

Synergistic effects of feeding and eyestalk ablation on ovarian maturation of the black tiger shrimp



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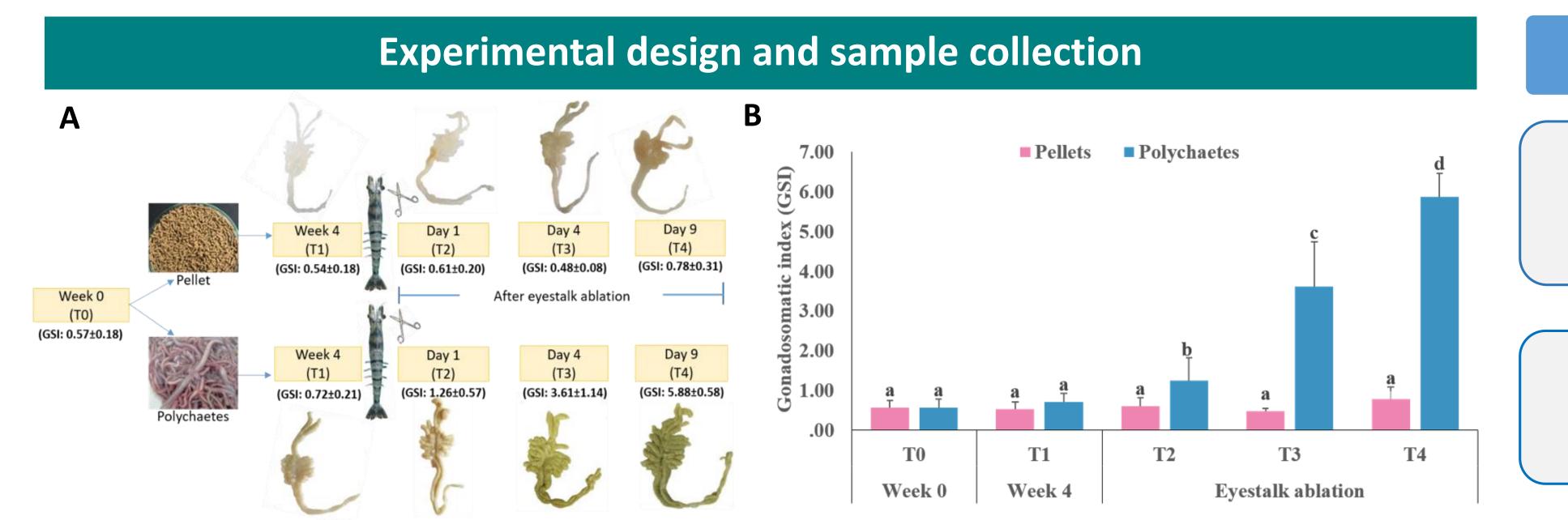
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Abstract

Unilateral ablation of eyestalk in female black tiger shrimp Penaeus monodon is known to induce ovarian maturation. However, without feeding the female broodstock with live feeds, the inductive effect cannot be realized. Thus, the synergistic effects of feeding with live feeds and the ablation must be elucidated. This study was not available, thus it constructed the first high-quality ovarian reference transcriptome in P. monodon using short-read Illumina RNA sequencing and long-read Pacific Biosciences (PacBio) isoform sequencing (Iso-seq). This transcriptome assembly allowed us to dissect the effects of feeds and eyestalk ablation and reveal their synergistic effects at the transcriptomic level. We found the regulation of important genes involved in fatty acid regulation, energy production, and hormone-mediated oocyte maturation pathways. These findings shed to means to induce ovarian maturation without having to perform eyestalk ablation in the future.



RNA extraction

Iso-seq Pooled T0, T1, T2, T3, T4 (N=1/each group)

RNA-seq **Individual T0, T1, T2, T3, T4** (N=2/each group)

(C)

I. 52 features in pellet-fed

II. 390 features in polychaetes-fed (a=7, b=215, c=71, d=97)

I. Responses in pellet-fed group

II. Responses in polychaetes-fed group

Collagen alpha-1V chain-like Heme-binding protein 2-like

Histone H1.3-like 3-phosphoinositide-dependent protein kinase 1-like

eiosis regulator and mR stability factor 1-like listone deacetylase complex subunit SAP30 homolog

Mitochondrial pyruvate carrier 1-like

TP synthase F0 subunit 6 mitochondrion

Cytochrome c oxidase subunit II mitochondrion

tochrome c oxidase subunit I mitochondrion stochrome c oxidase subunit III mitochondrion

NADH dehydrogenase subunit 4 mitochondrion

NADH dehydrogenase subunit 2 mitochondrion

NADH dehydrogenase subunit 3 mitochondrion ADH dehydrogenase subunit 1 mitochondrion

litelline membrane outer layer protein 1 homolog

Mitochondrial basic amino acids transporter-like

longation of very long chain fatty acids protein 6

grase core domain containing protein

as-related GTP-binding protein A isoform X2

ibril-forming collagen alpha chain-like

Iitogen-activated protein kinase kinase

ogestin membrane receptor component 1

Guanine nucleotide-binding protein-like 1

Crustacean hyperglycemic hormone 3 precursor CDK5 and ABL1 enzyme substrate 1-like

Mitochondrial uncoupling protein 4-like isoform X1

Calcium-activated chloride channel regulator 2-like

DNA damage-inducible transcript 4-like protein

Phosphatidylinositol 4-phosphate 3-kinase C2 Multiple inositol polyphosphate phosphatase 1-like Importin subunit alpha-4

Sphingomyelin phosphodiesterase-like

Phosphatidate phosphatase LPIN3-like

Calcineurin-binding protein cabin-1-like

Transcription factor E2F4-like

Kinesin-like protein KIF18A

NPC intracellular cholesterol transporter 2 homolog a-like

Solute carrier organic anion transporter family member 5A1

biquitin-protein ligase E3C-like

ntegrin beta-PS-like isoform X1 ollagen alpha-3IX chain-like

nnexin inx2-like isoform X2

itellogenin receptor

Kinesin-like protein

Protein FAM8A1-like

Transport protein Sec24C

15 kDa calcium-binding protein-like

Na- and Cl-dependent transporter XTRP3A-like isoform X1

ransport and golgi organization protein 6-like isoform X3

Stearoyl-CoA desaturase 5-like

vclin-I-like, partial

Fatty acid synthase

Cation-dependent mannose-6-phosphate receptor-like Mitotic checkpoint protein BUB3-like isoform X3

Ubiquitin-like protein ATG12 Inosine-5'-monophosphate dehydrogenase 1b-like isoform X1

3rd screening Clustered by their expression patterns 442 features

Tyrosine—tRNA ligase, mitochondrial Dynein heavy chain 5, axonemal-like

Library preparation

SMARTer PCR cDNA Synthesis Kit (Clontech, Mountain View, USA)

Illumina NovaSeq™ 6000 instrument (150 bp pair end read)

Sequencing

PacBio RS II instrument

TruSeq stranded Library Preparation kit (Illumina, San Diego, USA)

Data analysis

- Transcriptome assembly and annotation
- Mapping and differential gene expression analysis

Fig 1. Ovarian maturation after unilateral eyestalk ablation in the shrimp fed with pellet or polychaetes. (A) Representatives of ovaries from different time points. (B) Gonadosomatic Index (GSI) of the shrimp at different time points. Error bars represent standard derivation. Different letters indicate significant differences among time points of the same feed group (p<0.05, ANOVA Duncan test). Asterisks indicate significant differences between the two feed groups at the same time point (p<0.05, *t-test*).

(A)

RNA assembly and annotation

1st screening

11,964 features

· High quality of full length

transcript from 8 cells

Longest orf from Trinity

Cluster at 95% identity

(33,277 transcripts)

Annotate with NCBI nr, GO,

KEGG database

(15,482 features)

Remove duplicate feature

(11,694 features)

2nd screening

1,559 features

east 1 time point of 8 time points

Significant differences (p-adjusted <0.05) in at

Polychaetes-fed

T1 T2 T3 T4

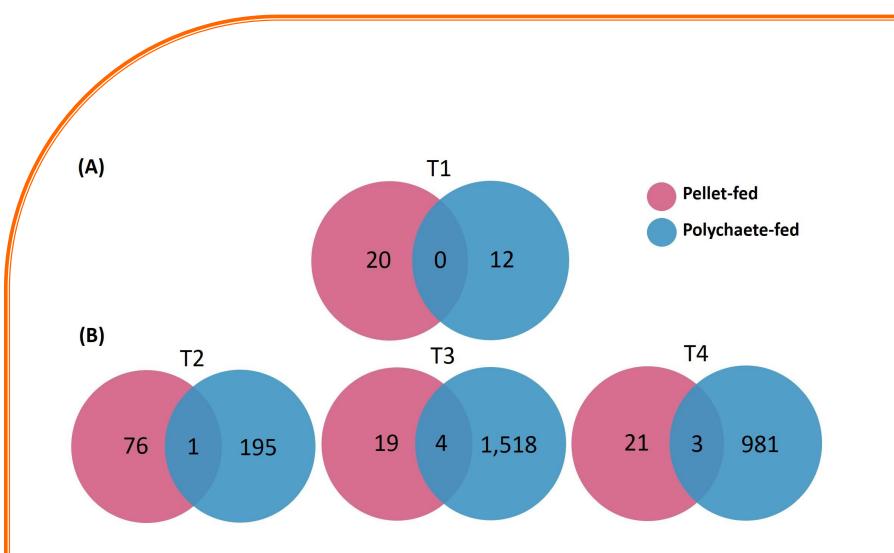


Fig 2. Venn diagram of DEGs of the pellet-fed shrimp and the polychaete-fed shrimp (A) after the 4-week feeding trial and before eyestalk ablation (T1) and (B) after eyestalk ablation at Day 1, 4, and 9 (T2, T3, and T4, respectively).

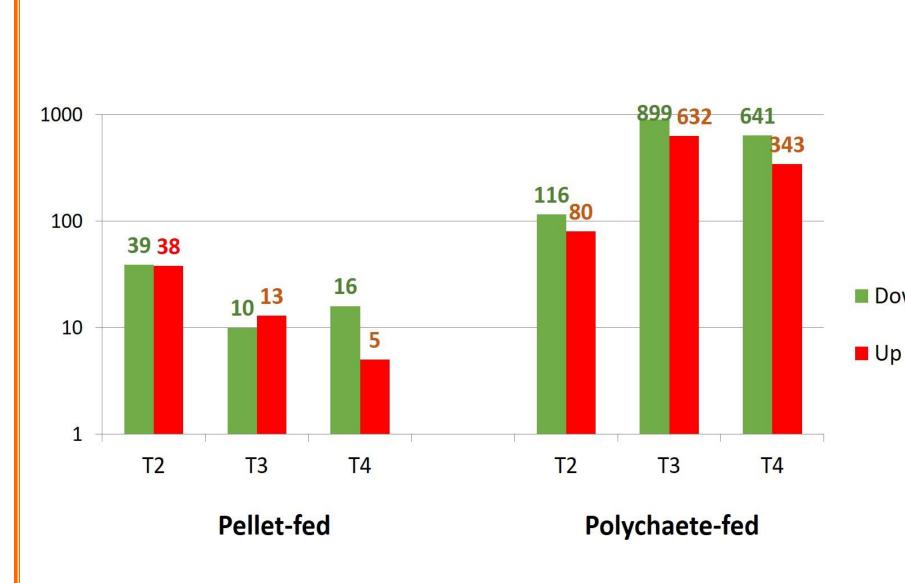
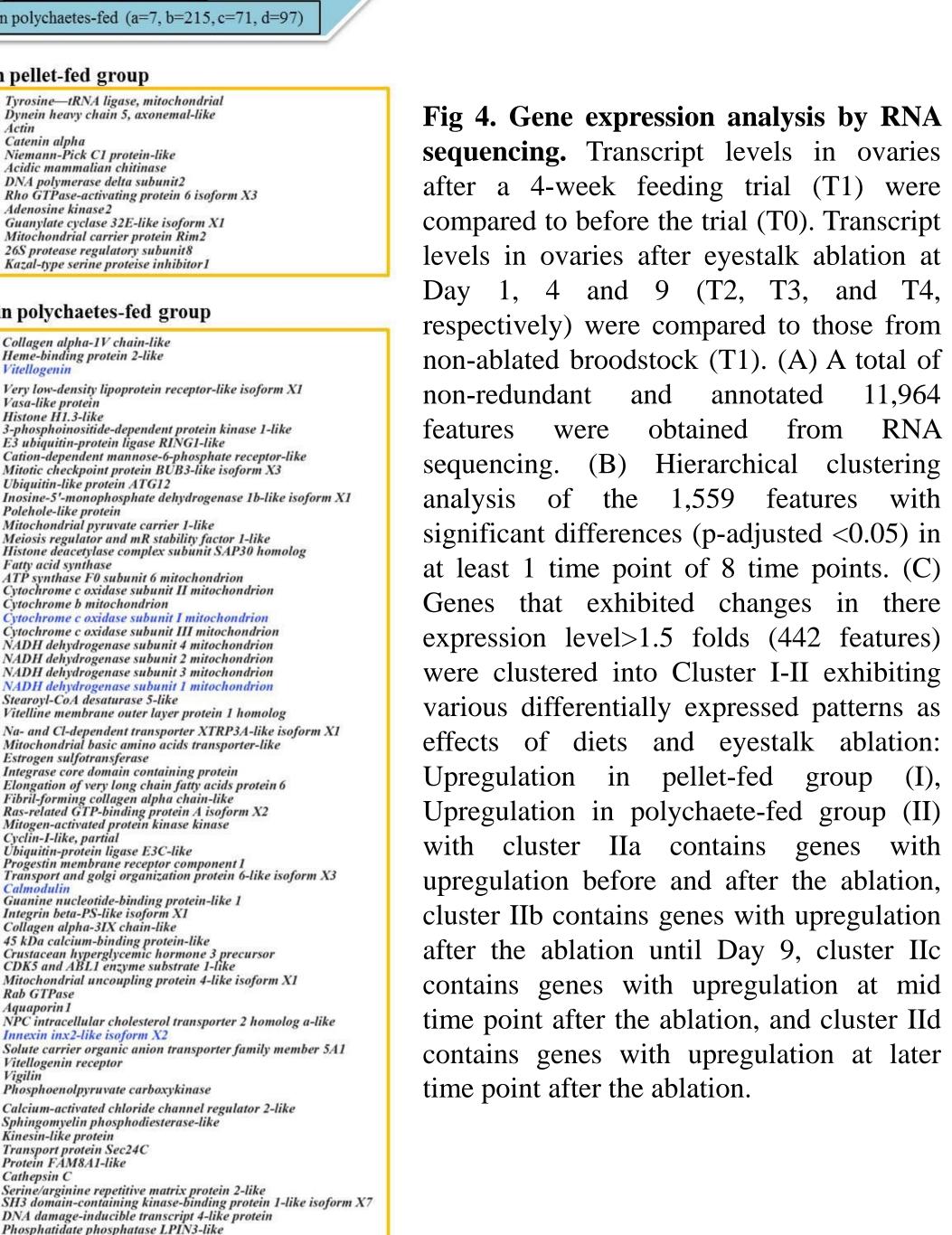


Fig 3. Numbers of DEGs after eyestalk ablation of the pellet-fed shrimp and the polychaete-fed shrimp at Day 1, 4, and 9 (T2, T3, and T4, respectively). Red and green bars indicate up- and downregulated DEGs. A base-10 log scale is used for the Y axis.





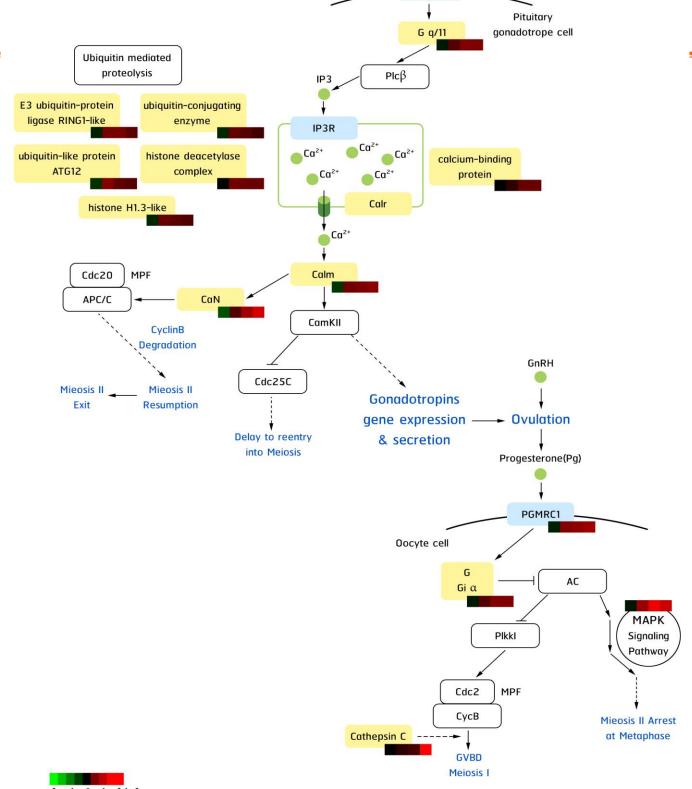


Figure 5. Differential expressed genes in the hormone-mediated ovarian maturation pathways in the polychaete-fed group. Light blue box, yellow box and white box represent receptor genes, genes were found in this study, and genes known to be in this pathway, respectively. The heatmap bars under each gene in the pathway reflect expression change from RNA-seq result after a 4-week feeding trial (T1) compared to before the trial (T0), after eyestalk ablation at Day 1, 4 and 9 (T2, T3, and T4, respectively) compared to those from non-ablated broodstock (T1).

Conclusion

- · RNA-seq results strongly suggest that there are synergistic effects between the polychaete feeding and the eyestalk ablation in the process of ovarian maturation in black tiger shrimp
- The eyestalk ablation is still necessary to perhaps manipulate the female endocrine of the black tiger shrimp, but this technique was synergist with nutrition diet in polycheates
- The synergy seems to be the induction of important groups of genes, namely energy production genes, endocrinological genes, fatty acid regulatory genes, and oogenesis
- These findings shed to light on molecular mechanisms and key molecular pathways that lead to successful ovarian maturation

Sittikankaew, K., Pootakham, W., Sonthirod, C., Sangsrakru, D., Yoocha, T., Khudet, J., Nookeaw, I., Uawisetwathana, U., Rungrassamee, W., Karoonuthaisiri, N., Transcriptome analyses reveal the synergistic effects of feeding and eyestalk ablation on

ovarian maturation in the black tiger shrimp (*Penaeus monodon*), Scientific report., ttps://doi.org/10.1038/s41598-020-60192-2

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