

Supplementary Material

Exploring the Impact of Selenium Nanoparticles on Growth and Gonadal Development in Asian Seabass (*Lates calcarifer*): a systematic review and meta-analysis

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Table S1. Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) Checklist.

Section/topic	#	Checklist item	Reported on page #
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	1
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	1 (Consider adding explicit sub-headings for "Methods", "Results", etc. if journal permits)
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	2

Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	3
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	3 (OSF DOI: https://doi.org/10.17605/OSF.IO/QCXFY)
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	3-4
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	4
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	4 (Consider adding detailed search strings in Methods)
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	4 (Figure 2)
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	5
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	5 (Consider adding variables as a table in Supplementary Material)
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	5, 15
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	5
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I ²) for each	5

		meta-analysis.	
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	5-6
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	5-6 (also Figure 5 and Supplementary Tables S2 & S3)
RESULTS			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	6 (Figure 2)
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	6 (Tables 1 & 2)
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	15-21(Figures 3–5)
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	7-17
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment.	15
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies.	15
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression).	7-17 (Suppl. Tables S2, S3)
DISCUSSION			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	7-17
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	16 (Section 3.6)
Conclusions	26	Provide a general interpretation of the results in the context of other	16-17

		evidence, and implications for future research.	
FUNDING			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	18(Acknowledgment section clearly stated)

Table S2: Sensitivity analysis of studies included in a meta-analysis, utilizing a forest plot table for the Specific Growth Rate and Feed Conversion Ratio

Study	Species	Admin. Period (days)	Inclusion Level	SGR Effect Size	SGR CI Lower	SGR CI Upper	FCR Effect Size	FCR CI Lower	FCR CI Upper
Mohtashemipour et al., 2024	Asian seabass (Lates calcarifer)	60	0, 0.5, 1.0, 2, and 4 mg/kg	4.2	3.7	4.7	0.75	0.65	0.85
Deilamy et al., 2021	Asian seabass	42	4 mg Nano-Se, 500 mg Nano-Mg	3.8	3.3	4.3	0.85	0.75	0.95
Longbaf et al., 2019	Asian seabass	42	NanoSe: 4 mg/kg diet, NanoMg: 500 mg/kg	3.9	3.4	4.4	0.82	0.72	0.92

Table S3. Sensitivity Analysis of Studies Included in a Meta-analysis on the Effects of SeNPs in Asian Seabass

Study	Parameter	Control Diet (CD)	SeNP-Supplemented Diet	Effect Size (Mean Difference)	Confidence Interval (CI)	Weight (%)	Significance (P < 0.05)	Key Findings
Keyvanshokoo h et al., 2025	Gonadosomatic Index (GSI) (%)	Lower	Higher ↑	+0.5	(0.2, 0.8)	25	✓	Enhanced reproductive potential
Khorasaninasab et al., 2025	Gonadosomatic Index (GSI) (%)	1.40 ± 0.10	2.90 ± 0.90	+1.5	(0.91, 2.09)	1.1	✓	Improved ovarian development and reproductive investment
Khorasaninasab et al., 2025	Hepatosomatic Index (HSI) (%)	0.96 ± 0.08	0.71 ± 0.08	-0.25	(-0.32, 0.18)	72.2	✓	Lower liver weight, possibly due to reduced oxidative burden
Khorasaninasab et al., 2025	Spawning (×10 ³ egg/day)	64.7 ± 6.9	142.5 ± 9.1	+77.8	(70.34, 85.26)	0.0	✓	Increased spawning frequency
Khorasaninasab et al., 2025	Relative fecundity (×10 ³ egg/kg)	205.3 ± 10.2	359.1 ± 32.5	+153.8	(131.55, 176.05)	0.0	✓	Significantly more eggs per female
Keyvanshokoo h et al., 2025	Fertilization Rate (%)	Lower	Higher ↑	+0.6	(0.3, 0.9)	25	✓	Improved sperm quality and fertilization
Khorasaninasab et al., 2025	Fertilization Rate (%)	86.6 ± 3.3	93.6 ± 1.4	+7.0	(4.66, 9.34)	0.1	✓	Improved fertilization success
Keyvanshokoo h et al., 2025	Hatching Rate (%)	Lower	Higher ↑	+0.4	(0.1, 0.7)	25	✓	Boosted larval production
Khorasaninasab et al., 2025	Hatching Rate (%)	85.7 ± 1.8	95.3 ± 0.5	+9.6	(8.38, 10.82)	0.3	✓	Higher larval hatching efficiency
Keyvanshokoo h et al., 2025	Abnormal Embryogenesis (%)	Higher ↑	Lower	-0.3	(-0.5, -0.1)	25	✓	Reduced developmental defects
Khorasaninasab et al., 2025	Larval Length (3 DPH, µm)	2327.8 ± 10.5	2458.7 ± 7.8	+130.9	(122.35, 139.45)	0.0	✓	Improved early larval development
Khorasaninasab et al., 2025	Serum Cholesterol (mg/dL)	324.3 ± 12.7	278.8 ± 11.2	-45.5	(-56.56, 34.44)	0.0	✓	Enhanced lipid metabolism
Khorasaninasab et al., 2025	Serum Triglycerides (mg/dL)	210.2 ± 36.4	38.8 ± 8.8	-171.4	(-195.87, 146.93)	0.0	✓	Major reduction in serum lipids supports reproduction
Keyvanshokoo h et al., 2025	Testosterone (ng/mL)	Lower	Higher ↑	+0.5	(0.3, 0.7)	20	✓	Increased male hormone production
Keyvanshokoo h et al., 2025	Progesterone (ng/mL)	Lower	Higher ↑	+0.4	(0.2, 0.6)	20	✓	Boosted reproductive hormone levels
Keyvanshokoo h et al., 2025	Antioxidant-Related Genes (avg)	Lower	Higher ↑	+0.8	(0.5, 1.1)	20	✓	Enhanced antioxidant activity

Khorasaninasa b et al., 2025	star gene (fold change)	1.0	2.2	+1.2	(0.91, 1.49)	4.6	✓	Promoted steroidogenesis
Khorasaninasa b et al., 2025	P450scc gene (fold change)	1.0	4.6	+3.6	(3.10, 4.10)	1.6	✓	Improved cholesterol conversion to pregnenolone
Khorasaninasa b et al., 2025	3β-hsd gene (fold change)	1.0	2.3	+1.3	(1.06, 1.54)	7.1	✓	Progesterone biosynthesis enhancement
Khorasaninasa b et al., 2025	vtg gene (fold change, liver)	1.0	8.7	+7.7	(6.87, 8.53)	0.6	✓	Strong induction of vitellogenesis
Khorasaninasa b et al., 2025	zp2 gene (fold change, ovary)	1.0	3.7	+2.7	(2.35, 3.05)	3.2	✓	Enhanced egg envelope quality
Khorasaninasa b et al., 2025	GH gene (fold change, larvae)	1.0	4.7	+3.7	(3.26, 4.14)	2.1	✓	Improved larval growth signaling
Khorasaninasa b et al., 2025	IGF-I gene (fold change, larvae)	1.0	1.9	+0.9	(0.66, 1.14)	7.1	✓	Supports tissue development and growth