RNAi-Coupled Transcriptome Bioinformatics Maps *VgH*-Dependent Networks in *Artemia parthenogenetica*

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Abstract. *Artemia* is a major live-feed resource for aquaculture, but networks linking reproduction, metabolism, and immune resilience remain incompletely defined. We combined RNA interference and transcriptome bioinformatics to characterize hemocyte responses to *VgH* depletion. LPS screening identified *VgH* as the most inducible of eight vitellogenin-family genes. Injection of 2000 ng/μL dsVgH reduced expression by 75.2% at 24 h without lowering survival relative to dsEGFP. Four RNA-seq libraries were analyzed with an FDR-controlled workflow, followed by GO/KEGG enrichment and qRT-PCR. Knockdown identified 2,482 differentially expressed genes (1,300 up- and 1,182 down-regulated). Enriched modules included endoplasmic-reticulum protein processing, oxidative phosphorylation, cell adhesion, Hippo/polarity signaling, calcium entry, and immune-associated pathways. qRT-PCR supported selected transcript directions but did not validate proteins or pathway activity. This exploratory map identifies candidate pathways for *Artemia* stress robustness and aquaculture biotechnology; larger cohorts and pathway-specific, protein-level, and production-level validation under aquaculture conditions are required.

Keywords: *Artemia*, Aquaculture biotechnology, RNA interference, Transcriptome bioinformatics, Systems biology

1 Introduction

Artemia nauplii are widely used as live feed in fish and crustacean hatcheries, and the species also offers opportunities for linking hypersaline biological resources with circular aquaculture production [5, 6]. Molecular knowledge of stress tolerance, reproduction, and immune competence can therefore support both resource utilization and live-feed biotechnology.

Vitellogenin (Vg) is best known as a yolk precursor that transports nutrients to developing oocytes [1, 2], but Vg proteins also contribute to antioxidant and antimicrobial functions in invertebrates [3, 4]. Parthenogenetic *Artemia* is suitable for functional genomics because clonal reproduction reduces genetic heterogeneity. Its hemocytes include phagocytic cells and lipid-rich adipohemocytes, creating a cellular context in which nutrient transport and innate immunity intersect [7, 8]. Silencing the Vg receptor

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alters ovarian development and fecundity [9], but the non-reproductive roles of individual Vg paralogues remain unresolved.

RNA interference and emerging gene-editing approaches provide gene-specific evidence in aquaculture species [10, 11], whereas RNA sequencing can map downstream networks. We therefore screened the Vg family after LPS stimulation, silenced the most responsive member, *VgH*, and used a quality-controlled bioinformatics workflow to identify metabolic, proteostatic, structural, and immune modules. The objective was to generate a biologically interpretable molecular map relevant to the ISAEB themes of agricultural molecular biology, bioinformatics, and systems biology without overstating transcriptomic associations as validated production traits.

2 Materials and Methods

2.1 Animals, LPS Screening, and RNAi

Diploid parthenogenetic *A. parthenogenetica* cysts from Ebinur Lake, Xinjiang, China, were hatched at 30 g/L salinity, 28 °C, continuous aeration, and approximately 2000 lux. Nauplii were transferred after 24 h to 70 g/L brine at 200 individuals/L. Hemocyte samples were obtained after removal of the intestine, ovary, and brain and centrifugation of the remaining tissue at 6000 rpm for 20 min at 4 °C [7, 9]. Total RNA was extracted with TRIzol, assessed by agarose electrophoresis and A260/A280, treated to remove genomic DNA, and reverse-transcribed.

Adult animals were exposed for 24 h to 1 mg/L LPS or saline. Each treatment had three biological replicates of 30 animals. *VgA-VgH* expression was quantified by qRT-PCR; LPS is a common surrogate for bacterial challenge in crustaceans [12]. T7-flanked *VgH* fragments were transcribed in vitro, and dsEGFP served as the negative control. Animals received 1 μL of dsVgH or dsEGFP at 1000 or 2000 ng/μL. Knockdown, persistence, and survival were assessed at 24 h and, where applicable, 1, 3, and 5 d.

2.2 RNA-Seq and Bioinformatics

Two libraries per treatment were analyzed: dsVgH H1/H2 and dsEGFP E1/E2. Poly(A) RNA was enriched, fragmented, converted to cDNA, ligated to adaptors, size-selected at approximately 370-420 bp, amplified, and quality checked. Raw reads were inspected with FastQC, trimmed with Trimmomatic [24], aligned to the available *Artemia* reference with HISAT2 [25], reconstructed and quantified with StringTie and FeatureCounts [26, 27], and tested with DESeq2 [28]. Differentially expressed genes satisfied $|\log2 \text{ fold change}| \geq 1$ and FDR < 0.05. GO and KEGG enrichment organized genes into functional modules [29, 30]. Recent chromosome-scale assemblies strengthen annotation-based analysis in *Artemia* [13, 14].

2.3 qRT-PCR and Statistics

qRT-PCR assessed selected transcripts using three biological and three technical replicates; this validated transcript directions only. Expression was calculated by 2^(-ΔΔCt). Two-group tests used two-sided t-tests; stages used ANOVA with Duncan's test ($p < 0.05$; $p < 0.01$).

3 Results

3.1 VgH Selection and Knockdown

LPS induced *VgB*, *VgF*, and *VgH*, with *VgH* showing the largest increase (Fig. 1A). Ovarian *VgH* expression rose strongly at stages III-IV and declined at stage V (Fig. 1B), indicating reproductive activity together with an inducible hemocyte response. At 24 h, 1000 and 2000 ng/μL dsVgH reduced *VgH* expression by approximately 42% and 75.2%, respectively (Fig. 2A). Suppression remained 71% at 3 d and decreased to 46.3% at 5 d (Fig. 2B). Survival data in Table 1 showed no disadvantage of 2000 ng/μL dsVgH relative to its dsEGFP control.

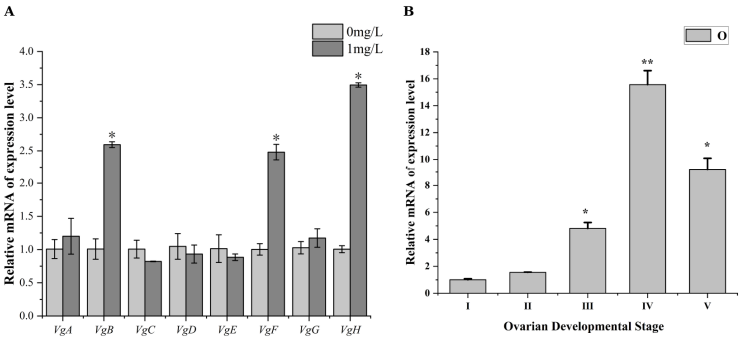


Fig. 1. (A) Vg-family expression after 24 h of LPS exposure. (B) Ovarian *VgH* expression across developmental stages. Data are mean ± SD; a single asterisk indicates $p < 0.05$ and a double asterisk indicates $p < 0.01$.

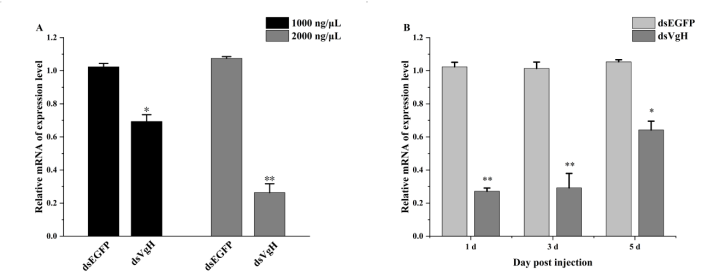


Fig. 2. RNAi efficiency. (A) Dose response at 24 h. (B) *VgH* expression at 1, 3, and 5 d after injection. A single asterisk indicates $p < 0.05$ and a double asterisk indicates $p < 0.01$.

Table 1. Injection conditions and survival at 24 h.

Group	Conc. (ng/μL)	Volume (μL)	Injected	Survivors	Survival (%)
dsEGFP	1000	1	30	26	87
dsVgH	1000	1	30	24	80
dsEGFP	2000	1	30	24	80
dsVgH	2000	1	30	25	83

3.2 Sequencing Quality and Differential Expression

The libraries retained 41.5-44.4 million clean reads and 6.23-6.67 Gb clean bases. Q30 values were 96.79%-97.59%, and GC content was 39.13%-39.99% (Table 2). At |log2 fold change| ≥ 1 and FDR < 0.05, the exploratory comparison identified 2,482 differentially expressed genes, comprising 1,300 up-regulated and 1,182 down-regulated genes (Fig. 3A).

Table 2. RNA-seq quality summary.

Sample	Raw reads	Raw Gb	Clean reads	Clean Gb	Q30 (%)	GC (%)
H1	44,743,106	6.71	43,746,780	6.56	96.86	39.39
E1	46,010,398	6.90	44,438,508	6.67	96.79	39.26
H2	43,022,096	6.45	42,071,522	6.31	97.49	39.99
E2	42,469,512	6.37	41,526,946	6.23	97.59	39.13

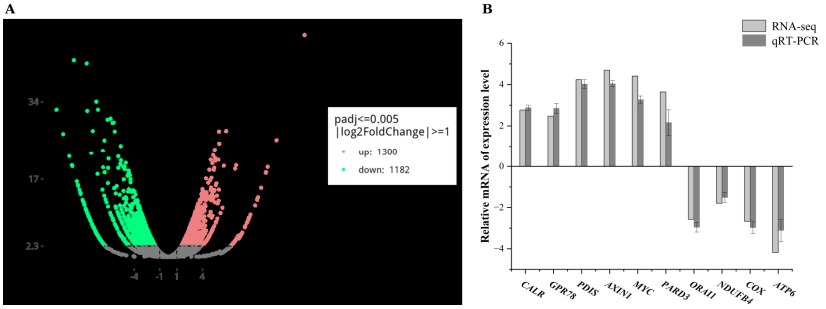


Fig. 3. (A) Volcano plot of differentially expressed genes. (B) Directional comparison of RNA-seq and qRT-PCR for representative genes.

3.3 Functional Modules and Validation

GO terms emphasized metabolism, protein modification, mitochondrial and Golgi components, extracellular-matrix functions, oxidoreductase activity, and structural molecules. KEGG enrichment highlighted oxidative phosphorylation, thermogenesis,

protein processing in the endoplasmic reticulum, cell adhesion, Hippo signaling, and immune-associated modules (Fig. 4). Disease-named KEGG terms were interpreted as conserved gene sets, not diagnoses in *Artemia*. qRT-PCR supported the RNA-seq directions: *CALR*, *GRP78*, *PDIS*, *AXIN1*, *MYC*, and *PARD3* increased; *ORAI1*, *NDUFB4*, *COX*, and *ATP6* decreased (Fig. 3B).

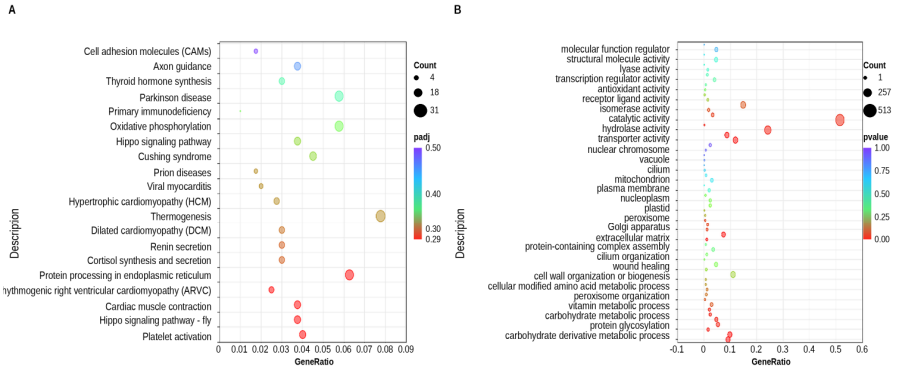


Fig. 4. Representative KEGG pathways and GO terms enriched after *VgH* knockdown. Bubble size denotes gene count and color denotes adjusted probability.

4 Discussion

The non-uniform LPS response and broad knockdown phenotype support functional diversification of the *Artemia* Vg family. Similar member-specific expression occurs in whiteleg shrimp, freshwater giant prawn, and *Exopalaemon carinicauda* [15-17]. *VgH* retained stage-dependent ovarian expression but also influenced hemocyte networks, suggesting a dual reproductive and stress-associated role. The data do not establish direct transcriptional regulation; reduced delivery of a secreted lipid-carrying protein may instead perturb nutrient transport, receptor signaling, extracellular interactions, and secretory demand.

Mitochondrial genes, including *NDUFB4*, *COX*, and *ATP6*, decreased, whereas ER chaperone genes increased. This pattern is consistent with altered protein-folding demand [18-20], and reduced *NDUFB4* with changes in complex I organization [21]. However, transcript and qRT-PCR data do not demonstrate impaired respiration, ER stress, protein abundance, or pathway activity. Energy supply and proteostasis remain priorities for functional testing. In hemocytes, these systems may influence phagocytosis, ion transport, biosynthesis, and recovery from environmental or infectious challenge.

Changes in cell-adhesion, Hippo/polarity, and calcium-entry modules indicate altered cellular organization. Hippo signaling responds to mechanical and growth cues [22], while Orai channels mediate store-operated calcium entry [23]. Because

phosphorylation, protein localization, calcium flux, and pathogen resistance were not measured, pathway activation and immune impairment remain hypotheses requiring functional and protein-level validation.

For aquaculture biotechnology, the main contribution is a perturbation-to-pathway framework and candidate modules for *Artemia* health research. However, two RNA-seq libraries per treatment limit statistical power and dispersion estimates. Future work should include larger independent cohorts, *VgH* rescue, live-pathogen challenge, cell-type-resolved profiling, proteomics, respiration and calcium assays, and production-level validation under aquaculture conditions linked to growth, reproduction, cyst quality, nutrition, and hatchery performance. Until then, these genes remain research candidates rather than breeding or production biomarkers.

5 Conclusion

VgH was the most LPS-responsive Vg paralogue, and its knockdown altered 2,482 transcripts across mitochondrial, ER, adhesion, polarity, calcium-signaling, and immune-associated modules. qRT-PCR supported selected transcript directions. This exploratory map connects reproductive biology with metabolic-immune regulation in *Artemia* and identifies candidate pathways for biological-resource utilization and live-feed resilience. Mechanistic and production applications require larger phenotype-linked cohorts and pathway-specific, protein-level, and aquaculture-condition validation.

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Disclosure of Interests

The authors have no competing interests to declare. ChatGPT (OpenAI, accessed July 2026) was used only for English-language editing and document formatting; the authors verified all scientific content, values, figures, analyses, and references and take full responsibility for the manuscript.

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