NOTE: Please save this file locally before filling in the table, DO NOT work on the file within your internet browser as changes will not be saved. Adobe Acrobat Reader (available free here) is recommended for completion.

The ARRIVE guidelines 2.0: author checklist

The ARRIVE Essential 10

These items are the basic minimum to include in a manuscript. Without this information, readers and reviewers cannot assess the reliability of the findings.

Item		Recommendation	Section/line number, or reason for not reporting
Study design	1	For each experiment, provide brief details of study design including: a. The groups being compared, including control groups. If no control group has been used, the rationale should be stated. b. The experimental unit (e.g. a single animal, litter, or cage of animals).	Section 2.1.1, lines 120–136; Section 2.1.2, lines 138–154; dual-control rationale lines 155–162. Groups compared: 0 mg/L control and six SSC treatments (276, 286, 296, 326, 376, 426 mg/L) for transcriptomics; the physiological experiment used 276 mg/L as control. Control groups were included, and the rationale for dual controls is stated in lines Experimental unit: replicate 60-L aquarium (n = 3 per treatment), each containing 40 clams (lines 131–136, 145–154). For molecular analyses, the sampling unit was individual clam tissue, with 3 biological replicates per
Sample size	2	a. Specify the exact number of experimental units allocated to each group, and the total number in each experiment. Also indicate the total number of animals used. b. Explain how the sample size was decided. Provide details of any *a priori* sample size calculation, if done.	Section 2.1.1, lines 131–136; Section 2.1.2, lines 145–154; Section 2.2, lines 167–169. Each treatment had 3 replicate 60-L aquaria with 40 clams each. Transcriptomic experiment: 7 treatments × 3 aquaria × 40 clams = 840 clams No a priori sample size calculation was performed. According to general scientific experimental conditions, the minimum number of scientifically meaningful replicates (n = 3 per treatment per tissue) was set, in line with the 3Rs principle to reduce animal
Inclusion and exclusion criteria	3	a. Describe any criteria used for including and excluding animals (or experimental units) during the experiment, and data points during the analysis. Specify if these criteria were established *a priori.* If no criteria were set, state this explicitly. b. For each experimental group, report any animals, experimental units or data points not included in the analysis and explain why. If there were no exclusions, state so. c. For each analysis, report the exact value of *n* in each experimental group.	no exclusions Transcriptomics: n = 3 biological replicates per treatment per tissue (Section 2.2, lines 167–169; Section 3.1.1, lines 270–279). qRT-PCR: n = 3 biological replicates per treatment, with 3 technical replicates each
Randomisation	4	a. State whether randomisation was used to allocate experimental units to control and treatment groups. If done, provide the method used to generate the randomisation sequence. b. Describe the strategy used to minimise potential confounders such as the order of treatments and measurements, or animal/cage location. If confounders were not controlled, state this explicitly.	Aquaria were randomly assigned to the control and SSC treatment groups using a random number generator Not reported.
Blinding	5	Describe who was aware of the group allocation at the different stages of the experiment (during the allocation, the conduct of the experiment, the outcome assessment, and the data analysis).	Not reported.
Outcome measures	6	a. Clearly define all outcome measures assessed (e.g. cell death, molecular markers, or behavioural changes). b. For hypothesis-testing studies, specify the primary outcome measure, i.e. the outcome measure that was used to determine the sample size.	Section 2.2–2.7, lines 163–243; Section 3.1–3.7, lines 270–630. Outcomes assessed: RNA-seq quality/assembly/annotation, DEGs, GO/KEGG enrichment, expression pattern clustering, and qRT-PCR validation of Not specified. No primary outcome measure is identified, and no outcome measure was used to determine sample size.
Statistical methods	7	a. Provide details of the statistical methods used for each analysis, including software used. b. Describe any methods used to assess whether the data met the assumptions of the statistical approach, and what was done if the assumptions were not met.	Not reported.
Experimental animals	8	a. Provide species-appropriate details of the animals used, including species, strain and substrain, sex, age or developmental stage, and, if relevant, weight. b. Provide further relevant information on the provenance of animals, health/immune status, genetic modification status, genotype, and any previous procedures.	Section 2.1.1, lines 120–128. Species: adult Meretrix meretrix. Strain/substrain: not reported. Sex: not reported. Age/developmental stage: adult. Weight: not reported. Recommend providing these details if available Section 2.1.1, lines 122–128. Provenance: intertidal clam farming area adjacent to the Tongzhou Bay pipeline construction site, Jiangsu, China. Health status: healthy and active. Genetic modification status: not
Experimental procedures	9	For each experimental group, including controls, describe the procedures in enough detail to allow others to replicate them, including: a. What was done, how it was done and what was used. b. When and how often. c. Where (including detail of any acclimatisation periods). d. Why (provide rationale for procedures).	Section 2.1.1, lines 120–136; Section 2.1.2, lines 138–154; Section 2.2, lines 163–190; Section 2.7, lines 231–243. Procedures: SSC exposure in 60-L glass aquaria with wire mesh platforms; wave pumps for sediment Section 2.1.1, lines 124–136; Section 2.1.2, lines 145–154. Timing/frequency: 7-day acclimation; feeding twice daily (09:00 and 22:00); daily seawater renewal; sediment suspension 12 h/day followed by 11 h Section 2.1.1, lines 124–128. Where: 60-L glass aquaria; acclimatisation for 7 days prior to the experiment. Section 2.1.1, lines 124–128. Where: 60-L glass aquaria; acclimatisation for 7 days prior to the experiment.
Results	10	For each experiment conducted, including independent replications, report: a. Summary/descriptive statistics for each experimental group, with a measure of variability where applicable (e.g. mean and SD, or median and range). b. If applicable, the effect size with a confidence interval.	Section 3.1–3.7, lines 270–630; Tables 1–4; Figures 1–7. DEG numbers are reported per group; qRT-PCR data are presented as mean ± SD (n = 3) in Table 4; gene expression distributions are shown in Figure 1. Effect sizes are reported as log2 fold changes (RNA-seq) and relative expression/fold changes (qRT-PCR). Confidence intervals are not reported.

The Recommended Set

These items complement the Essential 10 and add important context to the study. Reporting the items in both sets represents best practice.

Item		Recommendation	Section/line number, or reason for not reporting
Abstract	11	Provide an accurate summary of the research objectives, animal species, strain and sex, key methods, principal findings, and study conclusions.	Abstract, lines 13–31. Summarizes research o
Background	12	a. Include sufficient scientific background to understand the rationale and context for the study, and explain the experimental approach. b. Explain how the animal species and model used address the scientific objectives and, where appropriate, the relevance to human biology.	Introduction, lines 42–99 and 101–119. Provides scientific background on SSC increases, dredging/coastal construction, bivalve filter-feeding, and the need for transcriptomic study. Introduction, lines 42–64. Explains why M. meretrix is an appropriate model: commercially important coastal bivalve, obligate filter-feeder, gill directly contacts suspended particles. Relevance to human biology is not applicable; this is an
Objectives	13	Clearly describe the research question, research objectives and, where appropriate, specific hypotheses being tested.	Introduction, lines 109–119. Four specific obje
Ethical statement	14	Provide the name of the ethical review committee or equivalent that has approved the use of animals in this study, and any relevant licence or protocol numbers (if applicable). If ethical approval was not sought or granted, provide a justification.	To be completed in Institutional Review Board Statement, lines 911–913. Use: "The animal study protocol was approved by the Animal Ethics Committee of [full institution name] (protocol code [XXX] and date of approval [YYYY-MM-DD])." Please insert the exact committee name, protocol number, and approval date from your ethics approval form.
Housing and husbandry	15	Provide details of housing and husbandry conditions, including any environmental enrichment.	Section 2.1.1, lines 120–136. Reports 60-L gla
Animal care and monitoring	16	a. Describe any interventions or steps taken in the experimental protocols to reduce pain, suffering and distress. b. Report any expected or unexpected adverse events. c. Describe the humane endpoints established for the study, the signs that were monitored and the frequency of monitoring. If the study did not have humane endpoints, state this.	Section 2.1.1lines 139–141Clams were handled minimally, and no invasive procedures were performed before terminal sampling. At the endpoint, clams were dissected immediately after collection. No expected or unexpected adverse events were observed during the 30-day exposure. Not reported.
Interpretation/ scientific implications	17	a. Interpret the results, taking into account the study objectives and hypotheses, current theory and other relevant studies in the literature. b. Comment on the study limitations including potential sources of bias, limitations of the animal model, and imprecision associated with the results.	Discussion, Sections 4.1–4.3, lines 634–829. Interprets tissue-specific responses, energy metabolism, ribosome biogenesis, cell death pathways, immune/inflammatory responses, and oxidative stress defence in relation to study objectives and literature. Discussion, Section 4.4, lines 830–866. Limitations are discussed: laboratory-based single-time-point design, only two tissues, low annotation rate, and RNA-seq/qPCR platform discordance.
Generalisability/ translation	18	Comment on whether, and how, the findings of this study are likely to generalise to other species or experimental conditions, including any relevance to human biology (where appropriate).	Discussion, Section 4.4, lines 856–866; Conclusions, lines 870–882. Findings are likely generalisable to other filter-feeding bivalves under SSC stress and to coastal environmental monitoring, but not directly to human biology.
Protocol registration	19	Provide a statement indicating whether a protocol (including the research question, key design features, and analysis plan) was prepared before the study, and if and where this protocol was registered.	Not reported.
Data access	20	Provide a statement describing if and where study data are available.	Section 2.2, lines 188–190: raw RNA-seq read
Declaration of interests	21	a. Declare any potential conflicts of interest, including financial and non-financial. If none exist, this should be stated. b. List all funding sources (including grant identifier) and the role of the funder(s) in the design, analysis and reporting of the study.	The authors declare no conflicts of interest. Funding: This study was funded by research grants from the National Natural Science Foundation of China (No. 41706142).