Indicate significant differences (P< 0.005). *Pm COX*= Cyclooxygenase; *Pm CRP*= Cortical Rod Protein; *Pm CYP4*= Cytochrome P450 enzyme 4; *Pm Elovl4*= Fatty acid elongase 4; *Pm FAS*= Fatty Acid Synthase; *Pm Nvd*= Neverdeland; *Pm RXR*= Retinoid X Receptor; *Pm Vgt*= Vitellogenin; *Pm VgR*= Vitellogenin receptor.

In the hepatopancreas of newly spawned females (stage 0), no differences in gene expression were observed between wild and domesticated animals. Similarly, no differences were observed between good and poor spawners. There was a significant correlation between hepatosomatic index and the expression of *Pm FABP* (P=0.03), that is involved in fatty acid biosynthesis (Table 5). Genes that encode for enzymes involved in cholesterol biosynthesis, *Pm CYP4* (P=0.05), also showed a significant correlation

with total lipid content (Table 5). No significant correlation was found between hepatopancreas gene expression and the reproductive performance parameters. Nevertheless, the highest levels of correlation were found between *Pm CRP* (0.176) and *Pm CYP4* (0.117) expression and relative fecundity, and between *Pm RXR* (0.208) and *Pm Nvd* (0.181) expression and hatching rate (Table 5).

Table 5. Pearson's correlation coefficient between the different parameters analyzed in this study (hepatopancreas biomass expressed as hepatosomatic index – HIS, total lipid content – TL and reproductive performance expressed as relative fecundity – RF and hatching rate – HR) and gene expression in the hepatopancreas from spawned *Penaeus monodon* females (stage 0).

Genes	Gene Abbreviation	HSI	TL	RF	HR
Ovarian Development					
Vitellogenin	*Pm Vtg*	0.006	0.001	0.009	0.000
Cortical Rod Protein	*Pm CRP*	0.067	0.068	0.176	0.003
Lipid Metabolism					
Fatty acid elongase 4	*Pm Elovl4*	0.262	0.012	0.083	0.019
Fatty Acid Biding Protein	*Pm FABP*	0.532 (P=0.03)	0.307	0.010	0.011
Steroid Hormone Biosynthesis					
Cyclooxygenase	*Pm COX*	0.004	0.022	0.023	0.117
Cytochrome P450 enzyme 4	*Pm CYP4*	0.418 (P=0.11)	0.499 (P=0.05)	0.117	0.143
Neverland	*Pm Nvd*	0.216	0.419 (P=0.11)	0.029	0.181
Transcriptional Regulation					
Retinoid X Receptor	*Pm RXR*	0.128	0.229	0.006	0.208
Small ubiquitin like modifier	*Pm SUMO*	0.164	0.129	0.028	0.006

Probability has been indicated when P<0.1

4. Discussion

Six new genes were identified in *P. monodon* across a range of biosynthetic pathways such as lipid metabolism, steroid hormone biosynthesis and transcriptional regulation. One of these is the Neverland (*Pm Nvd*) gene, which is an evolutionally conserved Rieske-domain protein essential for ecdysone synthesis and insect growth while involved in cholesterol trafficking (Yoshiyama et al., 2006).

In previous studies it has been proved that wild broodstock presented better reproductive performance than domesticated animals (Arnold et al., 2013, Coman et al., 2013, Marsden et al., 2013). Broodstock accumulate lipids in the ovary prior to spawning concomitant with the decrease in the total or specific lipids in the hepatopancreas during maturation (Wouters et al., 2001). In this study, genes related to fatty acid and eicosanoid synthesis pathways were differentially expressed between wild and domesticated mature females, while the expression of genes related to steroid metabolism were the closer correlated with reproductive performance. We also observed increased expression of *Pm Elovl4*, *Pm COX* and *Pm SUMO* in the hepatopancreas of domesticated animals (Fig 1), genes involved in fatty acid metabolism and transcriptional regulation. In addition, we observed a decrease in the expression of *Pm Elovl4* in the ovary of domesticated animals prior to spawning (Fig 2). This suggests that domesticated animals may be attempting to increase their lipid metabolism compared with wild females in order to produce long chain fatty acids and in particular arachidonic acid (ARA), which are known to be required for ovaries to reach full maturity (Mourente and Odriozola, 1990, Mourente, 1996, Hoa et al., 2009). This process may be impaired in the ovary, through lower *Pm Elovl4* expression or lower abundance of metabolic pathway intermediates. In wild females, it has been previously observed that the amounts of prostaglandin F synthase gene transcripts and prostaglandin F2α, related to ARA abundance, decreased as the ovaries matured (Wimuttisuk et al., 2013). It could be expected that in domesticated females these genes will be also overexpressed, but have not yet been investigated. The expression of the genes related to ovarian development was not different between wild and domesticated females, suggesting that *Pm Vgt*, *Pm VgR* and *Pm CRP* were expressed, independently of the broodstock condition, to be able to reach maturation.

In females that had spawned, the expression of genes in the hepatopancreas related to fatty acid metabolism and sterol metabolism was correlated with HSI and lipid content. These results suggest that TL and HSI were linked with the expression of some genes involved in fatty acid biosynthesis and steroid hormone synthesis, and imply a link between TL content in the hepatopancreas and overall condition. However, no correlation was observed between TL levels, HSI or reproductive performance measures, potentially due to the small number of samples in this study. Despite the relationship not being significant, the expression of *Pm CYP4C* and *Pm FABP* showed a strong positive correlation with HSI, and the expression of *Pm CYP4* and *Pm Nvd* with TL (Table 5). These genes are known to play a critical role in cholesterol biosynthesis (cytochrome P450) and trafficking (Neverland) from their interaction long chain fatty acids (Miller, 2005). The significant relationship between *Pm CYP4* and TLs outlined above may translate to an overall improvement in HSI, as shown by the strong correlation between *Pm CYP4* and HSI. On the basis of these results, further investigation of these genes in assessing reproductive performance would be justified.

In relation to the reproductive performance, although our results were not significant, we observed that relative fecundity showed the highest correlation with the expression of *Pm CRP* and *Pm CYP4* and hatching rate and the expression of *Pm Nvd* and *Pm RXR* (Table 5). RXR is also related to ecdysteroid metabolism forming a dimer with the ecdysteroid receptor (EcR–RXR) and regulating the transcription of target genes through ecdysteroid responsive elements on the DNA (Brown et al., 2009), while *Pm CRP* encodes cortical rod proteins that form an acellular vitelline envelope at nearing the completion of oogenesis (Okumura et al., 2006).

Although our results are still preliminary, the present study shows that genes encoding several metabolic pathway regulators may be related to reproductive

performance in shrimps. We have shown that the expression of lipid metabolism genes as *Pm Elovl4* and *Pm COX* that belong to the fatty acid and ecosanoid biosynthesis pathways, respectively, might be good biomarkers for maturation. Similarly, *Pm CYP4*, *Pm Nvd* and *Pm RXR* that belong to the sterol biosynthesis might be good biomarkers for studying reproductive performance.

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