Transcriptomic Analysis of the Reproductive Effects on Immunity in the Clam Meretrix Petechialis During the Breeding Season

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Abstract

Background: Immune defense and reproduction are both physiologically demanding processes, therefore the trade-off is expected between them in breeding season. Although this balance strategy has been extensively studied in many species, it is still rarely noticed in mollusc. Moreover, summer mortality of marine bivalve often occurs during breeding season and reproduction is suspected to play a significant role for the mass death.

Results: To address if spawning affects immunity to cause clam death, we performed transcriptome and gene expression analyses in the clam *Meretrix petechialis* pre-/post-spawning. DEGs enrichment analysis revealed important immune signaling pathways and key genes changed after spawning. Further analysis showed females up-regulated genes involved in apoptosis, TLR signal pathway and heat shock, whereas males down-regulated complement-related genes after spawning. Additionally, both genders of clams up-regulated its immune response level to against *Vibrio* infection after spawning revealed by the changes of four immune-related DEGs.

Conclusions: The spawning indeed affected the clam immune resistance. The immune and defense related genes and pathways obtained here provide important insights into the molecular mechanisms of physiological acclimation and immune response under reproduction-influence and offers some clues to explain the possible reason for clam summer mortality.

Background

In natural condition, the resources acquired by an organism are usually not enough to satisfy its needs in growth, reproduction and survival, which will lead to trade-off of resource allocation [1]. It means that organisms flexibly invest their energy to different priorities [1-5]. One commonly observed trade-off occurs between reproduction and immune due to they are both physiologically demanding processes [6]. Recent works in this field can be divided into two broad categories: the immune impacting on reproduction and reproductive efforts affecting immunity, which also allows us to understand this trade-off by a comprehensive perspective. Luong and Polak [7] reported the selected lines of *Drosophila nigrospiracula* showed a decreased fecundity after increased their resistance against an ectoparasite mite. Similar effects were also founded in other species such as insect and fish [8, 9]. On the other hand, investigation on the reproductive cycle suggested that reproductive process can weak the immunocompetence for a seasonally reproducing fish [6]. Furthermore, the trade-off is also known to be affected by factors such as sex, body size, seasonal changes and parasite infection[10].

As we know, mass mortality of marine bivalve often occurs during the summer breeding season [11-13]. Spawning is considered to be a key cause of this result, because gametogenesis is suspected to be a period of intensive physiological change and most of the energy acquired is used for the production of gametes [14, 15]. Although the trade-off between reproduction and immune has been deeply studied in the invertebrate model organisms, *Drosophila* and *Caenorhabditis*, it remains understudied in mollusc.

Fortunately, mollusc shares the conserved immune components and pathways with the above invertebrate model organisms, such as the immune effectors and immune signal transduction cascades, etc [16]. They all display a highly effective immune response that consists of interconnected cellular and humoral components, although lacking an adaptive immune response comparable to vertebrates[17-19].

To date, the association of summer mortality and immunity in mollusc has been extensively studied. For example, a review in oyster *Crassostrea gigas* summarized the omics approaches to investigate host–pathogen interactions in mass mortality outbreaks [20]. Besides, pathogene caused mass mortality were widely reported in scallop *Chlamys farreri* [21], clam *Chamelea gallina* [22] and abalone *Haliotis gigante* [23]. In our laboratory, we have investigated the immune response in the clam *Meretrix petechialis* to pathogenic bacteria associated with clam mass mortality in recent years [24, 25]. However, the relationship study between reproduction and immunity from a global perspective remains rare in mollusc because the weak research background and the lack of feasible method. RNA-seq as an efficient and high-throughput technique is commonly used to investigate the transcription of genes including immunity, reproduction and growth, etc. [26-28], thus, it provides a possible way for the correlation research of reproduction and immunity [29].

In the present study, we selected the clam *M. petechialis,* an important commercial mollusc cultured in China, to investigate the changes of immune characteristics affected by production in breeding season by RNA-seq. Moreover, we further examined the reproductive effects on the clam immune response against pathogen invasion. These results will help us to understand the trade-off of reproduction and immune in breeding season, and provide insights into the causes of clam summer mortality.

Methods

Experimental animals

Matured clams were collected from one cultured population in Wenzhou, China. The clams were acclimated in a 2000 L tank with aerated seawater and fed with *Isochrysis galbana* for five days at 28 °C. Then we took the pre-spawning individuals, identified the males and females by dissecting the gonads and grouped “pre_M” and “pre_F” for the male and female respectively. Parental clams were induced spawning by running seawater while post-spawning individuals were classified as “post_M” and “post_F” respectively, according to their germ cell morphology. Then the 9 clams from each group were dissected, and 3 hepatopancreas were mixed as one replicate and total of 3 replicates for RNA-Seq.

RNA-Seq and data analysis

For RNA-Seq, the hepatopancreas were sent to BGI (Shenzhen, China). After total RNA isolation, cDNA libraries preparation, RNA-seq was performed by BGISEQ-500 platform. Raw data (raw reads) were filtered by SOAPnuke, removing reads containing adapters, more than 5% unknown nucleotides and low-quality reads (more than 50% bases with Q-value ≤ 15) [30]. Then the assembled de novo transcriptome [24] was

used as the reference database and the clean reads were aligned with the reference database using bowtie2 (v2.2.5) [31]. The expression level of genes and transcripts was calculated using RSEM [32]. For gene annotation, assembled unigenes were aligned with the TFs (AnimalTFDB), Nr (NCBI non-redundant protein sequences), GO (Gene Ontology) and KEGG (Kyoto Encyclopedia of Genes and Genomes) database.

Differentially expressed genes (DEGs) analysis

Differential expression analysis between two groups, including pre_F vs. post_F, pre_M vs. post_M, pre_F vs. pre_M and post_F vs. post_M was performed by DESeq R package software (1.10.1). Adjusted P value (Q-values) of 0.01 and log2 (fold_change) of 2 were set as the threshold for significantly differential expression. DEGs were classified to signaling pathways by KEGG pathway analysis, while GO enrichment analysis was used to describe DEGs functions by mapping to functional categories. Based on the GO and KEGG annotation results and the official classification, the functions of DEGs were performed by the phyper R package, and enrichment was considered for FDR corrected p-values (Qvalue) less than 0.05.

Vibrio challenge and sample collection

The *Vibrio* challenge experiment was conducted with immersion method as described in Yue et al. [33] and *Vibrio parahaemolyticus* strain (MM21) isolated from *M. petechialis* was used in the challenge. Clams were divided into four groups as the groups in RNA-seq (pre_F, post_F, pre_M and post_M) and immersed in seawater with 1×10^7 CFU ml^{-1} *V. parahaemolyticus*. The seawater through sand filter and *V. parahaemolyticus* is renewed every day. The hepatopancreas tissues from 5 clams in each group were sampled at 0, 12, 24 hours post-immersed and then frozen in liquid nitrogen for RNA extraction.

Quantitative real-time PCR analysis

In order to verify the expression pattern obtained by RNA-seq, ten immune-related DEGs were randomly selected and detected by qPCR. The template used in the qPCR was the cDNA applied to RNA-seq. Primer premier 5 was used to design the primer pairs to amplify the selected genes. The sequences of primer pairs were listed in Additional file 1 Table S1. The mean value of β-actin and EF1α were employed as the internal references to normalize the relative expression levels among samples [34]. Bio Rad CFX 96 Real time PCR system of SYBR premix (TaKaRa, Japan) was used in qPCR and the PCR process were 95 °C for 30 s and 40 cycles of 95 °C for 5 s, 60 °C for 30 s, followed by the melting curve determination. Similarly, the expression patterns of four immune related DEGs were examined by qRT-PCR in the clams under *Vibrio* immersion challenge. The relative gene expression was analyzed using the $2^{-\triangle\triangle CT}$ method [35]. All data were expressed as the mean ± standard deviation and applied to F text. Through analysis by t-test using software SPSS version 22.0, the difference was considered statistically significant when $P< 0.05$.

Western blot analysis

The protein expression of MpDOUX and MpMITF were detected using Western blots. Protein Analysis Kit (BestBio, China) was used to extract the total protein from the hepatopancreases of clams. The polyclonal antibody used in this study included DOUX polyclonal antibody (catalog no. A8583, ABclonal, USA) and MITF polyclonal antibody (catalog no. A1255, ABclonal, USA). The detailed description of the western blot method was referred to Yue et al. [36]. In detail, the samples at each time point (12 h, 24 h) after the *Vibrio* challenge were mixed by protein from 3 individuals. 12% SDS-PAGE was used to separate each equal amounts of protein and then protein was transferred from gel onto a PVDF membrane. After blocked with 5% non-fat milk for 2 h, this membrane was incubated with the primary rabbit anti-DOUX or anti-MITF antibody or rabbit anti-β-actin antibody (ABclonal, USA) at 4℃ overnight. Then the membrane was washed 3 times for 10 min with TBST and incubated with the secondary goat anti-rabbit IgG-HRP antibody (ZSGB-BIO, China) for 1 h. Finally, after washing 3 more times, the protein on membrane was a visualization using Sparkjade ECL super (Cat # ED0015-B, Sparkjade).

Results

Sequencing assembly and annotation

A total of 263.21M raw reads for reference database was generated based on BGISEQ-500 sequencer and 262.05M clean reads were remained after filtering out the low quality reads. For the clean reads, more than 97.89% of the bases had a phred value > 20, and more than 89.35% of them had a phred value > 30. An overview of the assembly results was provided in Additional file 2 Table S2. The raw data were uploaded to the NCBI sequence read archive database with the accession number PRJNA632458.

In total, 48,339 unigenes were generated and annotated by searching the sequences against the NR, KEGG, GO and TFs databases, which produced 17,737 (36.69%), 13,336 (27.59%), 6,128 (12.68%) and 1,756 (3.63%) hits respectively (Table 1). There were only 1,752 (3.62%) were annotated in all of the databases, and 17,847 (36.92%) were annotated in at least one database. Among these, GO classification analysis showed that 48,339 unigenes were categorized into 21 biological processes, 16 cellular components and 9 molecular functions. The KEGG classification analysis showed that total unigenes were assigned to 6 KEGG categories in the top level: 12 metabolisms, 11 human diseases, 10 organismal systems, 4 cellular processes, 4 genetic information processing and 3 environmental information processing.

DEGs analysis and immune related pathway identification

DEGs analysis among the four groups Pre_F, Post_F, Pre_M and Post_M was applied to identify gene changes pre/post-spawning. All DEGs with the absolute value of fold change > 2, adjust P-value < 0.01 are showed in Fig.1. A total of 1,805 DEGs were detected between the Pre_F vs. Post_F, of which 722 and 715 unigenes were up-regulated and down-regulated respectively. For the Pre_M vs. Post_M comparison,

2,488 DEGs were detected, of which 772 and 1716 unigenes were up-regulated and down-regulated respectively. As shown in Fig. 1, the number of up-regulated genes were basically equal between male groups (Pre_M vs. Post_M) and female groups (Pre_F vs. Post_F), but the number of down-regulated genes in male was more than double in female after spawning.

To further explore the function of the identified DEGs, GO classification analyses were conducted and 7 categories attracted our attention, which were cellular process, metabolic process, biological regulation, cell, organelle, binding and catalytic activity. In these categories, while the number of DEGs in male was at least 40% more than that of DEGs in female, the proportion of down-regulated genes in male was at least 50% more than that of down-regulated genes in female. These categories were marked in Figure 2, and a list of DEGs numbers was provided in Table 2. Additionally, in the majority of KEGG classification categories, the number of DEGs in both genders were approximately in the same, except “human diseases” that the number of DEGs in male was 80%（256/141）more than that of DEGs in female, and the proportion of down-regulated genes in male was 171% (192/39) more than that of down-regulated genes in female. The function of the more down-regulated DEGs in male is to participate in metabolism, catalytic activity, cell function and disease, which implied male reduced more these competences after spawning.

GO term and KEGG pathway enrichment analyses were performed to explore the DEGs related to immune regulation. We screened differential genes on GO term by keywords “immune” in Pre_F vs. Post_F and Pre_M vs. Post_M comparisons, and enriched 11 and 4 differential genes, respectively. Here, both quantity of DEGs and the type of GO enrichment were more in females than in males (Additional file 3, Fig.S1). To better understand the immune-related pathways changes pre/post-spawning, we evaluated significant enriched pathways about “Immune system” of secondary level of KEGG pathway enrichment, and enriched 19 and 20 differential genes in female and male respectively (Fig. 3). Interestingly, common pathways with high value enriched in different genders included IL-17 signaling pathway, NOD-like receptor signaling pathway, TLR signal pathway, RIG-I-like receptor signaling pathway, which played an important role in innate immunity. The results here implied that immune-related cytokines and inflammation were indeed affected by spawning.

Differences expression of immune-related genes between reproductive states

Based on the DEGs enriched by KEGG pathway in the Section 3.2 (19 and 20 genes in female and male respectively), 22 genes were further screened by Nr annotation. Based on their function in pathway, we classified them into seven categories: complement and coagulation cascades, apoptosis, TLR signal pathway, NFκB signal pathway, leukocyte transendothelial migration, MAPK signal pathway and others (Fig. 4). The expressions of genes in complement and coagulation cascades are almost 2/3 significantly down-regulated after spawning in males, whereas the analysis showed the expressions of genes in apoptosis, TLR signal pathway, NFκB signal pathway and HSP family are significantly up-regulated after

spawning in females. It means that male decreases immune-competent of complement, while female improves immune function in apoptosis, antimicrobial peptides and heat shock.

To validate the reliability of DEGs identified by RNA-Seq, 10 out of these 22 genes were selected for further relative expression quantified by qRT-PCR. These genes belong to NFκB signal pathway (*IKK*, *DTHD1*), TLR signal pathway (*TLR4*), apoptosis (*BIRC7*, *IAP* and *CREM*), MAPK signal pathway (*ARAF-like*, *MAPKK3*), complement and coagulation cascades (*CTRP2*) and others immune-related genes (*PSME3*). The expressions of these genes pre/post-spawning showed in Fig.5. Compared their expression in RNA-seq (Fig. 4), 8/10 genes had a common expression pattern, only *BIRC7* and *IAP* are not consistent in male.

Gene expression changes pre/post-spawning under *Vibrio* challenge

The transcriptome analysis revealed that immune-related genes changes pre/post-spawning in clam *M. petechialis* in natural condition. Here, we want to know how these genes expression pre/post-pawning under the pathogen challenge. Four immune-related DEGs (*ARAF-like*, *CREM*, *TLR4* and *TBK1*) were selected for further examination. As shown in Fig. 6, without *Vibrio* challenge (0h), the expression patterns of these DEGs were consistent with the changes revealed by RNA-seq under natural condition (Fig. 4), that was, after spawning their expressions were significantly increased in females but there was no significant difference in males. Under *Vibrio* challenge, the four genes also showed up-regulated expression in females at 12h or 24h; in male, the non-differentially expressed genes showed a significant up-regulated expression after spawning at 24h. These results indicated that the response of these immune-related genes became more sensitive and their expression changes more drastically after spawning under the pathogen challenge. It means pathogen reinforced the expression differences of immune-related genes between pre-spawning groups and post-spawning groups.

Assessment of immune status of clam under *Vibrio* challenge

To further verify the effects of spawning, we chose two previously reported genes, *DOUX* and *MITF* both involving the immune defense of *M. petechialis* [24, 37], to detect the immune changes of the clams pre/post- spawning. As shown in Fig. 7, under bacterial immersion challenge, the mRNA expression of *MpDOUX* and *MpMITF* were significantly up-regulated after spawning in both genders. We also tested the protein changes of MpDOUX and MpMITF in female clams by western blot because we perceived that many immune genes had more significant differences after spawning in female based on transcriptome data (Fig. 4). As shown on Fig. 8 and Additional file 4, the protein expressions increased at 24 h for MpDOUX and at 12 h for MpMITF in the post-spawning individuals under *Vibrio* challenge. The up-regulation at the transcription and protein levels of these maker genes further confirmed our conclusion that pathogen reinforced the expression differences of immune-related genes between pre-spawning and post-spawning groups.

Discussion

The existence of temporal changes in immunocompetence associated with reproduction is widely accepted [38]. The purpose of present study was to examine the effect of spawning on molluscan immunity. Through transcriptome analysis, we compared and analyzed the globle changes of genes in clams pre/post-spawning. According to the annotation and enrichment analysis, we obtained immune-related pathways and key genes and they showed significant changes after spawning. Further *Vibrio* challenge experiment clarified that immune-related genes expression changed after spawning during pathogen invasion.

Previous studies demonstrated the trade-off between reproduction and immunity can influenced by sex [39, 40], and here the transcriptome analysis showed gene expression differences between male and female in the clam *M. petechialis*. In detail, the number of down-regulated genes in male is more than double in female after spawning. Through GO and KEGG enrichment, we found these decreased genes were distributed in metabolism, catalytic activity, disease and so on. The effect of reproduction on metabolism caused researchers attention very early, Williams [5] reported high production efficiency resulted in some undesirable effects that mostly related to metabolic imbalance. In our results, the decrease in metabolic capacity occurred primarily in males, possibly due to the differences in hormone levels between males and females, as Zera and Zhao [41] suggested that hormonal tight controlled of metabolism.

Immunological performance may be subject to rapid temporal changes due to possible resource re-allocation between the immune system and reproduction, or through immunomodulation by reproductive hormones [42]. In our study, major immune-related pathways and genes were explored by enrichment analysis. The pathways changed pre/post-spawning in both genders were IL-17 signaling pathway and three classic PRRs-related pathways (NOD-like receptor, TLR and RIG-I-like receptor pathways), which involved in cytokine secretion, inflammation and apoptosis [43-46]. By further analysis of up/down-regulated genes we found the up-regulated DEGs in apoptosis, TLR signal pathway and heat shock protein after spawning only occurred in females. However, DEGs in complement and coagulation cascades were down-regulated after spawning in males. Similarly, a positive relationship between antimicrobial capacity and reproductive investment in female was found in insect during breeding season [10], moreover, the latest research in sea cucumber revealed that the complement activation were weakened after spawning [47]. All these results suggested that females improve their immunity (like apoptosis, antimicrobial peptides and heat shock), whereas males weaken part of their immunity (such as the complement system) after spawning. One explanation in vertebrates for the down-regulation of male immune defense is that testosterone weakens immunity [48], but it was unclear what mechanisms in invertebrate.

Mollusc usually reproduce in summer and they are threatened by more enriched pathogens in the water simultaneously, resulting in mass mortality in breeding season. Therefore, we further focused on the response of immune pathways and genes to pathogen stress before and after spawning. In this study,

Vibrio was selected as pathogen to challenge this clam, and four immune DEGs from different pathways were used to test their expression differences between pre/post-spawning groups under *Vibrio* challenge. Compared to pre-spawning individuals, the four genes were all up-regulated expression in the post-spawning clams under *Vibrio* challenge. This result indicated pathogen could reinforce the differences between different reproductive state. The expression changes of these genes that involved in apoptosis**,** TLR signaling pathway and MAPK signaling pathway [49-51] can reflect the immune status, however, in this clam these genes have not been well characterized before, and we need more marker genes to verify our finding in *M. petechialis*. In our previous works, several immune genes have been identified from *M. petechialis* and their expression could indicate the immune response of this clam to *Vibrio* infection [52, 53]. Among them, microphthalmia-associated transcription factor (MITF) is a basic helix-loop-helix-leucine zipper protein that plays a key role in cell proliferation and immune defense, and Dual oxidase (DUOX) is a main ROS source and plays role in host resistance against infection by diverse pathogens [37, 54]. In present study, we used MpMITF and MpDOUX as makers to evaluate the immune response changes of this clam. Their up-regulation expression at the transcription and protein levels after spawning under the *Vibrio* challenge further verified that spawning can strengthen immune response to pathogen invasion.

Although part of our results differ from the theory of trade-off, they coincide with the findings in fish that the changes between pre-spawning and post-spawning showed different trends under differential measure parameters of the immune system [38]. These results further support the idea that the trade-off could be affected by factors such as sex, individual condition and parasite infection [10]. Anyhow, we investigated the trade-off between reproduction and immune in natural condition and pathogenic stress environment, which can help us to understand the interaction between these two complex physiological processes. The combination of the current results and previous research may give us some clues to explain the possible reason for clam summer mortality. As an important finding of this study, the down-regulate genes related to the complement system in males will reduce their ability to withstand adversity stress. For females, although immune function are enhanced after spawning, the large investment of immunization and reproduction consume huge energy and direct somatic damage by the immune response can also be responsible for the impact on survival [55].

Conclusions

We compared the overall genes differences in *M.petechialis* before and after spawning using transcriptomes analysis and found male decreased more genes were distributed in metabolism, catalytic activity, disease and so on. In natural condition, the females up-regulate immune function related to apoptosis, antimicrobial peptides and heat shock, whereas males weaken the majority of complement-related genes after spawning. Under the pathogen challenge, immune genes were up-regulated in both genders after spawning and pathogen could reinforce the differences between two reproductive-states. Our study provides a new insight into the understanding reproductive effects on immunity in *M. petechialis* and offers some clues to explain the possible reason for clam summer mortality.

Abbreviations

BIRC7: baculoviral IAP repeat-containing protein 7; IAP: inhibitor of apoptosis isoform X1; IKK: inhibitor of nuclear factor-kappaB; CREM: cAMP-responsive element modulator isoform X1; DTHD1: death domain-containing protein 1-like; TLR4: toll-like receptor 4; MAPKK3: dual specificity mitogen-activated protein kinase kinase 3 isoform X1; ARAF-like: serine/threonine-protein kinase A-Raf-like isoform X2; CTRP2: complement C1q tumor necrosis factor-related protein 2 isoform X2; PSME3: proteasome activator complex subunit 3; CDC42: cell division control protein 42 homolog; RTase: RNA-directed DNA polymerase from transposon BS; RhoJ: rho-related GTP-binding protein RhoJ; BIRC2_3: baculoviral IAP repeat-containing protein 2/3; SCC4: short-chain collagen C4-like; Muc2: mucin-2; A2ML1: alpha-2-macroglobulin-like isoform X1; FGA: fibrinogen alpha chain; F11: coagulation factor XI; HSP70: heat shock protein 70; HSP20: small heat shock protein 24.1; TBK1: TANK-binding kinase 1; MITF: microphthalmia-associated transcription factor; DUOX: Dual oxidase.

Declarations

Ethics approval and consent to participate

This study does not involve endangered invertebrates. According to the national regulation (Fisheries Law of the People's Republic of China), no permission is required to collect the animals and no formal ethics approval is required for this study.

Consent for publication

Not applicable.

Availability of data and material

The datasets analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

BL designed the project, interpreted the data and revised the manuscript. DW performed the experiment and contributed to data interpretation rafted the manuscript. All authors read and approved the final manuscript.

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Tables

Table 1. The percentage of annotated genes in different database.

Databases	Number of Unigenes	Percentage (%)
Nr	17,737	36.69
KEGG	13,336	27.59
GO	6,128	12.68
TFs	1,756	3.63
Annotated in all Databases	1,752	3.62
Annotated in at least one Databases	17,847	36.92
Total Unigenes	48,339	100

Table 2. The number of DGEs in gender difference GO classification

Category	Female	Male
Cellular process	49(30.61%)	96(86.46%)
metabolic process	33(42.42%)	49(79.59%)
biological regulation	25(16%)	36(77.78%)
cell	37(32.43%)	88(81.82%)
organelle	21(23.81%)	65(81.54%)
binding	82(21.95%)	151(79.47%)
catalytic activity	62(32.26%)	105(86.67%)

Note: Number in “()” means the ratio of down/ total DGEs in the significant different categories.

Figures

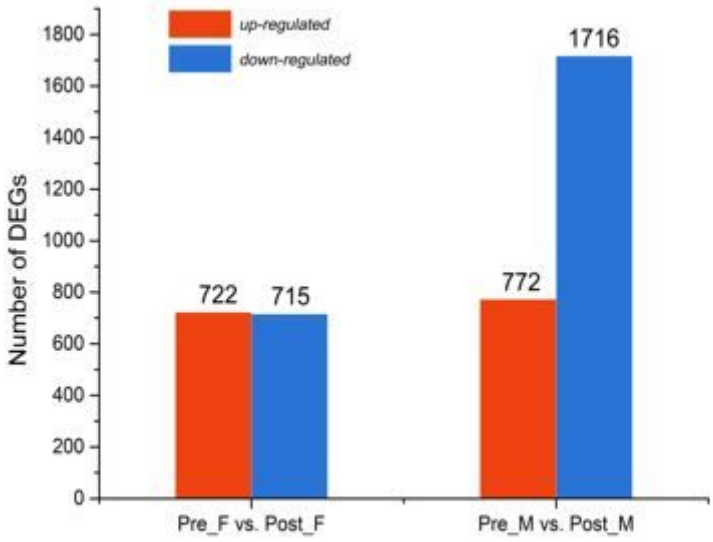

Figure 1

The numbers of DEGs in Pre_F vs. Post_F and Pre_M vs. Post_M in the clam M. petechialis

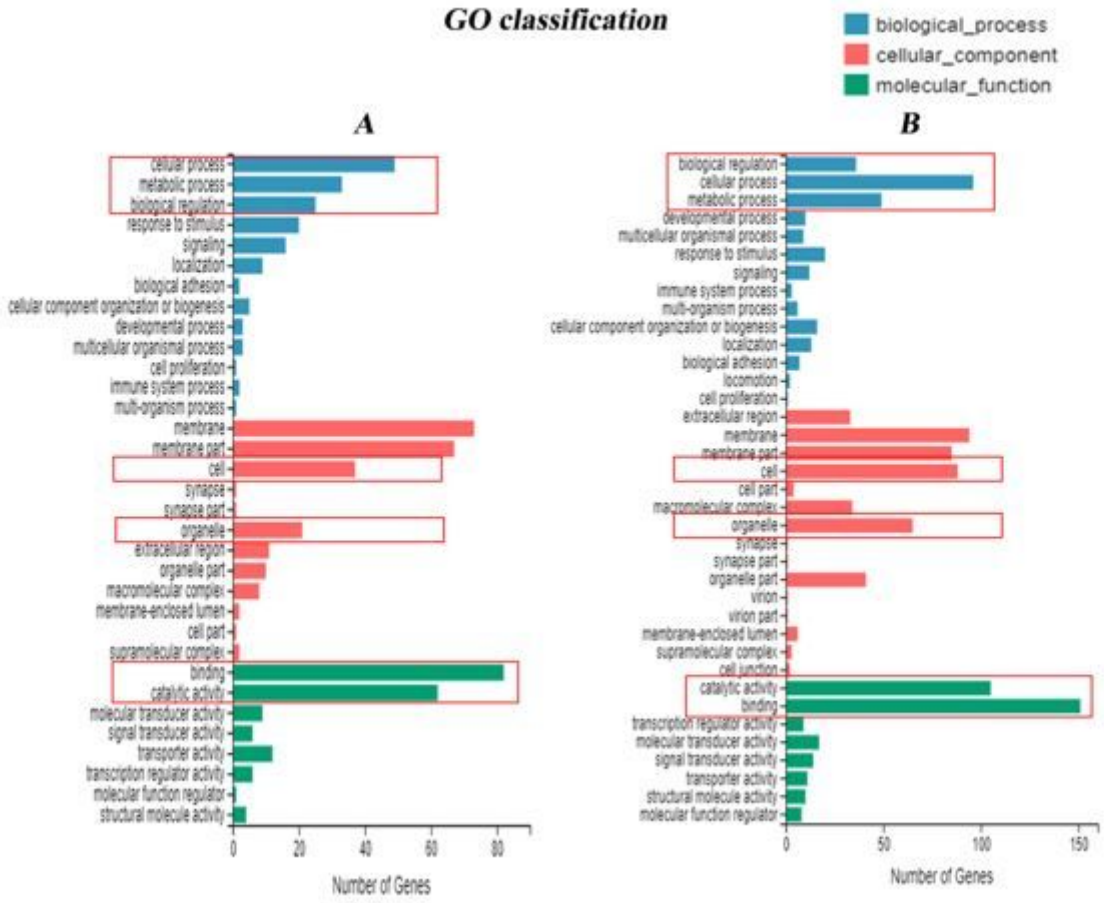

Figure 2

The GO classification analyses of DEGs in Pre_F vs. Post_F (A) and Pre_M vs. Post_M (B) in M. petechialis

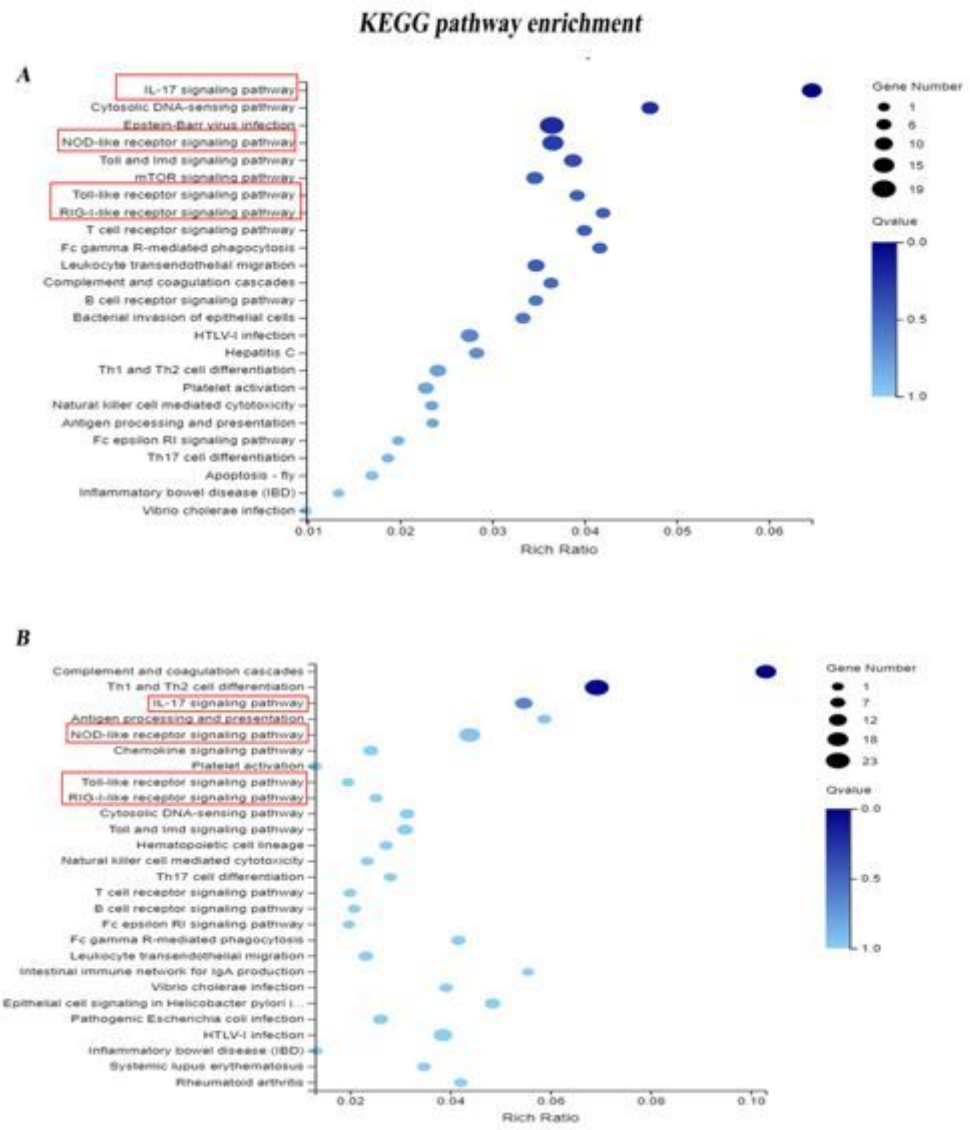

Figure 3

The KEGG pathway enrichment analyses of DEGs in Pre_F vs. Post_F (A) and Pre_M vs. Post_M (B) in M. petechialis

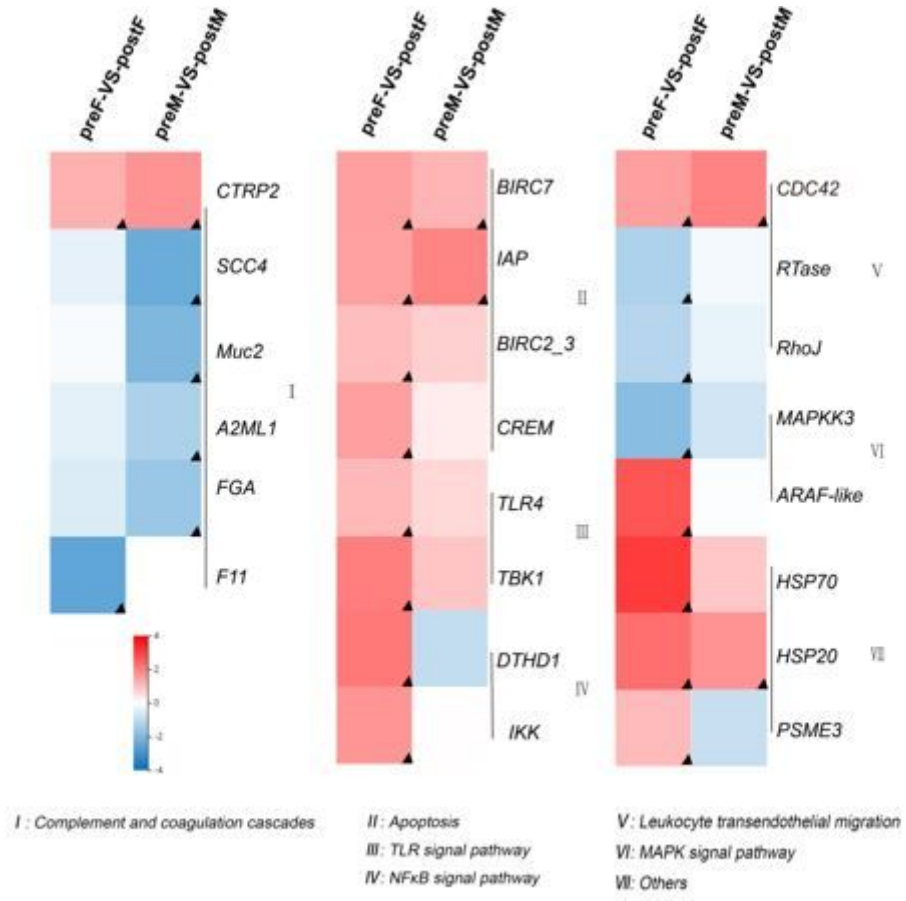

Figure 4

DEGs associated with immune function in the hepatopancreas of M. petechialis. The color of each cell represents log2fold change in the genes expression level of each comparison. Red is up-regulated and blue is down-regulated after spawning compared to pre-spawning, but only genes with a |log2FoldChange| > 2 and a p-value threshold after FDR correction of 0.05 were considered significantly differentially-expressed genes marked by ▲.

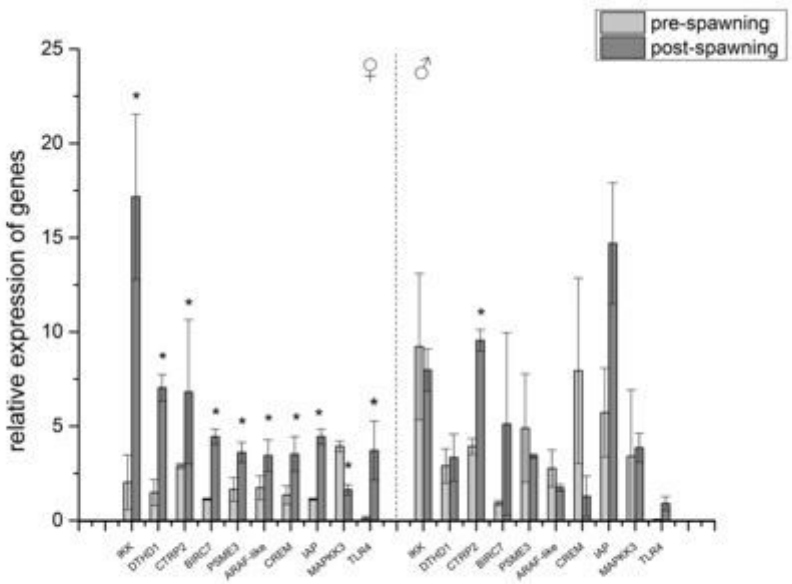

Figure 5

Relative mRNA expression of DEGs in the hepatopancreases of M. petechialis pre/post-spawning by qRT-PCR. Error bars represent the SD. The asterisk (*) represents significant differences in pre-/post- spawning ($P < 0.05$).

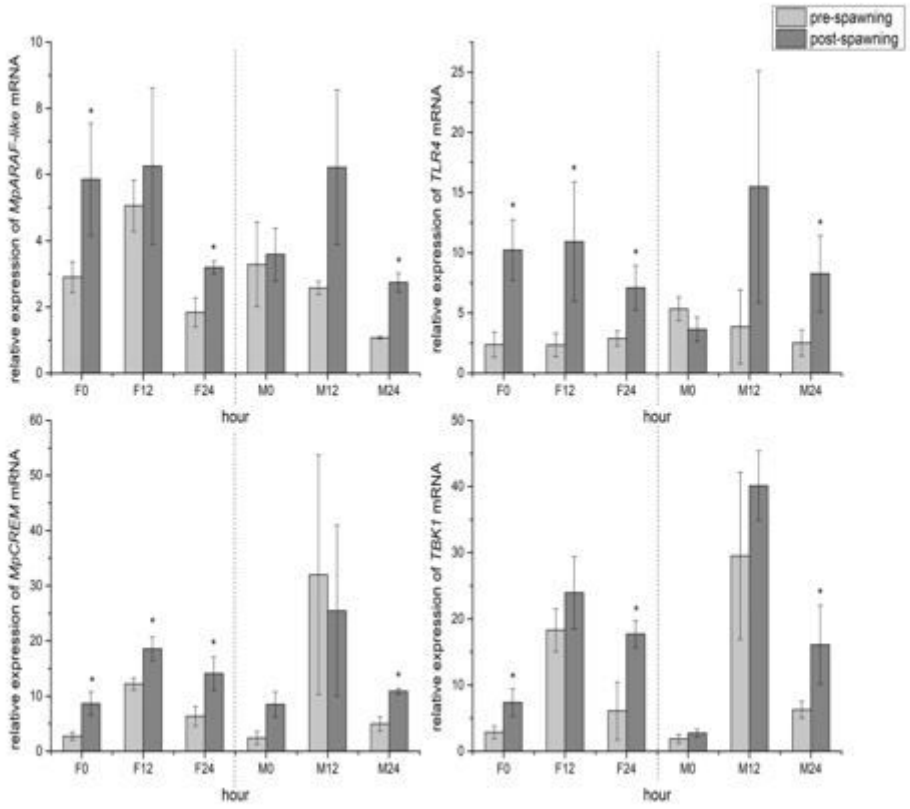

Figure 6

Relative mRNA expression of DEGs in the hepatopancreases of M. petechialis at 0–24 hour after immersion in V. parahaemolyticus by qRT-PCR. Error bars represent the SD. The asterisk (*) in post-spawning clam represents significant differences found when compared to pre-spawning one at each time point (P < 0.05). “F” and “M” means “female” and “male” respectinely.

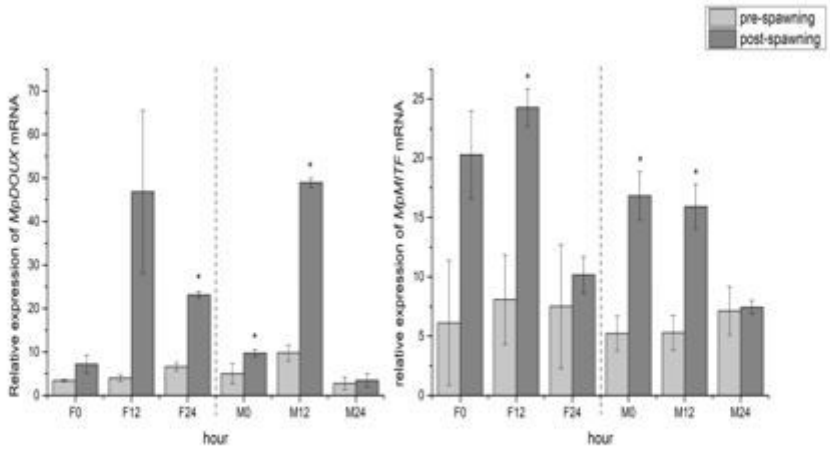

Figure 7

The relative expression of MpDOUX and MpMITF in the hepatopancreases of M. petechialis at 0–24 hour after immersion in V. parahaemolyticus by qRT-PCR. Error bars represent the SD. The asterisk (*) represents significant differences between pre-spawning and post-spawning at each time point ($P < 0.05$). “F” and “M” means “female” and “male” respectinely.

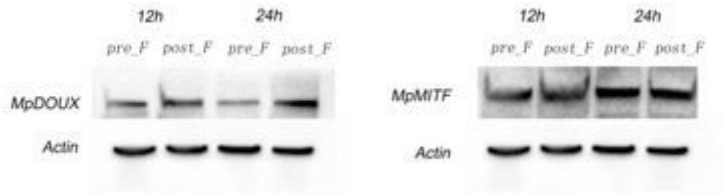

Figure 8

MpDOUX and MpMITF protein expression in the hepatopancreases of M. petechialis at 12 h and 24 h during Vibrio challenge by western blot. β-actin was used to confirm that equal amounts of protein were run on gel. The original, unprocessed gel images of this figure are available in additional file 4.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Additionalfile1TableS1.docx