

**Effects of polystyrene nanoplastics on hormonal regulation
and glucose metabolism of Pacific whiteleg shrimp
(*Litopenaeus vannamei*)**

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1 Experimental animals and PS-NPs

To minimize the occurrence of cannibalism during molting, 3–5 polyvinyl chloride tubes were placed in each tank for shelter. Dead shrimps and residual feed were removed daily.

Images of the PS-NPs obtained by Fourier Transform Infrared Spectroscopy, Dynamic Light Scattering, Scanning Electron Microscopy, and Transmission Electron Microscopy are consistent with our previous published work (Li et al., 2024a).

2 Chronic exposure to NPs

Dead shrimps, feces, and residual feed were removed daily and half water was changed every two days. After the 28-day experimental period, the shrimps were fasted for 24 h and used an ice bath to minimize pain before sampling. The hemolymph and hepatopancreas (Shrimp alive in all treatment groups) were collected and stored at -80°C for biochemical, enzyme activity and gene expression analyses. Hemolymph was removed from the ventral sinus of *L. vannamei* using a 1 mL sterile syringe. And the hemolymph samples were centrifuged (7100 g, 10 min, 4°C) and the supernatant was immediately stored at -80°C.

3 Statistical analysis

One-way analysis of variance, principal component analysis (PCA), and Pearson's correlation analysis were performed with Origin software (OriginLab Corporation, Northampton, MA, USA). The Kruskal–Wallis test was used to compare differences of data without normal distributions or homogeneity of variance, and the Dunn–Bonferroni post hoc test was used for pairwise comparisons.

Table S1

PS-NP concentration (mg/L)	Parameters					
	SR (%)	BLGR (%)	WGR (%)	SGR (%/d)	HSI (%)	FCR (%)
0	93.33±3.33 ^a	162.55±6.67 ^a	226.61±6.58 ^a	4.23±0.07 ^a	6.04±0.4 ^a	1.36±0.04 ^a
0.1	86.67±3.33 ^a	130.73±17.33 ^b	216.3±13.64 ^a	4.11±0.16 ^a	5.46±0.34 ^b	1.43±0.09 ^a
1	77.78±5.09 ^b	102.08±10.94 ^c	163.25±9.38 ^b	3.45±0.12 ^b	5.22±0.69 ^b	1.89±0.1 ^b
5	68.89±5.09 ^c	73.15±8.59 ^d	128.38±10.06 ^c	2.95±0.16 ^c	4.77±0.21 ^c	2.42±0.19 ^c
10	63.33±3.33 ^c	64.47±8.67 ^d	94.92±16.18 ^d	2.37±0.29 ^d	4.62±0.29 ^c	3.33±0.53 ^d

Note: Data were expressed as mean ± SEM (standard error of the mean) (n=3 in SR, n=10 in all others). Different lowercase letters indicate significant difference among the groups ($P < 0.05$). Abbreviations: SR, survival rate; BLGR, body length growth rate; WGR, weight gain rate; SGR, specific growth rate; HSI, hepatosomatic index; FCR, feed conversion ratio.