Original Research Articles

Behavioral characteristics and transcriptome analysis of *Mizuhopecten yessoensis* in response to *Neptunea arthritica cumingii* predation during laboratory bottom-sowing culture

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In this study, we investigated the effects of predation by the whelk *Neptunea arthritica cumingii* on the culture and survival of scallops (*Mizuhopecten yessoensis*) during bottom culture. The behavioral characteristics of three sizes (small, medium, and large) of scallops were assessed in response to exposure to *N. cumingii*. We also measured the activities of superoxide dismutase, catalase, arginine kinase, and octopine dehydrogenase in the gill, adductor muscle, and mantle tissues of scallops before and after exposure to predation. We found that scallops escaped from *N. cumingii* predation by continuous shell closure or movement. The shell closure force increased with the scallop size, and scallops of the same size that were stimulated by the presence of *N. cumingii* closed their shells more frequently than control scallops. The shell closure ability of scallops of all sizes decreased as the duration of the experiment continued. The enzyme activities in the three scallop tissues changed significantly after continuous stimulation, and the trend was particularly obvious for the enzyme activities in the adductor muscles of medium-sized scallops. Transcriptome analysis of the adductor muscles from medium-sized scallops detected 405 differentially expressed genes (172 upregulated and 233 downregulated), where the expression levels of RYK, APC, BAMBI, WNT16, CACYBP, and RUVBL1 changed after stimulation by N. cumingii. The sequencing results were verified by quantitative real-time PCR. We hypothesize these genes may be related to muscle movement and regulation in *M. yessoensis* after stimulation.

INTRODUCTION

Mizuhopecten yessoensis (Mollusca, Bivalvia, Pteromorphia, Pterioida, Pectinidae) is a large, cold water, filter-feeding shellfish with double shells and fan-shaped shells, which mostly lives in high salinity and freshwater-free inner bays. *M. yessoensis* is native to Japan and South Korea, and has rich nutritional value. The taste of this scallop is delicious and unique, and thus, it has high economic benefits, making it strongly favored by breeding enterprises and consumers. *M. yessoensis* was introduced into China in the 1980s, and it is now extensively farmed and one of the most important shellfish species in northern China.[1]

In China, the two main methods for breeding *M. yessoensis* are raft farming and bottom-sowing farming, where bottom-sowing farming is the main breeding mode.[2] Due to the expanded scale of shrimp and scallop cultivation, problems have occurred, such as a low recapture rate and high mortality rate during the bottom sowing cultivation

process, severely affecting the healthy development of scallops.[3] In particular, predation by enemy organisms is one of the main causes of the high mortality rate for scallops. *N. cumingii* is mainly distributed in the Yellow and Bohai Seas in northern China, and its living conditions are similar to *M .yessoensis*. *N. cumingii* mostly feeds on bivalves and other shellfish and is one of the main predators of scallops. During the bottom sowing and breeding process for *M. yessoensis,* significant economic losses were caused by *N. cumingii*. Thus, predation by enemy organisms significantly impacts the cultivation and survival of scallops.

The behavior of animals determines their survival, growth, and successful breeding.[4] Thus, understanding the behavior of scallops can help to ensure targeted breeding, healthy development, and economic benefits. When encountering stimuli due to predators, scallops will behave by closing their shells, swimming, and jumping to avoid predators.[5] Blood (coelom fluid) is an important biological component for assessing the responses of aquatic animals

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to external stimuli. The activities of various immune-related enzymes in the blood can be used as important physiological indicators to measure aquatic animals' health status and immune capacity, which directly reflect their adaptability to the external environment.[6]

So far, few reports have reported *M. yessoensis* responding to predator predation. In this paper, we studied the behavior characteristics and ability of *M. yessoensis* to avoid enemy when *N. cumingii* stressed different specifications of *M. yessoensis* in the laboratory, compared the changes in enzyme activity of *M. yessoensis* tissues after no enemy organism stimulation and continuous enemy organism stimulation, and conducted deep transcriptome sequencing for tissues with significant differences, and randomly selected genes with significant differences were verified by qRT-PCR. The results of this study contribute to the in-depth understanding of *M. yessoensis*' response mechanism to predation pressure, especially at the genetic and molecular levels, filling the gap in the biological response mechanism of *M. yessoensis* to avoid the enemy. At the same time, the results could provide data support and theoretical references for the prevention and control strategy of *M. yessoensis* enemies and the improvement of *M. yessoensis* bottom-sowing farming technology.

MATERIALS AND METHODS

EXPERIMENTAL MATERIALS

M. yessoensis were caught in Longwangtang Bay (Lvshunkou District, Dalian, China) in November 2022 and transported back to the Key Laboratory of Mariculture and Stock Enhancement in North China's Sea, Ministry of Agriculture in a wet state using a holding tank. Scallops were rinsed clean and temporarily cultured in tanks. They were fed with Spirulina powder at regular intervals every day. After 7 days of culture, scallops that could close naturally and were healthy and undamaged were selected for use in the experiment.

N. cumingii were also caught in Longwangtang Bay in November 2022. Healthy *N. cumingii* were cultured without feeding for 7 days. Individuals without obvious trauma and able to feed normally were selected for use in the experiment.

The shell length, height, and width of the scallops were measured using Vernier calipers (Marr Precision Measuring Instruments Co., Ltd., Suzhou, China) with an accuracy of 0.01 mm. The wet weight of each scallop was measured using electronic scales (Changshu Shuangjie Testing Instrument Factory, Jiangsu, China) with an accuracy of 0.01 g. Scallops were classified into three size groups: shell length, height, and width. **Table 1** shows the basic biological indexes for the specimens.

Generally, the *M. yessoensis* release specifications are generally 30-60mm in shell length in China, and which can reach the market specifications after 2-3 years, so we chose 60,90 and 120mm three specifications in this experiment, which can more fully reflect the ability of *M. yessoensis* bot-

tom to avoid enemy organisms at different stages after sowing.

BEHAVIORAL OBSERVATIONS OF SCALLOPS

We filmed each experiment described below for 72h using a webcam (Model: KS-X6-QG4, Police Vision Guard, Jiangsu, China). The camera was installed 3m above the tank, allowing filming under light-free conditions. For each group, a 1h window was randomly selected to count the number of swimming events (shell opening and closing > 3 times consecutively) and jumping events (shell opening and closing ⩽ 3 times consecutively). During the experimental period, the experimental conditions were the same for all groups, and the water was minimally aerated to minimize other disturbances.

N. cumingii-scallop Experiment I consisted of one control group and three experimental groups. Control group: put 15 scallops (5 large scallops, 5 medium scallops and 5 small scallops) into the tank, without *N. cumingii* (I-L0). Experimental group: after 15 scallops were put into the experiment, small size *N. cumingii* (I-sL), medium size *N. cumingii* (I-mL) and large size *N. cumingii* (I-lL) were put into the experiment respectively. Three replicates per experiment were set to reduce errors.

In *N. cumingii*-scallop experiment II: Five scallops of the same size put into their respective tanks, to which different size *N. cumingii* was added for predation stimulation small (II-sLsB, II-sLmB, and II-sLlB), medium (II-mLsB, II-mLmB, and II-mLlB), and large (II-lLsB, II-lLmB, and II-lLlB). Control groups without a crab present were set up for each size class (II-LsB0, II-LmB0, and II-LlB0). Each group had three replicates.

FORCE OF CLAP MEASUREMENT

The scallop shell closure force was measured following a previously described force gauge method.[4] The experimental setup included a digital force gauge (Nscing SH-III-100N, digital dorce gauce, Suce, Nanjing, Jiangsu, China), water tank, and stand (**Figure 1**). The lower valve of a scallop was fixed on the experimental table in the water tank, and the upper valve was able to open and close freely. The force meter was fixed with a bracket. One end consisted of a hooked rod fixed between the upper and lower valve, which could accurately capture each time the scallop closed its valve, and the other end was a data port connected to a personal computer using a USB port. Soft-SH software was used to collect and store the data.

The scallops were fixed to the test bench, and the force measuring device was installed to assess the movement state of different sizes of scallops in their natural state and during continuous stimulation by the presence of an *N. cumingii*. The contraction of the adductor muscles is mainly divided into two stages.[4] Phasic contractions (i.e., the rapid opening and closing of the shell) are generated by the contraction of the transverse striated muscle in the adductor muscles; in the force output diagram, every peak value represents one clap of the scallop. The force generated by the process is the force of the clap (F_{clap}), and the tense con-

Table 1. The basal biological data of experimental animals

	M. yessoensis			*N. cumingii*		
	Large size	Middle size	Small size	Large size	Middle size	Small size
Shell length/mm	119.85 ± 3.23^a	89.24 ± 3.77^b	60.10 ± 3.23^c			
Shell height/mm	116.92 ± 6.02	87.46 ± 3.55	61.39 ± 6.02	111.21 ± 2.17^x	100.25 ± 1.08^y	89.81 ± 0.88^z
Shell width/mm	26.98 ± 2.98	26.98 ± 2.98	16.21 ± 2.63			

Note: experiment Ⅰ n=15, experiment Ⅱ n=5. Value are x̄± SD. The letters "a" "b" "c" indicates whether the shell length varies significantly for different scallop sizes, "x" "y" "z" indicates whether the shell heigth varies significantly for different *N. cumingii* sizes. Different letters indicate significant differences ($p < 0.05$).
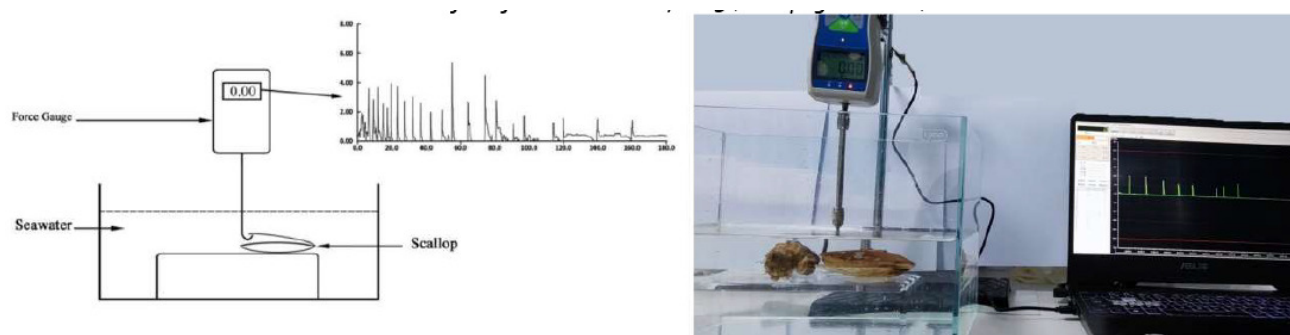

Figure 1. Schematic diagram of the experimental device for measuring the movement of M. yessoensis Note: Schematic diagram of the experimental device (left) and operation diagram (right)

traction (i.e., sustained for > 0.5 s) is the contraction of the shell. The second stage is tonic contractions (i.e., sustained for > 0.5 s), which result in slow opening and closing of the shell. They are generated by the contraction of the smooth muscles in the adductor muscles, and they show an obvious and sustained value in the output graph of the force measurement (**Figure 1**). The force generated by this process is the tonic contraction force (F_{tonic}). Typically, a scallop undergoes a cycle of motion that consists of rapidly clapping n times within a short period, followed by one more sustained slow contraction. The force generated by this process is called the phasic contractile force (F_{phasic}), which is the sum of F_{clap}.

According to Zhang et al.[4] and the results of a pre-test, *M. yessoensis* stops moving after 3 min of continuous stimulation by a predator, and a period of recovery time is needed before it can attempt to evade again. Therefore, the measurement time for each scallop in this experiment was set at 180.2±0.2 s. First, the movement of each scallop in each size class was measured in the natural state (i.e., no *N. cumingii*). After 48h, the shell closure of each scallop in each size class under continuous stimulation by the presence of *N. cumingii* was measured. The data indexes were the maximum force of clap (F_{max}), number of phasic contractions (T_{phasic}), number of tonic contractions (T_{tonic}), and frequency of shell closure (the number of times the scallop closes its shell per minute).

Because the initial response and strength of the scallop is important for its survival when it encounters a predator, the number of shell closures in the first 30 s as a percentage of the total number of shell closures (P_{30s}) was also calculated. In order to minimize inter-individual errors, 15 parallels were set up for each set of experiments.

ENZYME ACTIVITY ASSAY

The adductor muscles, gills, and mantle tissues were removed from three scallops in the unstimulated control group and three scallops from each size group after exposure to *N. cumingii*, frozen in liquid nitrogen, and preserved at −80℃ for subsequent enzyme activity assays. Activities of superoxide dismutase (SOD), catalase (CAT), octopine dehydrogenase (ODH), and arginine kinase (AK) activities in each tissue from each scallop were measured using the FANKEW ELISA kit.

STATISTICAL ANALYSIS

SPSS25.0 software was used to conduct one-way ANOVA on the motor behavior indicators of the same state (normal state or *N. cumingii* stimulated state) with different specifications or the same specifications with different states, and LSD was used to make a two-by-two comparison of the indicators with significant differences; two-way ANOVA was conducted to analyze the interactions between the specifications and the states, with P<0.05 regarded as the difference being significant, and P<0.01 regarded as the difference being extremely significant.

TRANSCRIPTOME ANALYSIS

Total RNA was used as input material for the RNA sample preparations. Briefly, mRNA was purified from total RNA using poly-T oligo-attached magnetic beads. Fragmentation was performed using divalent cations under elevated temperature in First Strand Synthesis Reaction Buffer(5X). First strand cDNA was synthesized using random hexamer primer and M-MuLV Reverse Transcriptase (RNase H-). Second strand cDNA synthesis was subsequently performed using DNA Polymerase I and RNase H. Remaining overhangs were converted into blunt ends via exonuclease/polymerase activities. After adenylation of 3' ends of DNA fragments, Adaptor with hairpin loop structure were ligated to prepare for hybridization. To select cDNA fragments of preferentially 370~420 bp in length, the library fragments were purified with AMPure XP system (Beckman Coulter, Beverly, USA). Then PCR was performed with Phusion High-Fidelity DNA polymerase, Universal PCR primers and Index (X) Primer. At last, PCR products were purified (AMPure XP system) and library quality was assessed on the Agilent Bioanalyzer 2100 system.All RNA clean data were submitted to the Short Read Archive (SRA) Sequence Database at the National Center for Biotechnology Information (NCBI) (Accession No. PRJNA1048362).

According to the manufacturer's instructions, the index-coded samples were clustered on a cBot Cluster Generation System using TruSeq PE Cluster Kit v3-cBot-HS (Illumia). After cluster generation, the library preparations were sequenced on an Illumina Novaseq platform, and 150 bp paired-end reads were generated.

The quality of the raw data was assessed using FastQC. Raw data (raw reads) were processed using Trimmomatic to remove adapters, reads containing ploy-N, and low-quality reads to obtain clean reads. Then the clean reads were mapped to the *M. yessoensis* genome (https://www.ncbi.nlm.nih.gov/genome/12193) using HISAT2. The FPKM (Fragments Per Kilobase of exon model per Million mapped fragments) value of each gene was calculated using cufflinks, and the read counts of each gene were obtained by the-count. DESeq was used to compare RNA-seq data between the mBJ and L-mBJ groups. Differentially expressed genes (DEGs) in mBJ versus L-mBJ were identified using the DESeq functions estimateSizeFactors and nbinom Test. The false discovery rate (FDR) control method was used to identify the p-value threshold in multiple tests to compute the significance of the differences. Threshold values were set as p < 0.05 and fold change >2. Gene ontology (GO) enrichment analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis were performed. The stress-related genes were selected, and the sequences of selected DEGs and annotated genes were verified and compared by BLAST to ensure their similarity. Beijing Novozymes Technology Co conducted the bioinformatic analysis.

To examine the reliability of the RNA-Seq results, 9 DEGs were randomly selected for qRT-PCR validation. They included C1QL4, CYP2C8, ZCCHC8, TRXL, RAD17, OTOF, C25B8.10, PROM1A, and β-GLB. The housekeeping gene Gapdh was used as the reference gene. The primers were designed by Primer Premier 6, with lengths of 18-27 bp, GC (ratio of two bases, guanine, and cytosine, to the total base) content of 45%–55%, and synthesized by Sangon Biotech Co., Ltd. (Shanghai, China). The RNA was reverse transcribed to cDNA using TIANGEN® FastKing gDNA Dispelling RT SuperMix (Tiangen Biotech, China). Suitable primers were designed using Primer 6 (**Table 2**) and synthesized by Sangon Biotech Co., Ltd. (Shanghai, China). qRT-PCR was performed with TIANGEN® Talent qPCR Pre-Mix (SYBR Green) (Tiangen Biotech, China) on a Roche LightCycler®96 (Roche Diagnostics, Switzerland) real-time PCR system according to the manufacturer's protocol. Samples from the same batch experiment of the RNA-seq were used for the confirmation experiment.

RESULTS

SCALLOP BEHAVIOR

Table 3 shows the predation of scallops by *N. cumingii* in Experiment I. The small *N. cumingii* group (I-sL) did not predate scallops, whereas the medium *N. cumingii* group (I-mL) and large *N. cumingii* group (I-lL) exhibited relatively more predation of scallops. The number of motor behaviors by scallops was slightly higher in the presence of predators than in the control group, but the differences were insignificant (**Figure 2**).

Scallops were also exposed to different *N. cumingii* group sizes in the farming environment (*N. cumingii* predation of scallops in experiment II). The small N. cumingii group predated no scallop, whereas the medium N. cumingii group predated three small scallops and one medium scallop. In addition, four small scallops were predated by the large-sized *N. cumingii* group (**Table 4**). **Figure 3** shows the movement behaviors of the scallops of different sizes. In the presence of small and medium-sized *N. cumingii* groups, the frequency of movement behaviors by scallops was significantly greater than that of the control group. Still, the change was not significant with the large *N. cumingii* group.

FORCE OF SHELL CLOSURE

After observing images of the shell closure force by *M. yessoensis* of different sizes under natural conditions and under stimulation by *N. cumingii* (**Figure 4**), the results showed that the shell closure force was greater under stimulation by *N. cumingii* than the control group for scallops of the same size, and the number or duration of shell closure was also higher. The response patterns of scallops with different sizes to stimulation were slightly different. Under stimulation, the T_{phase} values for the small and medium-sized scallops were significantly higher than the natural state (**Figure 4; L-sB, L-mB**), and the Ttonic values were longer for larger scallops (**Figure 4**; L-lB). The shell closure capacities of scallops with various sizes tended to decrease over time.

The F_{max}, T_{phase}, T_{tonic}, t_{tonic}, closure frequency, and P30s values for each group of scallops are shown in (**Figure**

Table 2. Primers used for the qRT-PCR validation

Gene ID	Gene name	Description	Primer name	Primer sequences (5'-3')
	Gapdh	glyceraldehyde-3-phosphate dehydrogenase	*Gapdh*-F	TGGTATGGCTTTCCGTGTGC
			Gapdh-R	TCCTCTGTGTAACCAAGGAACC
110455157	*C1QL4*	complement C1q-like protein 4	*C1QL4*-F	TTGTCGTGTCTGCACAGGAAGG
			C1QL4-R	GCCGTCCGTTGAAGTCAGGTAG
110459241	*CYP2C8*	Cytochrome P450 2C8	*CYP2C8*-F	GTTGCTCCTCTTGGCGTTCCT
			CYP2C8-R	GGCGACCGACAGAGAATGCT
110464105	*ZCCHC8*	zinc finger CCHC domain-containing protein 8-like	*ZCCHC8*-F	ACCACCACTGCCAATCAACACTC
			ZCCHC8-R	CCATCACCTGTAGCTCCACCTCT
110449874	*TRXL*	thioredoxin, mitochondrial-like	*TRXL*-F	TGTCTACAACACCCGCCAGAAT
			TRXL-R	ACACCACGAAGCATGGAAGTC
110466699	*RAD17*	cell cycle checkpoint protein RAD17	*RAD17*-F	ACGAGTCGGAGTTGTGGTCTG
			RAD17-R	TGCCTGTGCCTTGAGATGTGT
110467166	*OTOF*	otoferlin-like	*OTOF*-F	GTTGACGGACTCGGACGACATC
			OTOF-R	GCCTTCAGCACTCGCACAGT
110461631	*C25B8.10*	uncharacterized methyltransferase-like C25B8.10	*C25B8.10*-F	GTTGAGCTTGGAGCTGGAACAG
			C25B8.10-R	GCCACCACAGTCCTAACAGAGT
110449776	*PROM1A*	prominin-1-A	*PROM1A*-F	GGTTTGGCTTGGGATGGTGTCT
			PROM1A-R	GCGTGGCTGACCTTGTTGCT
110454035	*β-GLB*	beta-galactosidase-like	*β-GLB*-F	TGCCTGCGACCATACCTACAC
			β-GLB-R	CCAGTAATCCAGCCAGCCAGT

Table 3. Number of scallop prey items eaten in experiment I

	Number of scallop prey eaten (pieces/group)			
	24h	48h	72h	total
I -L0	—	1	—	1
I -sL	—	—	—	—
I -mL	—	1	1	2
I -IL	1	2	—	3

Notes: "L" denotes *N. cumingii*; the lowercase letter "s," "m," and "l" denote the small, medium, and large groups, respectively; and the subscript "0" denotes the control group (no *N. cumingii*).
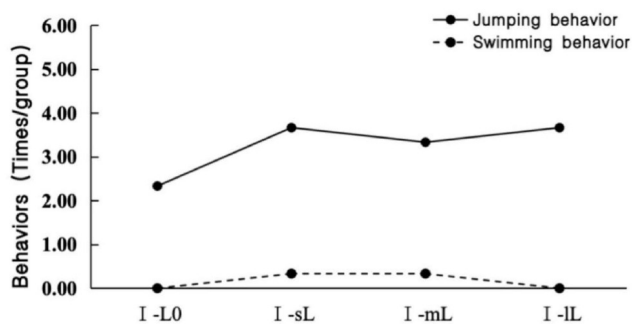
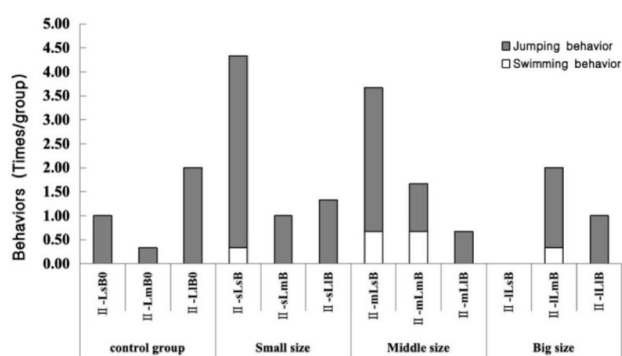**Figure 2. Behaviors of *M. yessoensis* in experiment I.**

5). As the size of the scallops increased, F_{max} tended to increase, and the values were slightly higher for the control group, which was the same size as the experimental group.

However, the difference was not significant (**Figure 5-A**). Compared with the control group, the T_{phase} and T_{tonic} levels tended to increase in small and medium scallops after stimulation, whereas the changes were insignificant in large scallops. The t_{tonic} values increased significantly, with extremely significant increases in both small and large scallops, and the duration of stimulation for large scallops was almost twice that in the natural state (**Figure 5-C**). The closure frequency increased significantly for small and medium shellfish, and it was twice that in large scallops (**Figure 5-D**), where the change in large scallops was insignificant. **Figure 5-E** shows that after stimulation, the P_{30s} values were relatively large for small scallops, but there were no significant differences between medium and large scallops.

Table 4. Number of predation items in experiment II

time group		Number of predation items			
		24h	48h	72h	total
control group	II -LsB0	–	–	1	1
	II -LmB0	–	–	–	–
	II -LlB0	–	–	–	–
Small size	II -sLsB	–	–	–	–
	II -sLmB	–	–	–	–
	II -sLlB	–	–	–	–
Middle size	II -mLsB	1	1	1	3
	II -mLmB	1	–	–	1
	II -mLlB	–	–	–	–
Big size	II -lLsB	2	1	1	4
	II -lLmB	–	1	–	1
	II -lLlB	–	–	1	1

Note: "L" denotes *N. cumingii* and "L" denotes *M.yessoensis*. The lowercase letters "s," "m," and "l" denote small, medium, and large groups, respectively. The subscript "0" denotes the control group (no *N. cumingii*).

Figure 3. Movement behaviors of *M. yessoensis* in experiment II.

CHANGES IN ENZYME ACTIVITIES IN TISSUES

Figure 6 shows changes in the enzyme activities (SOD, CAT, ODH, AK) in the gills, adductor muscle, and mantle of scallops under continuous stimulation by *N. cumingii*. The enzyme activities varied in different scallop tissues compared with those under no stimulation, with significant differences in the SOD activity in gill tissues and the SOD, CAT, and AK activities in the mantle (p < 0.05). The activities of the four enzymes changed significantly or extremely significantly in the adductor muscle under stimulation (p < 0.01).

Figure 7 shows the enzyme activities in the adductor muscles of scallops of various sizes. The ODH activities in the adductor muscles of largescale scallops after stimulation were 44.46 ± 0.69 U/mL in the control group and 37.53 ± 1.36 U/mL in the experimental group, and the difference was significant compared with the control group (p < 0.01). After stimulation, extremely significant differences (p < 0.01) were found in the activities of the four enzymes in the adductor muscles of medium-sized scallops. The activ-

ities of SOD, CAT, ODH, and AK in the control group were 36.82 ± 1.24 IU/mL, 8.46 ± 0.08 U/mL, 38.57 ± 0.28 U/mL, and 176.87 ± 2.89 U/L, respectively. The activities of SOD, CAT, ODH, and AK in the experimental group were 39.99 ± 0.80 IU/mL, 7.69 ± 0.32 U/mL, 43.38 ± 1.14 U/mL, and 158.82 ± 4.50 U/L, respectively. No significant differences were found in the CAT activities in small scallops. Still, significant differences were found in the SOD enzyme activities (41.28 ± 1.30 and 38.45 ± 1.11 IU/mL in the control group and experimental group, respectively), and extremely significant differences in the ODH and AK enzyme activities, where the activities in the control group and experimental group were 37.27 ± 0.37 and 42.73 ± 1.08 U/mL, and 187.92 ± 1.78 and 175.62 ± 1.70 U/L, respectively.

TRANSCRIPTOME RESULTS

The total RNA concentration, integrity, and purity were detected in the scallop samples. The RNA concentrations in mBJs and L-mBJs (L-mBJ1, L-mBJ2, and L-mBJ3) in the closed shell muscle tissues ranged from 61.000 ng/mL to 166.0000 ng/mL, and the total RNA contents ranged from 1.9520 mg to 5.8100 mg. An RIN value above 6.0 indicates that the sample quality met the quality requirements for library construction and sequencing. The total amounts satisfied the requirements for library construction in this study.

In total, 58.41–72.95 × 106 raw reads were obtained by transcriptome sequencing. After removing the connectors and low-quality reads, 55.62–71.19 × 106 clean reads were obtained, with a data volume of 56.63 G. The GC content ranged from 41.67% to 43.67%, and Q20 and Q30 exceeded 95.30% and 88.27% respectively (**Table 5**). Thus, the reliability of the sample data was high and they could be used for subsequent analyses.

The correlation coefficients were calculated between and within groups of samples based on the FPKM values for all
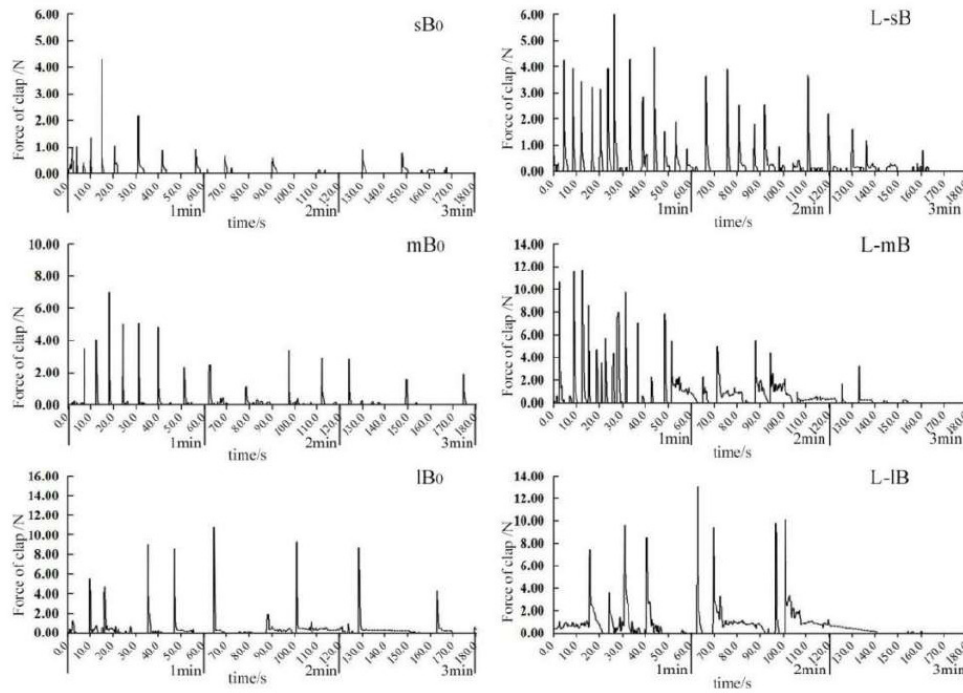
Figure 4. Typical forces recorded during shell closure responses by *M. yessoensis* with different sizes. sB_0: control group (small scallop), mB_0: control group (medium scallop), lB_0: control group (large scallop). L-sB_0: experimental group (small scallop), L-mB_0: experimental group (medium scallop), L-lB_0: experimental group (large scallop).
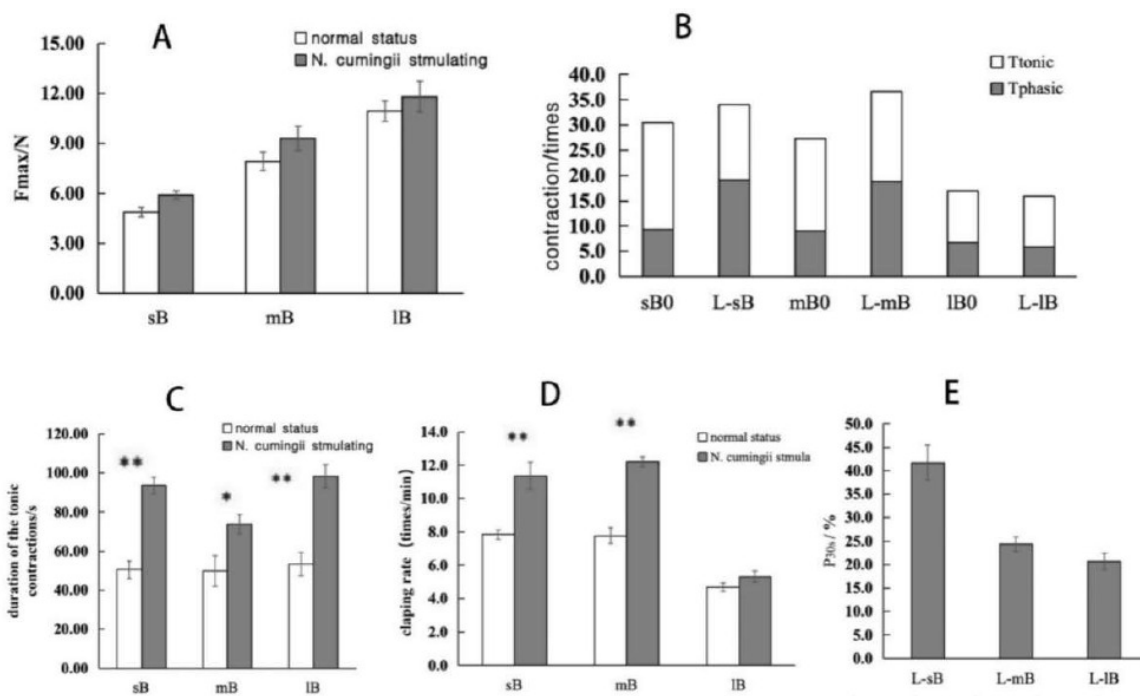

Figure 5. Escape response parameters related to phasic contraction.

Note: A, Maximum value of force. B, Contraction time. C, Duration of tonic contractions. D, Shell closure frequency. E, Percentage of first 30 s with total closed shells. "*", $p < 0.05$, "**", $p < 0.01$; n = 15, means ± standard deviations are shown

genes in each sample to draw a heat map illustrating the differences and similarities between samples. A correlation coefficient closer to 1 indicated higher similarity of the expression patterns between samples. According to the heat map, differences were found between the mBJ and L-mBJ groups (**Figure 8**, left). The RNAseq data obtained for the L-mBJ and mBJ groups were screened (DESeq2 p-value £ 0.05 log2FoldChange | ³ 0.0) and compared to obtain the distrib-
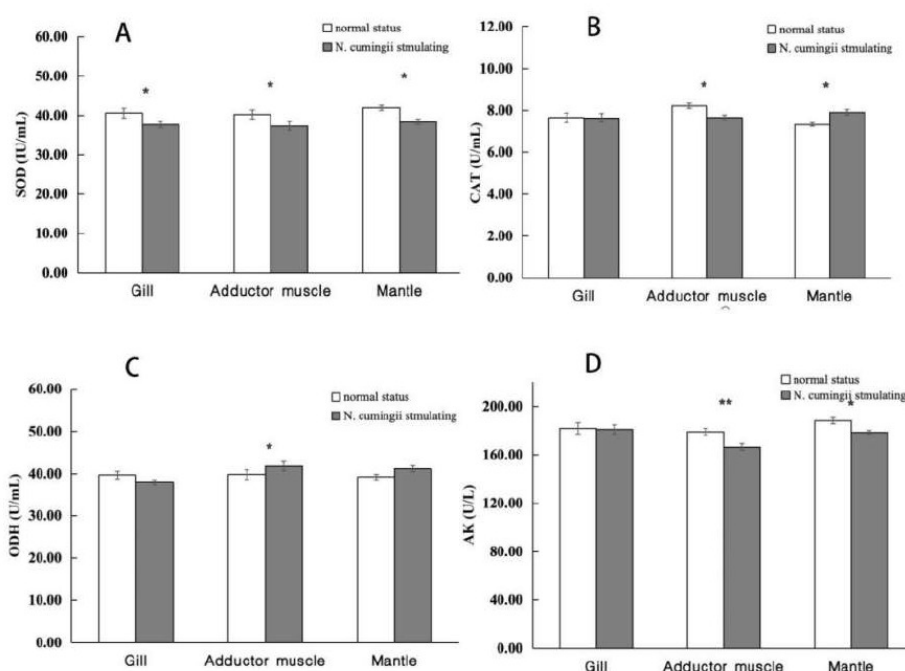
Figure 6. Comparison of enzyme expression levels in tissues of *M.yessoensis*.

Note: A, Superoxide dismutase activity. B, Catalase activity. C, Octopine dehydrogenase activity. D, Arginine kinase activity. "*", $p < 0.05$, "**", $p < 0.01$, and the same in other Figures; n = 9, means ± standard deviations are shown.
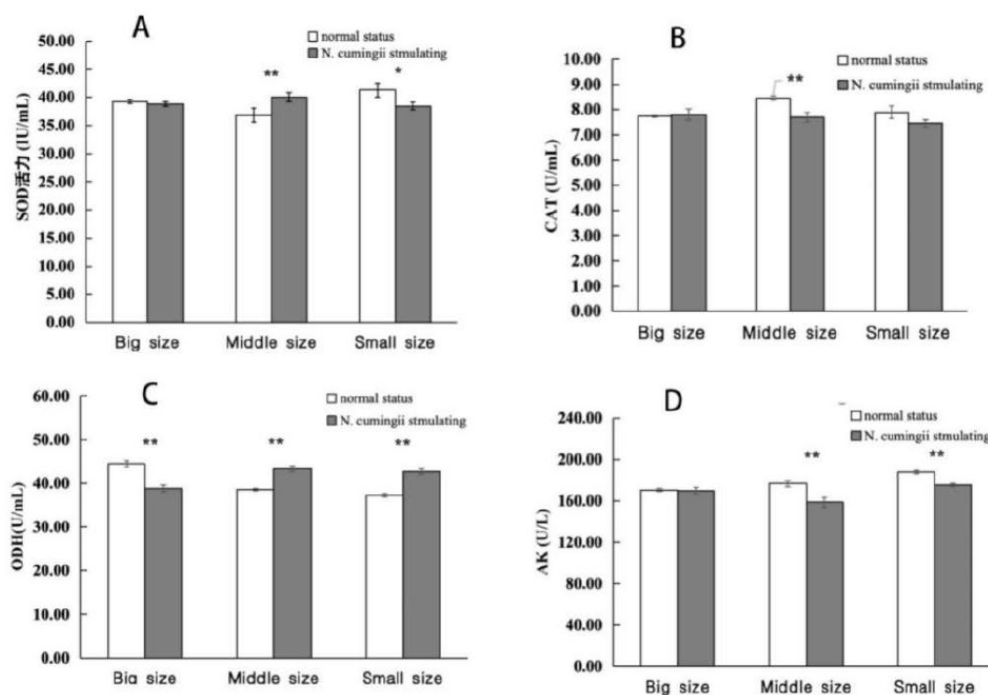

Figure 7. Comparison of enzyme expression levels in muscles of *M.yessoensis*; n = 3, means ± standard deviations are shown.

ution of DEGs in the adductor muscle of scallops after stimulation by *N. cumingii* (**Figure 8**, right). The results in the volcano plot in **Figure 8** show that 172 DEGs (red) were upregulated and 233 DEGs (green) were downregulated.

The GO database was used to classify DEGs, and 90, 50, and 149 DEGs were annotated as BP, CC, and MF processes.

The top 10 GO entries with enriched DEGs in each process are shown in **Figure 9**. Among BP processes, DEGs were mainly enriched in stimulating cell response to stimuli, signal transduction, and cell communication. Among CC processes, DEGs were mainly enriched in the cytoplasm, intracellular membrane-bound organelles, membrane-bound

Table 5. Summary of sample sequencing data quality

Sample	Raw reads	Raw bases	Clean reads	Clean bases	Q20 (%)	Q30 (%)	GC pct (%)
mBJ1	61741598	9.26G	60617904	9.09G	95.30	88.27	41.67
mBJ2	61358082	9.20G	58772844	8.82G	96.51	90.93	42.25
mBJ3	58408872	8.76G	55624608	8.34G	96.69	91.36	43.67
L-mBJ1	61061022	9.16G	58200216	8.73G	96.77	91.52	42.62
L-mBJ2	62048232	9.31G	59960498	8.99G	96.90	91.76	42.41
L-mBJ3	72953248	10.94G	71194850	10.68G	95.56	88.74	43.31

Notes: Sample: Sample name; Raw reads: number of reads in the raw data; Raw bases: number of bases in the raw data; Clean reads: number of filtered reads; Clean bases: number of filtered bases; Q20 and Q30: percentage of bases > 20 and 30, respectively; GC pct: percentage of G and C relative to four bases in clean reads.
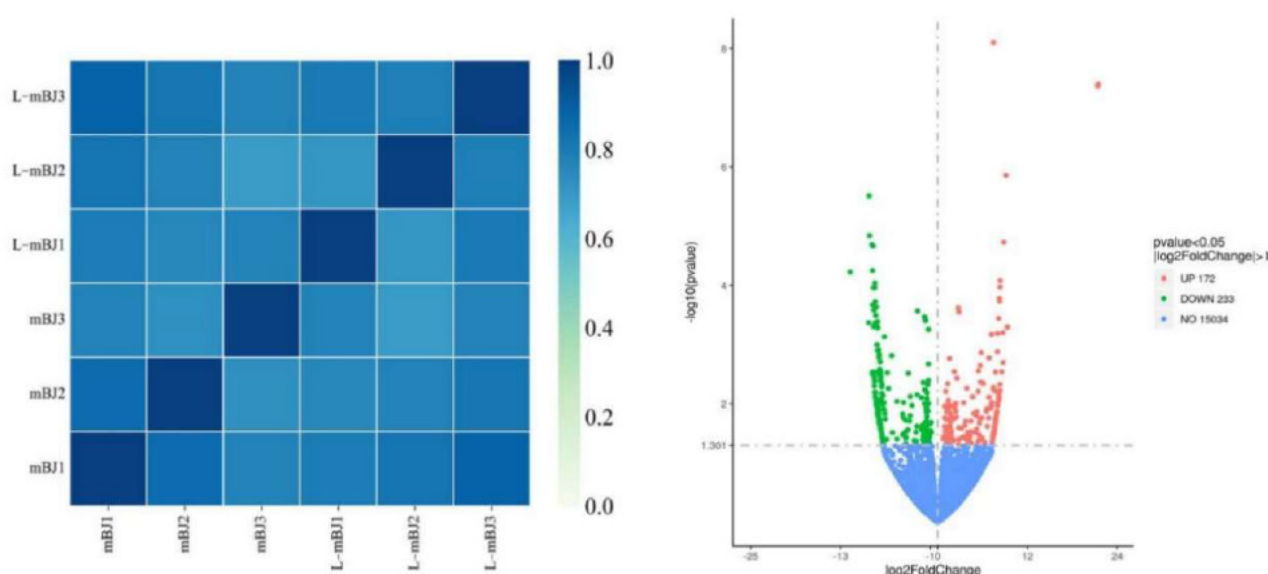
Figure 8. Heat map and volcano plot.

organelles, and protein-containing complexes. Among MF processes, DEGs were mainly enriched in oxidoreductase and transporter activity, transition metal ion binding, and other processes.

KEGG analysis was applied to understand further the biological functions of DEGs stimulated by *N. cumingii* in scallops. The results showed that 76 DEGs were enriched in 71 KEGG pathways, and the 20 most abundant pathways are shown in (**Figure 10**). In particular, the Wnt signaling pathway, Arachidonic acid metabolism, and Glycosphingolipid biosynthesis ganglion series. Other glycan degradation, Neuroactive ligand-receptor interaction, Drug metabolism cytochrome P450, and six other pathways were significantly enriched ($p < 0.05$).

Nine DEGs were subjected to qRT-PCR validation analysis, where the selected genes comprised C1QL4, OTOF, ZC-CHC8, RAD17, TRXL, CYP2C8, C25B8.10, β-GLB, and PROM1A. The expression patterns were consistent with the RNA-Seq results, and the high correlation ($R2 = 0.71$) confirmed the reliability of the transcriptome analysis results (**Figure 11**).

DISCUSSION

EFFECTS OF N. CUMINGII PREDATION ON THE CLOSED-SHELL FORCE OF SCALLOPS

The shell opening and closing behavior and mantle state of a bivalve can visually reflect the physiological condition of the shellfish, which is an effective behavioral indicator of the organism's response to environmental changes. For example, a scallop can produce slow or fast movements to move away from predators through the contraction or diastole of the adductor muscles.[7] In this study, the F_{max} of large, medium, and small scallops was measured using a dynamometer, and the T_{phasic}, T_{tonic}, F_{max}, and frequency of shell closure data were collected and analyzed. We found that the Fmax of scallops both with and without the presence of the predator increased with increasing scallop size and that the Fmax of scallops in the same size group produced a greater shell closure force in the presence of *N. cumingii*, Zhang et al reported similar results.[4] We found that scallops would jump or swim away from potential predators. However, we tested the effects of different sizes of *N. cumingii* on *M.yessoensis*. We found that the number of jumps made by scallops increased significantly with in-
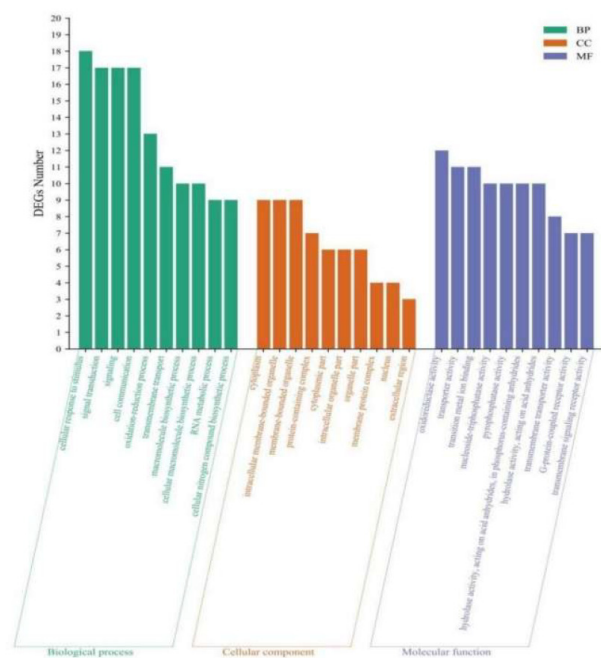
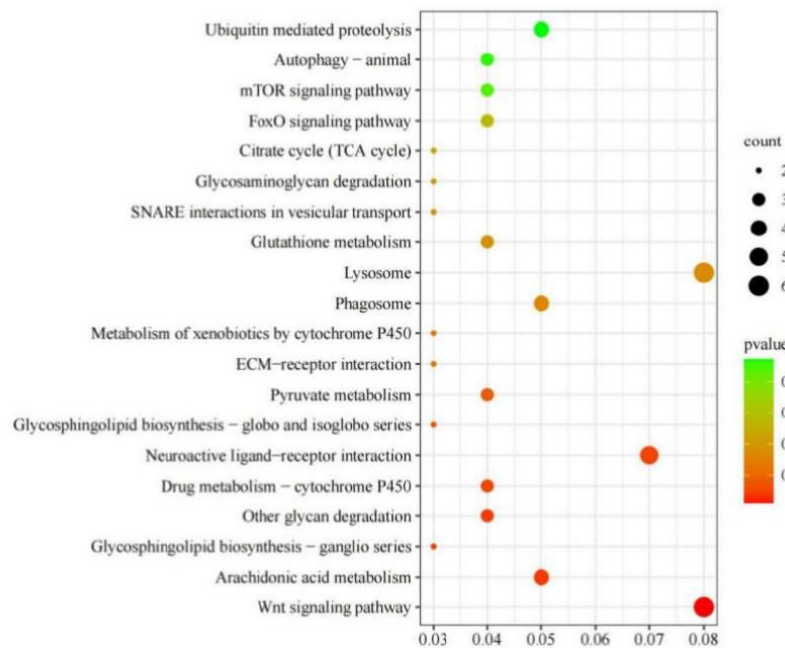**Figure 9. GO annotations for DEGs.**

Figure 10. KEGG bubble diagram.

creasing predator size, although increased swimming behavior was not obvious. Based on all of these findings, we speculate that scallops first choose the bitemporal contraction response when stimulated by predators (i.e., shells open and close quickly to produce jumping behaviors to escape from being eaten).

Scallops have two strategies to cope with predators: either they close their shells tightly or they jump quickly to escape. A thick, hard shell protects predators, but con-

versely, a heavy shell reduces the scallop's ability to swim. Therefore, there is a trade-off between mechanical protection and swimming ability when scallops face threats from predators, and their escape behavior in the face of predatory stimuli may change over time during individual development. We found that the T_{phasic} and T_{tonic} values of *N. cumingii*-stimulated scallops were higher than those of the control group for both small- and medium-sized scallops. Additionally, the frequency of shell closure increased sig-
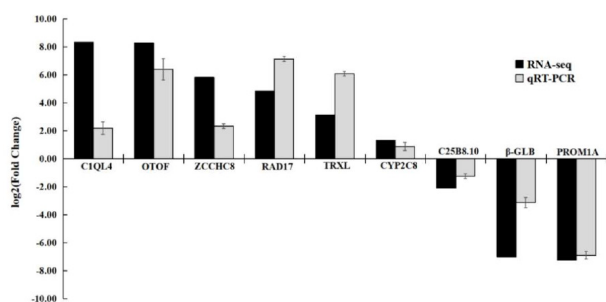
Figure 11. Comparison of gene expression levels according to RNA-Seq and qRT-PCR.

nificantly upon stimulation, and small-sized scallops closed their shells frequently in the first 30s after stimulation. In contrast, no significant changes in large-sized scallops' T_{phasic} and T_{tonic} values were detected after stimulation. The duration of shell closure in this experimental group was more than twice as long as that of the control group, and the increase in shell closure frequency was insignificant. These results suggest that small- and medium-sized scallops opted for locomotor behavior to escape from predators, whereas large-sized scallops preferentially used shell closure to escape from predators.

As scallops grow, the shell thickens and the ability to cope with predator attacks improves, but swimming ability may decline (Xia 2021). Changes in swimming behavior and ability with age and size vary among species. The locomotor ability (number of shell closures and magnitude of shell closure force) of all sizes of scallops tends to decrease with age. Tremblay et al. compared the swimming behavior of Amusium ballot, *Placopecten magellanicus*, Pecten format*s*, *Mimachlamys asperrima*, and *Crassadoma gigantea* and found that some scallops could avoid predation by staying still due to the advantage of their shell shape. In contrast, the more active scallops avoided predators by swimming.[8] In a study of *Aequipecten opercularis*, shell closure during the first and second escape responses was more frequent in smaller individuals than in larger ones.[9] This is consistent with our study of *M.yessoensis*. Therefore, we hypothesized that smaller-sized *M. yessoensis* were more active and avoided predation by *N. cumingii* through rapid shell closure. In contrast, larger scallops avoided predators by decreasing the frequency and increasing the duration of shell closure.

EFFECTS OF N. CUMINGII PREDATION ON THE ENZYME ACTIVITIES OF SCALLOPS

Enzyme activity is the fundamental driver of all chemical changes in living organisms, so the regulation of enzyme activity is one of the most important ways to realize the regulation of biological metabolism. The immunity of scallops is affected by exposure to stressors, including predators. SOD and CAT are two important immunoenzymes in the shellfish immune system, and they play an important role in maintaining the balance of the antioxidant system in shellfish (Gao 2016; Liu et al.[10]). CAT and SOD scavenge

and balance intracellular reactive oxygen radicals; thus they are important indicators of the immune defense ability of shellfish. SOD is also involved in defense against aging and biomolecular damage.[11]

In this study, we compared the SOD, CAT, ODH, and AK activities in the adductor muscles of control scallops and scallops exposed to *N. cumingii*. The SOD and CAT activities of different sized scallops were affected *N. cumingii* stimulation. Generally, stronger CAT and peroxidase activities are related to greater resistance and ability to eliminate free radicals. When the living environment of organisms changes, thereby putting them under stress, the activities of these two enzymes undergo changes to allow the organism to adapt to the new environment.[12] In our study, the CAT activity decreased most obviously in medium-sized scallops. In contrast, the SOD enzyme activity of medium-sized scallops increased significantly. We speculate that CAT was preferentially involved in the scavenging of reactive oxygen radicals when scallops perceived the threat of predation and that the regulatory stress experienced by the scallops was too high. However, this theory requires further study.

Putative ODHs (OpDHs) play an important role in the anaerobic metabolism of marine invertebrates, especially shellfish. The production of octopus enzyme by ODH is analogous to the production of lactic acid by lactate dehydrogenase in vertebrate muscles. In their studies, the elevated ODH levels correlated with an increase in swimming ability, as adenosine triphosphate (ATP) production in this activity was supported by hydrolysis of phosphoarginine, which was subsequently followed by the continuation of oxidation through ODH-catalyzed NADH and L-arginine to produce octopus alkali, NAD+, and water to ensure the intracellular redox balance.[13,14]

Our analysis of the adductor muscle activity of scallops revealed a highly significant difference in the ODH activity between control and *N. cumingii*-stimulated scallops of all three sizes. In particular, we detected a highly significant increase in the ODH activity of medium- and small-sized scallops in the groups exposed to predation. Therefore, we hypothesized that the up-regulation of ODH synthesis may be related to acute stress or sudden swimming activity in *M. yessoensis*. Strahl et al also showed that the escape and swimming behaviors produced by bivalves in response to a predator are supported by the trans-phosphorylation of phospho-L-arginine and by anaerobic glycolysis to obtain ATP to support this intense muscle activity.[15] During the escape response or subsequent recovery, bivalves restore phospho-L-arginine via anaerobic glycolysis, producing octopine. Octopine plays different roles in different tissues, and its level was higher in swimming scallops. The adductor muscle mass and ODH content *Argopecten ventricosus*, which are chronically exposed to predators, are high,[16] which is consistent with the results of our study of *M.yessoensis*.

AK was first isolated from *N. cumingii* muscle in 1935, and it is widely found in lower and higher invertebrates. AK is one of the most important enzymes regulating energy metabolism in invertebrates, and it plays a role in the re-

versible transfer of phosphate groups between ATP via the enzyme-specific guanidinium receptor to keep ATP at relatively stable levels.[17] It also can be directly or indirectly involved in related immune responses.[18] AKs may be involved in maintaining normal life activities, in an organism's defense against adverse external environments, and when an organism is subjected to environmental threats that result in locomotor behaviors.[19] Our study found that AK activity in different tissues of scallops of all sizes was down-regulated after *N. cumingii* stimulation. AK expression in the adductor muscles of medium- and small-sized scallops was significantly down-regulated, indicating that scallops experience muscle stress and undergo accelerated energy metabolism when is exposed to predators. This result suggests that AK plays an important role in the muscle movements of stressed scallops. Voncken et al. reported similar findings.[20] Predation stress causes scallops to produce explosive locomotor behavior, which requires much energy. Smits et al. (Smits et al. 2010) demonstrated that AK plays a role in vigorous muscle movements after analyzing the enzyme activity of scallop adductor muscles following escape or locomotor behavior. The decrease in AK expression under predation stress also reflects the increase in stress-related energy demand, and the elevated AK content accelerates the organism's energy metabolism. This process provides energy for the organism to cope with predation stress, which is conducive to the scallop's swimming ability and survival under predator attack.

EFFECTS OF N. CUMINGII PREDATION ON SCALLOP TRANSCRIPTOME

In this study, analysis of transcriptome sequencing data showed that scallops exhibited strong gene expression responses when stimulated by *N. cumingii*, where 405 significant DEGs were identified. These DEGs indicated that the predation stress on the scallops caused by *N. cumingii* stimulated a series of physiological activities, such as the significant upregulation of C1QL4. C1QL4 is a member of the C1q/TNF superfamily, and the presence of spherical C1q domains is a hallmark of the C1q/TNF superfamily.[21, 22] Members of the C1q family are involved in various important physiological functions, such as the innate immune response, insulin metabolism,[23] and synaptic the classical activation pathway. C1q can bind to antibodies in antigen–antibody complexes and activate C1r and C1s. Therefore, C1q is an important bridge connecting acquired immunity and innate immunity. C1q like protein has been found in animals such as lampreys, amphioxus, sea squirts, and sea urchins. In addition to playing a role in classic activation pathways, C1q participates in many immune processes,[24] such as clearing apoptotic cells to maintain immune tolerance,[25] B cells,[26] and T cells,[27] C1q also plays a role in development,[28] wound healing,[29] and other processes. A study of rodents found that the expression of C1QL4 was regulated by its developmental status and hormonal factors, and C1QL4 could activate steroids to produce acute reactions.[30] In the present study, C1QL4 was significantly upregulated in scallops after predation stimulation, suggesting that the immune system was affected

under predatory stress from *N. cumingii*. Predation stress could have caused changes in energy metabolism and cellular functions in scallops, and C1QL4 could have been involved in the response of scallops to predatory stimuli.

Transcriptome sequencing for the scallop *M. yessoensis* under stimulation by predation also indicated that significant changes occurred in the expression levels of genes such as glutathione S-transferase (GST), 15-hydroxyprostaglandin dehydrogenase (PGDH), and FMRFaR in the arachidonic acid pathway. In particular, in the arachidonic acid pathway, the GST and PGDH-related genes were significantly upregulated and downregulated, respectively. GST has functions in clearing lipid free radicals and blocking lipid peroxidation.[31] In addition, GST can promote the conversion of leukotrienes and prostaglandins. In previous studies, the oxidative stress caused by *Corbicula javanicus* and *Mytilus galloprovincialis* under different conditions led to increases in the GST enzyme activity.[32-34] In the present study, downregulation of the GST gene may have affected the contents of prostaglandins, thereby influencing oxidative stress in scallops. In summary, we suggest that under continuous stimulation by *N. cumingii*, oxidative stress affected scallops, and the upregulation of GST played a role in clearing free radicals from the body. In addition, GST affects the conversion of prostaglandins, and the downregulation of PGDH indicates that oxidative stress reactions were initiated in scallops. Neuropeptides are involved in neuroregulation, neural transduction, and hormone function.[35] Some neuropeptides have been shown to have strong inhibitory effects on muscle contraction in mollusks such as *Meretrix lusoria* and *Aplysia kurodai*.[36] FMRFaR is a neuropeptide, and it was also significantly upregulated in these scallops, which may have been related to the strong contraction of the adductor muscle when scallops avoided enemy attacks. In addition, studies have shown that FMRFamide is involved in energy metabolism and the tension and amplitude of rectal contraction in Crassostrea gigas and Sepia officinalis,[37] which are similar to our results. Prolactin-releasing peptide receptor (PrRP receptor) is a mediator of stress responses, and a study of rats showed that PrRP may regulate nociception through co-expression of its receptor.[38] The differential expression of genes related to the neural active ligand-receptor interaction signaling pathway in scallops under the predatory threat of N. cumingii may help to sense external threats but also lead to a stress response when danger is perceived to regulate stress levels.

This study's environmental conditions, such as water flow rate, temperature, and dissolved oxygen, are relatively stable as in a controlled laboratory environment, and the research results are ideal. However, under natural conditions, the changes in water flow, temperature, and dissolved oxygen may not be regular, which may be different from the results obtained in the laboratory. In our opinion, the results obtained from this study can still provide some technical reference for the bottom sowing and proliferation of scallops, especially when the size of the scallops exceeds 60mm. Their ability to avoid predators will be significantly

enhanced, and the recovery rate of scallops will be improved, so as to improve the breeding benefits.

CONCLUSION

In summary, physiological responses occurred when scallops responded to predatory stimulation by *N. cumingii*, with slightly different response modes by scallops of different sizes after stimulation. The shell closure force was generally greater when the scallops were larger. Escape from predation by *N. cumingii* involved shifting from multiple, rapid opening and closing of the double shells to extending the closure time. In addition, physiological activities such as enzyme activity levels, material metabolism, and cell proliferation in various body tissues responded to predatory stress through self-regulation.

AUTHORS' CONTRIBUTION

Conceptualization: Yang Liu (Lead). Writing – original draft: Yang Liu (Lead). Data curation: Danyang Li (Lead). Formal Analysis: Ying Tian (Lead). Investigation: Ying Tian (Lead). Methodology: Junxia Mao (Lead). Supervision: Xubo Wang (Lead). Writing – review & editing: Zhenlin Hao (Lead). Funding acquisition: Zhenlin Hao (Lead). Resources: Zhenlin Hao (Lead).

COMPETING OF INTEREST

The authors declare that they have no conflicts of interest.

ETHICAL CONDUCT APPROVAL – IACUC

All animal handling followed the guidelines for the care and use of animals for scientific purposes established by the Institutional Animal Care and Use Committee (IACUC) of Dalian Ocean University, China.

INFORMED CONSENT STATEMENT

All authors and institutions have confirmed this manuscript for publication.

DATA AVAILABILITY STATEMENT

The data supporting this study's findings are available from the corresponding author upon reasonable request.

REFERENCES

1. Cong HH, Gen WH, Zhu JW, et al. Patinopecten yessoensis Mantle Polypeptides Enhanced the Structural Stability of Myofibrillar Proteins from Silver Carp. *Meat Research*. 2022;36(09):13-19. doi:10.7506/rlyj1001-8123-20220428-048

2. Wang Y, Zhou L. Bottom sowing of proliferation of Patinopecten yessoensis yield research; case in Zhangzidao. *Chinese Fisheries Economics*. 2014;32(01):104-109. doi:10.3969/j.issn.1009-590X.2014.01.017

3. Gao ZK. *Effects of Environmental Stresses on Physiological, Immunological Parameters and Behavioral Characteristics of Patinopecten Yessoensis*. Shanghai Ocean University; 2016.

4. Zhang JH, Xia YY, Gao ZK, Liu Y, Wu WG, Zhang ZX. Force production during shell clap of scallop Pationopecten yessoensis and its response to predator starfish. *Journal of Fishery Sciences of China*. 2021;28(07):871-877. doi:10.12264/JFSC2020-0510

5. Xia YY, Zhang JH, Liu Y. Behavioral characteristics and physiological responses to hypoxic stress in Patinopecten yessoensis. *Journal of Fishery Sciences of China*. 2021;28(10):1319-1328. doi:10.12264/JFSC2021-0170

6. Duan LZ, Huang XY, Hu WB, et al. Comparison of Three Coelomic Fluid Enzymes Activities in Scallop Patinopecten yesoensis. *Chinese Agricultural Science Bulletin*. 2015;31(02):112-117.

7. Schalkhausser B, Bock C, Pörtner HO, Lannig G. Escape performance of temperate king scallop, Pecten maximus under ocean warming and acidification. *Marine Biology*. Published online 2014:2819-2829. doi:10.1007/s00227-014-2548-x

8. Tremblay I, Guderley HE, Himmelman JH. Swimming away or clamming up: The use of phasic and tonic adductor muscles during escape responses varies with shell morphology in scallops. *The Journal of Experimental Biology*. 2012;215(Pt23):4131-4143. doi:10.1242/jeb.075986

9. Schmidt M, Philipp EER, Abele D. Size and age-dependent changes of escape response to predator attack in the Queen scallop Aequipecten opercularis. *Marine Biology Research*. 2008;4(6):442-450. doi:10.1080/17451000802270346

10. Liu ZH, Mou HJ, Wang QY. Research progress of immune related enzymes in Mollusca. 2003;024(003):86-90. doi:10.3969/j.issn.1000-7075.2003.03.016

11. Yao CL, Wang WN, Wang AL. Progess of studies on superoxide dismutase in the body of aquatic animals. *Marine Sciences*. 2003;27(10):18-21. doi:10.3969/j.issn.1000-3096.2003.10.005

12. Ding RX, Huang XM, Zhao W, et al. Effects of pH acute stress on the behavior and immune enzyme activity of Babylonia Areolata. *Fishery modernization*. 2022;49(6):84-90. doi:10.3969/j.issn.1007-9580.2022.06.011

13. Zheng Y. *Octopine Dehydrogenase in the Adductor Muscle of Live Scallop and Its Changes during Postharves*. Dalian Ocean University; 2018.

14. Smits SHJ, Meyer T, Mueller A, et al. Insights into the Mechanism of Ligand Binding to Octopine Dehydrogenase from Pecten maximus by NMR and Crystallography. *PLoS ONE*. 2010;5(8):e12312. doi:10.1371/journal.pone.0012312

15. Strahl J, Dringen R, Schmidt MM, Hardenberg S, Abele D. Metabolic and physiological responses in tissues of the long-lived bivalve Arctica islandica to oxygen deficiency. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*. 2011;158(4):513-519. doi:10.1016/j.cbpa.2010.12.015

16. Guerra C, Zenteno-Savín T, Maeda-Martínez AN, Abele D, Philipp EER. The effect of predator exposure and reproduction on oxidative stress parameters in the Catarina scallop Argopecten ventricosus. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*. 2013;165(1):89-96. doi:10.1016/j.cbpa.2013.02.006

17. Morris S, Van Aardt WJ, Ahern MD. The effect of lead on the metabolic and energetic status of the Yabby, Cherax destructor, during environmental hypoxia. *Aquatic toxicology*. 2005;75(1):16-31. doi:10.1016/j.aquatox.2005.07.001

18. Yao CL, Wang ZY, Xiang JH. Structure and Function of Arginine Kinase in Crustacean. *Chinese Journal of Biochemistry and Molecular Biology*. 2008;24(3):203-208. doi:10.3969/j.issn.1007-7626.2008.03.004

19. Huang LN, Liu N, Zhao J, Ma J, Liu XN. The Research Prospect of Arginine Kinase. *Life Science Research*. 2015;19(05):452-456. doi:10.16605/j.cnki.1007-7847.2015.05.006

20. Voncken F, Gao F, Wadforth C, Harley M, Colasante C. The phosphoarginine energy-buffering system of Trypanosoma brucei involves multiple arginine kinase isoforms with different subcellular locations. *PLoS ONE*. 2013;8(6):e65908. doi:10.1371/journal.pone.0065908

21. Matsuda K, Yuzaki M. Cbln family proteins promote synapse formation by regulating distinct neurexin signaling pathways in various brain regions. *European Journal of Neuroscience*. Published online 2011:1447-1461. doi:10.1111/j.1460-9568.2011.07638.x

22. Uemura T, Lee SJ, Yasumura M, et al. Trans-synaptic interaction of GluRdelta2 and Neurexin through Cbln1 mediates synapse formation in the cerebellum. *Cell*. 2010;141(6):1068-1079. doi:10.1016/j.cell.2010.04.035

23. Yamauchi T, Kamon J, Waki H, et al. Globular Adiponectin Protected ob/ob Mice from Diabetes and ApoE-deficient Mice from Atherosclerosis. *Journal of Biological Chemistry*. Published online 2003:2461-2468. doi:10.1074/jbc.m209033200

24. Nayak A, Ferluga J, Tsolaki AG, Kishore U. The non-classical functions of the classical complement pathway recognition subcomponent C1q. *Immunology Letters*. 2010;131(2):139-150. doi:10.1016/j.imlet.2010.03.012

25. Nauta AJ, Trouw LA, Daha MR, et al. Direct binding of C1q to apoptotic cells and cell blebs induces complement activation. *European Journal of Immunology*. 2002;32(6):1726. https://doi.org/10.1002/1521-4141(200206)32:6%3C1726::aid-immu1726%3E3.0.co;2-r

26. Ferry H, Potte PK, Crockford TL, et al. Increased Positive Selection of B1 Cells and Reduced B Cell Tolerance to Intracellular Antigens in c1q-Deficient Mice. *The Journal of Immunology*. 2007;178(5):2916-2922. doi:10.4049/jimmunol.178.5.2916

27. Kobayashi H, Hirashima Y, Terao T. Human myometrial cells in culture express specific binding sites for urinary trypsin inhibitor. *Molecular Human Reproduction*. 2000;6(8):735-742. doi:10.1093/molehr/6.8.735

28. Naito NAT, Sumida T, Nomura S, et al. Complement C1q Activates Canonical Wnt Signaling and Promotes Aging-Related Phenotypes. *Cell*. Published online 2012:1298-1313. doi:10.1016/j.cell.2012.03.047

29. Bossi F, Tripodo C, Rizzi L, et al. C1q as a unique player in angiogenesis with therapeutic implication in wound healing. *Proceedings of the National Academy of Sciences*. Published online 2014:4209-4214. doi:10.1073/pnas.1311968111

30. Tan A, Ke S, Chen Y, et al. Expression patterns of C1ql4 and its cell-adhesion GPCR Bai3 in the murine testis and functional roles in steroidogenesis. *The FASEB Journal*. Published online 2019:4893-4906. doi:10.1096/fj.201801620rr

31. Zou QQ, Wang JH, Guo HJ, Sheng GR, Hong YJ. Expression of Glutathione S-Transferase Gene in Hyriopsis schlegerlii and H. cumingii. *Journal Of Zoology*. 2010;45(04):96-101. doi:10.13859/j.cjz.2010.04.025

32. Esterhuizen M, Buchenhorst L, Kim YJ, Pflugmacher S. In vivo oxidative stress responses of the freshwater basket clam Corbicula javanicus to microplastic fibres and particles. *Chemosphere*. Published online 2022:134037. doi:10.1016/j.chemosphere.2022.134037

33. Giannetto A, Maisano M, Cappello T, et al. Effects of Oxygen Availability on Oxidative Stress Biomarkers in the Mediterranean Mussel Mytilus galloprovincialis. *Marine Biotechnology*. Published online 2017:614-626. doi:10.1007/s10126-017-9780-6

34. Magara G, Elia AC, Dörr AJM, et al. Metal load and oxidative stress driven by organotin compounds on rainbow trout. *Environmental Science and Pollution Research*. Published online 2021:35012-35022. doi:10.1007/s11356-021-12984-w

35. Liu F, Baggerman G, Schoofs L, Wets G. The construction of a bioactive peptide database in Metazoa. *Journal of Proteome Research*. Published online 2008:4119-4131. doi:10.1021/pr800037n

36. Fujisawa Y et al. Te Aplysia mytilus inhibitory peptide-related peptides: identifcation, cloning, processing, distribution, and action. *J Neurosci*. 1999;19:9618-9634. doi:10.1523/JNEUROSCI.19-21-09618.1999. PMID:10531464

37. Bigot L, Beets I, Dubos MP, Boudry P, Schoofs L, Favrel P. Functional characterization of a short neuropeptide F-related receptor in a lophotrochozoan, the mollusk Crassostrea gigas. *Journal of Experimental Biology*. Published online 2014. doi:10.1242/jeb.104067

38. Lin SHS, Leslie FM, Civelli O. Neurochemical properties of the prolactin releasing peptide (PrRP) receptor expressing neurons: evidence for a role of PrRP as a regulator of stress and nociception. *Brain Research*. Published online 2002:15-30. doi:10.1016/s0006-8993(02)03183-9