

Effects of nonylphenol on histology, immunological and metabolomic responses of *Perna viridis*

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Introduction

- 4-nonylohenol (NP) is a typical endocrine-disrupting chemical, and has negative effects on marine organisms (Fig.1).
- *Perna viridis* is a world-wide distributed marine bivalve and economic important seafood. It is commonly used as a pollution monitoring animal (Fig.2).

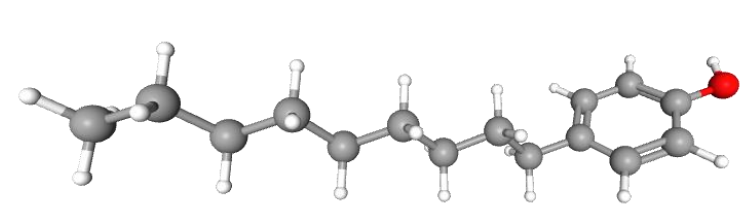


Fig.1 3D structure of NP
(From PubChem)



Fig.2 *Perna viridis*

Objectives

- Acute toxicity of NP on *P. viridis*.
- Sub-acute effects of NP on histology, non-specific immune system and metabolome of *P. viridis*.

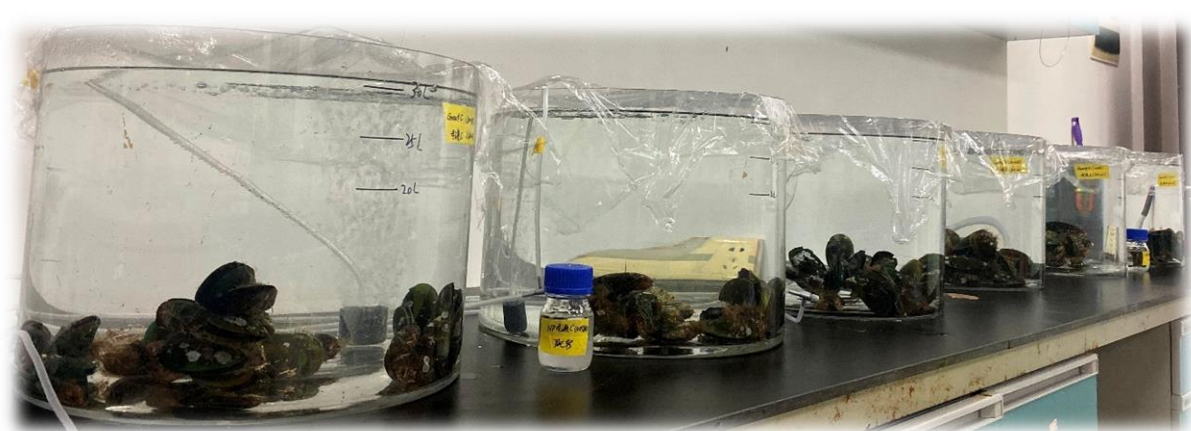
Materials and methods

❖ Experimental mussels

- Collected from Daya Bay, Shenzhen, China.
- (82.33 ± 2.16) mm in shell length and (29.12 ± 4.05) g in wet body weight (Mean ± SD).

❖ NP exposure experiment

Acute Toxicity				Sub-Acute Toxicity	
Groups	NP concentration (mg/L)	Groups	NP concentration (mg/L)	Groups	NP concentration (ug/L)
1	0.01	6	3.16	1	1.0
2	0.20	7	6.30	2	10.0
3	0.40	8	12.57	3	100.0
4	0.80	9	25.07		
5	1.58	10	50.00		



Histology analysis



Immunological responses



Metabolomic responses

Results

❖ Acute toxicity

- 50 mg/L NP exposure resulted in 100% mortality of mussel (Fig. 3), LC₅₀ (96 h) of NP on mussel was 2.75 mg/L.

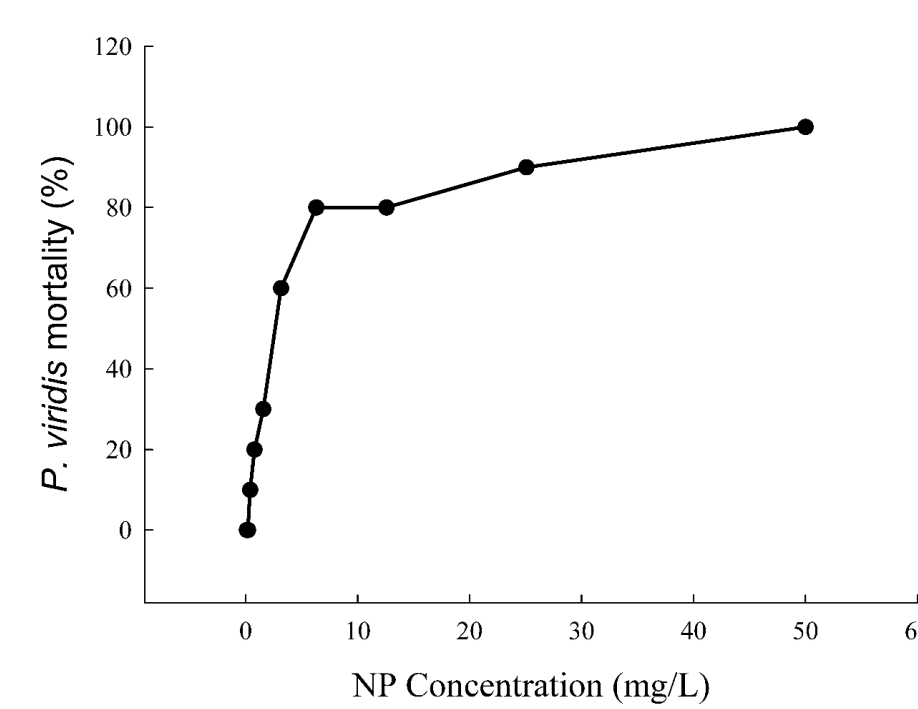


Fig. 3 Mortality of *P. viridis* exposed to NP for 96 h

❖ Sub-acute toxicity

- 10 ug/L and 100 ug/L of NP could trigger spawning of mussel, while no significant differences were observed among all groups in terms of mortality rates ($P > 0.05$) (Fig. 4).

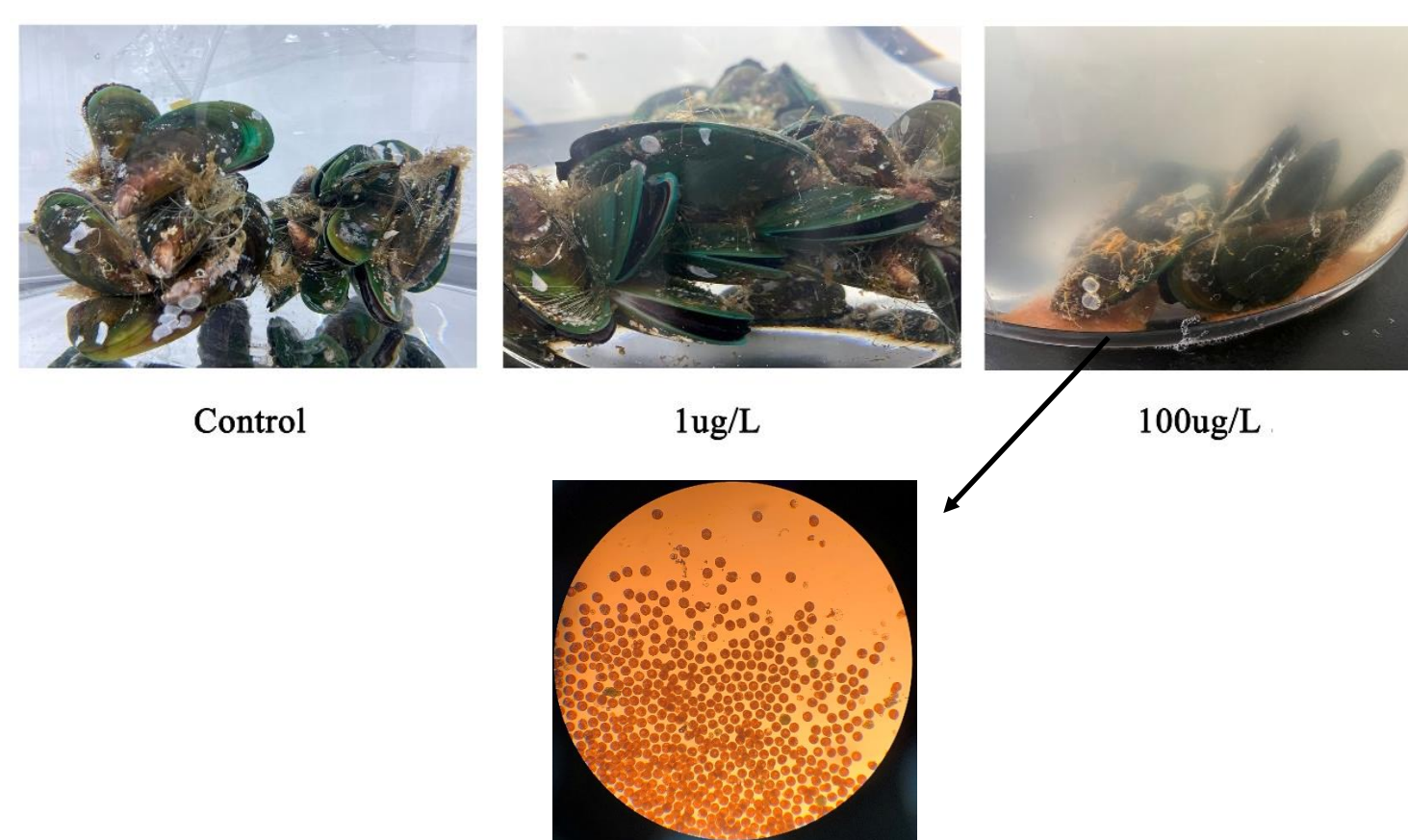


Fig. 4 spawning activity of *P. viridis* under NP exposure

- NP caused histological damages in both male and female gonads in a dose-dependent manner (Fig. 5).

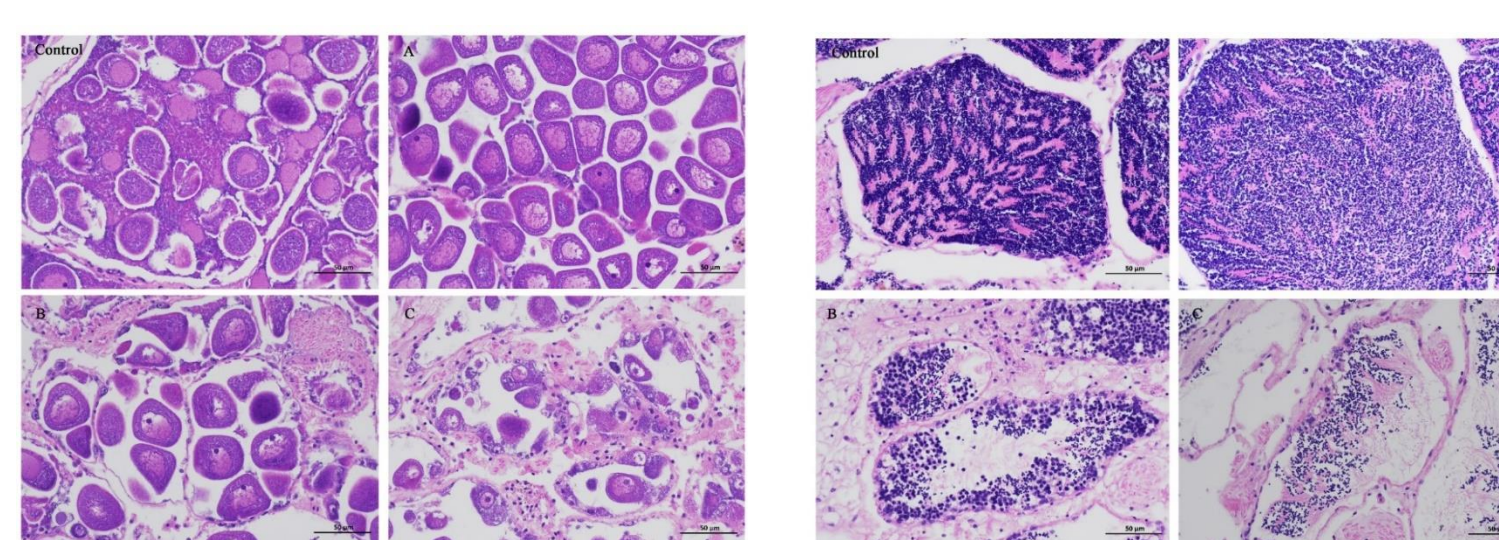


Fig. 5 Photomicrographs of transverse section of female (left) and male gonads of the *P. viridis*

Note: A, B and C represent 1ug/L, 10ug/L and 100ug/L group, respectively

- SOD, CAT, and GST activities, and MDA content were significantly higher than that of control in 10 or 100 ug/L groups ($P < 0.05$) (Fig.6).

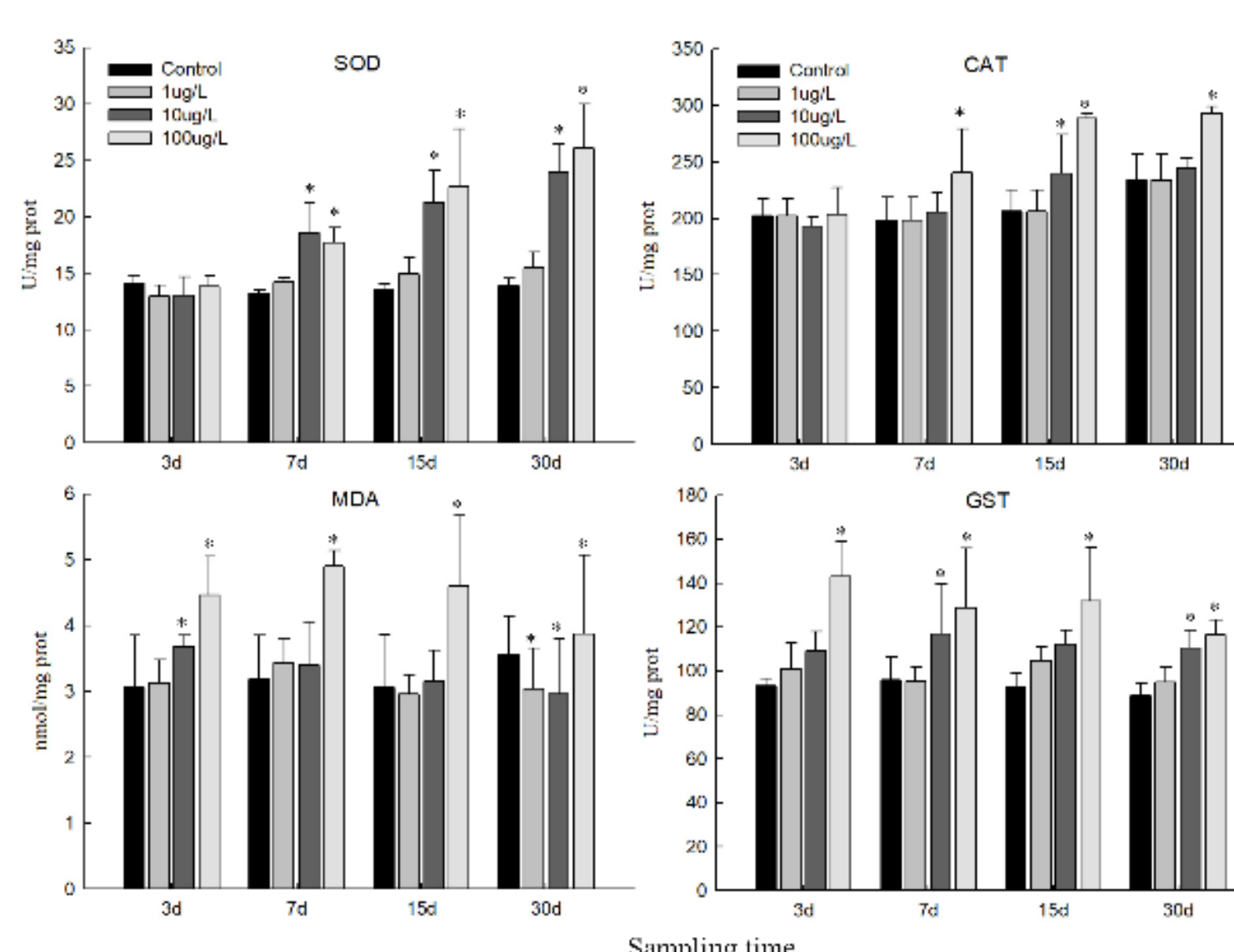


Fig. 6 Effects of NP on SOD, CAT, GST activities and MDA content in mantle of *P. viridis*

Note: * represents a significant difference from the control group ($P < 0.05$)

Results

- 117 significantly different metabolites (SDM) were determined among CK, LD and HD groups (Fig. 7).



Fig. 7 Metabolic alterations induced by NP exposure (n = 6)

Note: CK, LD and HD represent control, 10 ug/L and 100 ug/L group, respectively

- The relevant metabolic pathways were identified as taurine and hypotaurine metabolism, alanine, aspartate and glutamate metabolism, arachidonic acid metabolism, and pyrimidine metabolism (Fig.8).

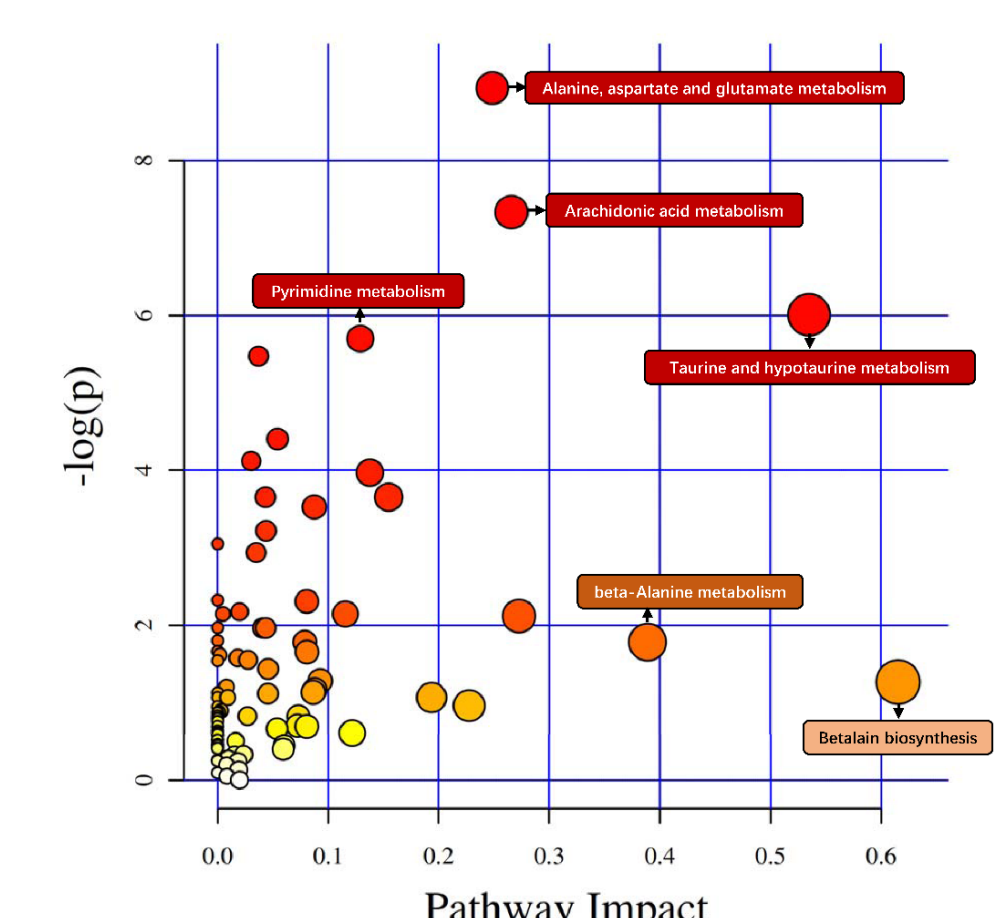


Fig.8 Metabolome view map of significant metabolic pathways characterized in hepatopancreas of *P. viridis* under NP stress

Note: This figure illustrates significantly changed pathways based on enrichment and topology analysis. The x-axis represents pathway enrichment, and the y-axis represents pathway impact. Larger sizes and darker colors represent greater pathway enrichment and higher pathway impact values, respectively.

Conclusions

- NP could affect spawning activity and gonad histology of *P. viridis*.
- 10 ug/L and 100 ug/L of NP may elicited oxidative stress in *P. viridis*.
- NP exposure caused alterations in hepatopancreas metabolome of *P. viridis*.

Acknowledgements

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