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升温与聚苯乙烯微塑料复合暴露对长牡蛎血细胞功能、免疫基因表达和能量代谢的影响*

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摘要 为阐明全球气候变暖和微塑料复合胁迫对长牡蛎(*Crassostrea gigas*)免疫应答、氧化应激和能量代谢的影响，本研究采用3个微塑料(microplastics, MPs)水平[无微塑料、小粒径聚苯乙烯微塑料(SPS-MPs, 6 μm)和大粒径聚苯乙烯微塑料(LPS-MPs, 50~60 μm)]和2个温度水平(20 ℃和25 ℃)对长牡蛎进行了为期21 d的单一和复合暴露，检测分析了各组长牡蛎血细胞功能[吞噬活性、活性氧(reactive oxygen species，ROS)含量]、糖原含量以及免疫相关基因表达的变化。研究结果表明，SPS-MPs暴露能增加长牡蛎血淋巴细胞中ROS含量，降低血细胞吞噬活性，揭示SPS-MPs毒性作用更强。升温与微塑料的协同作用增加了长牡蛎消化腺组织中的糖原含量。实时荧光定量PCR结果显示，升温与SPS-MPs复合暴露组长牡蛎消化腺组织通过上调热休克蛋白90 (heat shock protein 90, *HSP90*)、核因子κB抑制蛋白(inhibitor of NF-κB, *IκB*)和*p53*基因表达量进行免疫应答；升温与微塑料的拮抗作用增加了鳃组织*p53*基因表达量，揭示*p53*基因参与了鳃组织免疫调控。总之，升温与微塑料复合暴露能影响长牡蛎的氧化应激、免疫反应和能量代谢，升温与SPS-MPs长期暴露可能对长牡蛎的种群维持造成负面影响。

关键词 长牡蛎；微塑料；升温；免疫；能量代谢
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微塑料是指粒径小于5 mm的塑料碎片，是世界上最受关注的新兴污染物之一。直接加工形成的微塑料，通过自然和人为因素进入海洋环境，称为初级微塑料；塑料碎片还能经过光氧化、生物降解、热降解等方式，分解成为更小的塑料碎片，称为次级微塑料。很多研究发现，微塑料暴露能够导致海洋生物的组织损伤，并能影响其能量代谢、免疫和发育等过程(Bakir *et al*, 2014; Qiao *et al*, 2019; Bringer *et al*, 2020; Teng *et al*, 2021; 夏斌等, 2019)。例如，Teng等(2021)研究发现，微塑料暴露可以改变长牡蛎(*Crassostrea gigas*)的能量代谢并引发长牡蛎的炎症反应。Opitz等(2020)研究发现，环境相关浓度微塑料对贻贝(*Choromytilus*

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chorus)的能量平衡和生理指标的影响最小。小粒径微塑料暴露会导致菲律宾蛤仔(*Ruditapes philippinarum*)血淋巴细胞凋亡率升高(柳佳佳等, 2021)。此外，不同粒径大小的微塑料粒径对翡翠贻贝(*Perna viridis*)具有不同的毒性效应，大粒径(300~1 000 μm)的聚苯乙烯(PS)、聚丙烯(PP)和聚丁二酸丁二醇酯(PBS)微塑料与中等粒径(30~300 μm)和小粒径(<30 μm)微塑料相比，更可能导致翡翠贻贝死亡率升高(Phothakwanpracha *et al*, 2021)。

全球变暖使得海洋生物生活在更高的海水温度下。联合国政府间气候变化专门委员会(IPCC)预测，到 21 世纪末，温度将上升 1.4~3.1 ℃ (Pachauri *et al*, 2014)。海水温度升高会影响贝类的免疫反应、发育和能量代谢等多种生理过程(Rahman *et al*, 2019; Rahman *et al*,2021; Wu *et al*, 2021; Zhang *et al*, 2023; 吕旭宁等, 2018)。Coppola 等(2017)研究发现，温度升高会对紫贻贝(*Mytilus galloprovincialis*)产生更高的氧化损伤。也有研究表明，温度升高能够显著增加牡蛎(*Crassostrea virginica*)的细胞凋亡，并能引起热休克蛋白(heat shock protein 70, *HSP70*)基因 mRNA 表达量的升高(Rahman *et al*, 2021)。

在海洋和河口环境中，海洋生物经常暴露于高温、污染物等多种环境应激源中(Abe, 2021; Andrady, 2015; 高云涛等, 2022; 孔祥辉等, 2022)。以往的研究大多开展微塑料或温度变化对海洋生物的单一暴露实验(Paul-Pont *et al*, 2016; Pei *et al*, 2022; Rahman *et al*, 2021; 高振锟等, 2017)。升温和微塑料复合暴露的研究多集中在淡水生物(Kratina *et al*, 2019; Weber *et al*, 2020; Wen *et al*, 2018)。例如，聚苯乙烯微塑料和热刺激对淡水贻贝(*Dreissena polymorpha*)的复合暴露研究发现，热刺激对贻贝的影响大于微塑料(Weber *et al*, 2020)。Kratina 等(2019)研究表明，温度能够改变微塑料对蚤状钩虾(*Gammarus pulex*)代谢率的影响，在低温条件下代谢率随着微塑料浓度的增加而增加，而在较高温度条件下代谢率随着微塑料浓度的增加反而降低。Wen 等(2018)在探究升温和微塑料复合暴露对丽鱼(*Symphysodon aequifasciatus*)的研究中发现，升温与微塑料复合暴露对淀粉酶活性具有拮抗作用，而对脂肪酶活性无显著影响。有关升温和微塑料复合暴露对海洋生物的研究较少(Ferreira *et al*, 2016; Fonte *et al*, 2016)。例如，Ferreira 等(2016)在探究升温、金纳米颗粒(Au-NP)和微塑料复合暴露对海水虾虎鱼(*Pomatoschistus microps*)的研究中发现，在高温条件下，Au-NP 暴露对虾虎鱼个体和种群适应性产生不利影响的风险增加。因此，升温和微塑料复合暴露对海洋生物毒性效应研究亟待开展。本研究采用 3 个微塑料水平[无微塑料、小粒径聚苯乙烯微塑料(SPS-MPs, 6 μm)和大粒径聚苯乙烯微塑料(LPS-MPs, 50~60 μm)]和 2 个温度水平(20 ℃和 25 ℃)，探究升温和微塑料对长牡蛎血细胞功能、能量代谢和免疫基因表达的影响，以期为评估全球变暖背景下污染物对海洋生物的毒性效应提供数据支撑。

1 材料与方法

1.1 实验材料

2020 年 5 月于山东威海乳山长牡蛎养殖场购买 410 只长牡蛎(*Crassostrea gigas*) (壳长 6~8 cm)用于复合暴露实验。实验开始前，将长牡蛎在 40 L 的养殖缸中暂养 2 周[盐度为 32±0.4；温度为(20±0.2) ℃；pH 为 8.1±0.2]。暂养期间，每天用小球藻(*Chlorella*) (1×10^5 cells/mL)喂养牡蛎，养殖海水每 2 d 更换一次。

1.2 微塑料工作液制备

SPS-MPs (6 μm, 2.5% *w/v*, 10 mL)和 LPS-MPs (50~60 μm, 2.5% *w/v*, 10 mL)均购买于天津市倍思乐色谱技术开发中心。采用 0.22 μm 滤膜过滤的 Milli-Q 超纯水配制 SPS-MPs 工作液(浓度为 4×10^5 个/mL)。每次使用前均对原液和工作液进行超声处理，使其分散均匀。通过扫描电子显微镜(SEM, 日立 S-4800)检查微塑料的粒径和形态(图 1)。

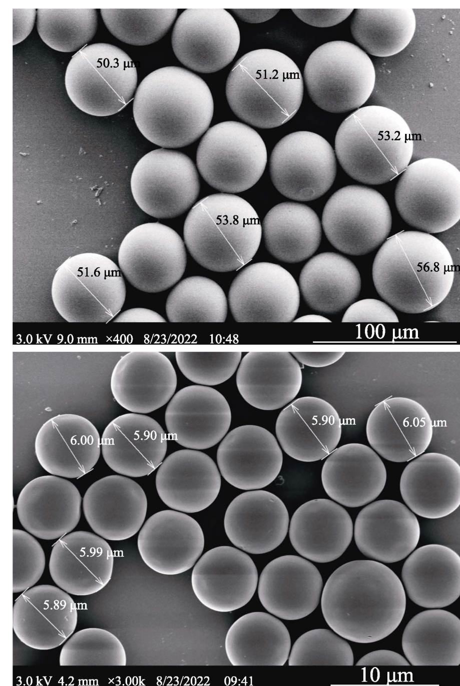

图 1 LPS-MPs(A)和 SPS-MPs(B)的扫描电子显微镜图
Fig.1 The SEM image of LPS-MPs (A) and SPS-MPs (B)

1.3　实验设计

长牡蛎暂养后，随机分为 6 组，分别采用 3 个微塑料水平[无微塑料、SPS-MPs (6 μm)和 LPS-MPs (50~60 μm)]和 2 个温度水平(20 ℃和 25 ℃)，共计 6 个处理组合，探究升温和 PS-MPs 复合暴露对长牡蛎的影响，暴露实验持续 21 d。每个处理组设 3 个水箱(40 L)作为重复，每个水箱养殖 20 只牡蛎。考虑到长牡蛎的物种适应性和环境最高水温(Sun *et al*, 2022)，25 ℃作为升温条件，20 ℃为实验期间环境实际水温。实验开始前，升温组的海水温度由环境温度(20 ℃)每天升高 2 ℃逐渐升高至 25 ℃，使长牡蛎逐渐适应 25 ℃的水温。微塑料暴露浓度设置为 1×10^4 个/L。各组别海水每天更换 1 次，并在微塑料暴露组中添加微塑料。于暴露第 21 天采样，收集各组长牡蛎的消化腺和鳃组织，液氮冷冻后，–80 ℃保存。

1.4　血淋巴细胞相关免疫指标

采用一次性注射器抽取长牡蛎血淋巴，经 300 目筛绢过滤后，迅速与等量的抗凝剂混合，将血淋巴样本分装 2 份各 500 μL 用于活性氧(reactive oxygen species，ROS)和吞噬活性的检测，采用台式冷冻高速离心机 4 ℃、2000×*g* 离心 10 min，弃上清液，加入等量 500 μL 的 PBS，再以 4 ℃、2000×*g* 离心 10 min 后，弃上清液，加入相应的缓冲溶液进行各指标的检测。

采用 2′,7′-二氯二氢荧光素二乙酸酯(2′,7′-dichlorofluorescein diacetate, DCFH-DA)荧光探针(Sigma)对血淋巴组织中的活性氧进行检测。向血淋巴细胞(500 μL)中加入 5 μL 荧光探针 DCFH-DA (0.01 mmol/L)，避光，在 18 ℃混合孵育 30 min。在激发波长为 488 nm、发射波长为 530 nm 的条件下，用流式细胞仪(BD Accuri™ C6 flow cytometer)对样本进行检测。上机前，采用 300 目筛绢过滤，根据 FL-1 通道的荧光强度的几何平均值，来表征血淋巴细胞 ROS 的含量。

采用荧光微球(YG 2.0 μm，Polysciences, 德国)对血细胞的吞噬活性进行测定。将 250 μL 的长牡蛎血淋巴与 2.3%的荧光微球进行混合，并避光放置 60 min，然后向混合液中加入福尔马林(15 μL)终止反应，经过 300 目筛绢过滤，采用流式细胞仪 FL-1 通道检测，采用摄入 3 个或更多荧光微球的血细胞占总的血细胞数目的百分比来估算血细胞吞噬活性。

1.5　糖原含量测定

长牡蛎消化腺组织中的糖原含量采用蒽酮显色法，并用肝/肌糖原检测试剂盒进行检测，购买自南京建成生物工程研究所。按照说明书的方法进行检测，单位为 mg/g 组织。

1.6　免疫和应激相关基因的 mRNA 表达

采集各实验组和对照组长牡蛎(*n*=6)的消化腺和鳃组织进行基因的 mRNA 表达检测，于–80 ℃保存。用 TRIzol 试剂(Invitrogen)分离提取总 RNA，Nanodrop 检测总 RNA 浓度。cDNA 用逆转录酶 M-MLV (Promega, 美国)合成。核因子 κB 抑制蛋白(inhibitor of NF-κB, *IκB*)基因、*p53* 基因和 *HSP90* 基因的 mRNA 表达量采用 StepOne Plus 实时荧光定量 PCR 仪(ABI 公司, 美国)进行检测。荧光定量 PCR 所用引物信息见表 1。选择转录延伸因子 1α (*EF1α*)作为内参基因。

1.7　微塑料镜检

为了观察长牡蛎是否摄入微塑料，采用显微镜进行镜检，由于 SPS-MPs 在体式显微镜下较难识别，只对 LPS-MPs 进行了镜检。首先，在复合暴露实验过程中收集长牡蛎粪便，并在显微镜下观察。然后，

表 1　荧光定量 PCR 引物序列

Tab.1　Primers used in real-time PCR

基因 Gene	正向引物 Forward primer (5′~3′)	反向引物 Reverse primer (5′~3′)	基因 Gene ID	参考文献 Reference
核因子 κB 抑制蛋白 *IκB*	CCCTTCACATTGCCAGTAG	ATTGGGAGATGGGTGTTCT	DQ250326.1	Zhang 等 (2011)
p53	ACCCAGCTCCGACTCATTT	TCATGGGGGATGATGACAC	AM236465	Farcy 等 (2008)
热休克蛋白 90 *HSP90*	AGCAGGGAAGTGGTTCAGTCG	TGACTTTGCACAATCCCTCGTAC	EF687776.1	Cao 等 (2018)
转录延伸因子 1α *EF1α*	ACCACCCTGGTGAGATCAAG	ACGACGATCGCATTTCTCTT	BQ426516	Sussarellu 等 (2012)

为了方便观察，将 LPS-MPs 采用 Shim 等(2016)的方法进行尼罗红染色，在 20 ℃和 25 ℃对长牡蛎进行复合暴露后，采集长牡蛎的鳃和消化腺组织，并加入 180 mL 10% KOH 和 20 mL 30% H_2O_2进行消解，60 ℃放置 24 h，采用 8 μm 滤膜(上海兴亚，中国)进行真空抽滤，采用体式显微镜(奥林巴斯 SZX10，日本)对 LPS-MPs 进行镜检(Munno *et al*, 2018)。

1.8 数据分析

结果均以平均值±标准误(Mean±SEM)表示。血细胞指标的数据通过 FlowJo 软件进行分析。数据的正态性检验采用 Shapiro-Wilk 检验，方差齐性检验采用 Levene 检验。对于不符合正态分布或方差齐性的数据，进行以 10 为底的对数变换(lg)。采用 SPSS 22.0 软件进行双因素方差分析(two-way ANOVA)，$P<0.05$ 被认为具有显著性。采用 LSD 检验(LSD test)进行多重比较分析。

2 结果

2.1 血细胞免疫指标

升温和微塑料复合暴露 21 d 后，各组别长牡蛎的血淋巴免疫指标如图 2 所示。ANOVA 分析表明，升温和微塑料复合暴露对长牡蛎血淋巴细胞中的 ROS 含量和吞噬活性无显著的交互作用($P>0.05$)(表 2)。总体而言，在各温度水平下，SPS-MPs 均可抑制长牡蛎血淋巴细胞吞噬活性，增加 ROS 产量。

2.2 糖原含量

升温和微塑料复合暴露 21 d 后，各处理组长牡蛎消化腺组织中糖原含量如图 3 所示。ANOVA 分析表明，升温与微塑料复合暴露对消化腺组织中糖原含量具有显著的交互作用($P<0.05$)(表 2)。升温能够增强微塑料对糖原含量的诱导作用，25 ℃+LPS-MPs 复合暴露组中长牡蛎消化腺组织中糖原的含量相比于升温和 LPS-MPs 单独暴露组显著增加($P<0.05$)(图 3)。

2.3 免疫相关基因表达量

升温和微塑料复合暴露 21 d 后，各处理组长牡蛎消化腺组织中免疫相关基因 mRNA 的表达量如图 4 所示。ANOVA 分析表明，升温与微塑料复合暴露对长牡蛎消化腺组织中 *HSP90*、*p53* 和 *IκB* 基因的表达量具有显著的交互作用($P<0.05$)(表 2)。25 ℃+SPS-MPs 复合暴露组长牡蛎消化腺中 *HSP90*、*p53* 和 *IκB* 基因表达量相较于 SPS-MPs 和升温单独暴露组均显著升高($P<0.05$)。微塑料单独暴露能够引起 *HSP90* 和 *IκB* 基因表达量相较于对照组上调。此外，25 ℃+LPS-MPs 复合暴露相较于 LPS-MPs 单独暴露能够显著降低 *IκB* 基因的表达量($P<0.05$)(图 4E)。

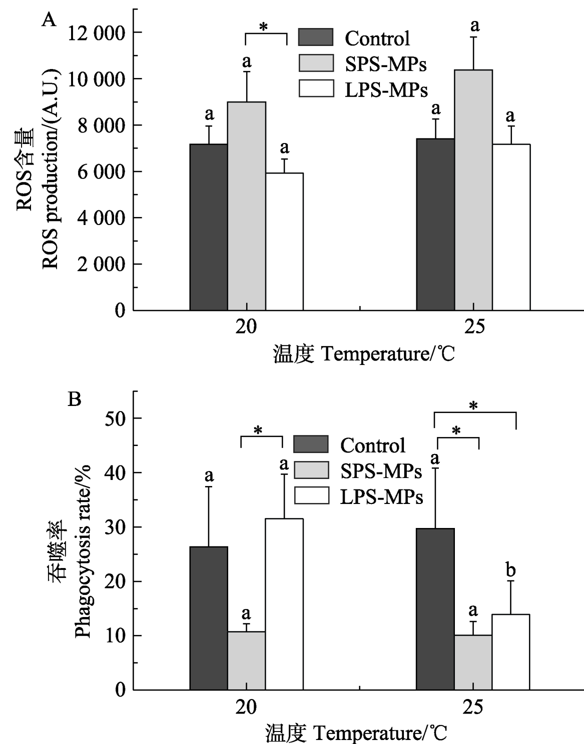

图 2 升温和微塑料暴露对长牡蛎血淋巴免疫指标的影响
Fig.2 Immune-related parameters in hemocytes of *C. gigas* exposed to elevated temperature and MPs

A：呼吸爆发(*n*=5)；B：吞噬活性(*n*=4~6)。不同字母表示相同微塑料水平下不同温度水平之间存在显著差异($P<0.05$)；星号(*)表示相同温度水平下不同微塑料水平之间存在显著差异($P<0.05$)。下同。
A: ROS (*n*=5); B: Phagocytosis (*n*=4~6). Different letters indicate significant differences between different temperatures within the same MPs level ($P<0.05$); asterisks indicate significant differences between different MPs levels within the same temperature ($P<0.05$). The same below.

升温和微塑料复合暴露 21 d 后，各处理组长牡蛎鳃组织中免疫相关基因 mRNA 的表达量如图 4 所示。ANOVA 分析表明，微塑料与升温复合暴露对长牡蛎鳃组织中 *p53*、*IκB* 和 *HSP90* 基因的表达表现出显著交互作用($P<0.05$)(表 2)。25 ℃+SPS-MPs 复合暴露组长牡蛎鳃组织 *HSP90* 基因的表达相较于 SPS-MPs 单独暴露组显著降低($P<0.05$)(图 4B)。此外，在 20 ℃条件下，微塑料暴露会抑制 *p53* 基因的表达量；而在 25 ℃条件下，微塑料暴露会诱导 *p53* 基因的表达量(图 4D)。微塑料和升温单独暴露相较于对照组能够显著增加 *IκB* 基因的表达量($P<0.05$)(图 4F)。

表 2　升温和微塑料暴露对长牡蛎血细胞功能、糖原含量和免疫基因表达的影响(双因素方差分析)

Tab.2　Effects of elevated temperature and MPs on hemocytes function, glycogen content, and the expression of immune related genes of *C. gigas* (two-way ANOVA)

	指标 Parameter	升温 Elevated temperature	微塑料 MPs	升温 × 微塑料 Elevated temperature × MPs
血细胞 Hemocytes	活性氧 ROS	*F* (1,24) = 1.255 *P* = 0.274	***F* (2,24) = 4.615** ***P* = 0.020**	*F* (2,24) = 0.202 *P* = 0.819
	吞噬活性 Phagocytosis rate	*F* (1,24) = 1.624 *P* = 0.215	***F* (2,24) =3.720** ***P* = 0.039**	*F* (2,24) = 2.036 *P* = 0.152
消化腺 Digestive glands	糖原 Glycogen content	***F* (1,18) = 17.589** ***P* = 0.001**	***F* (2,18) = 4.837** ***P* = 0.021**	***F* (2,18) = 5.246** ***P* = 0.016**
	HSP90	***F* (1,30) = 5.783** ***P* = 0.023**	***F* (2,30) = 11.005** ***P* < 0.001**	***F* (2,30) = 10.255** ***P* <0.001**
	IκB	*F* (1,30) = 0.208 *P* = 0.651	***F* (2,30) = 5.622** ***P* = 0.008**	***F* (2,30) = 6.946** ***P* = 0.003**
	p53	*F* (1,30) = 0.866 *P* = 0.359	*F* (2,30) = 1.461 *P* = 0.248	***F* (2,30) = 3.485** ***P* = 0.044**
鳃 Gills	*HSP90*	***F* (1,30) = 7.300** ***P* = 0.011**	*F* (2,30) = 0.281 *P* = 0.757	***F* (2,30) = 5.032** ***P* = 0.013**
	IκB	*F* (1,30) = 0.968 *P* = 0.333	*F* (2,30) = 1.865 *P* = 0.172	***F* (2,30) = 3.454** ***P* = 0.045**
	p53	*F* (1,30) = 0.281 *P* = 0.600	*F* (2,30) = 1.056 *P* = 0.360	***F* (2,30) = 8.721** ***P* = 0.001**

注：加粗字体表示具有显著性。

Note: Significances are highlighted in bold (*P*<0.05).

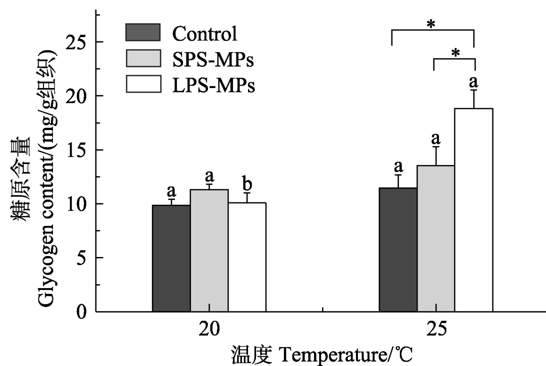

图 3　升温和微塑料暴露对长牡蛎消化腺组织中糖原含量的影响(n=4)

Fig.3　Glycogen content in digestive glands of *C. gigas* exposed to elevated temperature and MPs (n=4)

2.4　微塑料镜检

显微镜视野下，长牡蛎粪便中和组织消解后滤膜上的 LPS-MPs 如图 5 所示。镜检结果发现，在长牡蛎的粪便以及消化腺和鳃组织消解后的滤膜上均发现 LPS-MPs。

3　讨论

3.1　血细胞免疫指标

很多研究表明，微塑料暴露能够诱发海洋生物体内 ROS 的产生。ROS 包括过氧化氢(hydrogen peroxide，H_2O_2)、羟自由基(hydroxyl radical, ·OH)和超氧阴离子(superoxide anion，O^{2-})等，其作为细胞氧化代谢的有毒副产物，会破坏细胞结构，导致细胞膜系统损坏(Landis *et al*, 2005)。有研究表明，聚苯乙烯微塑料暴露可导致贻贝(*Mytilus* spp.)血细胞活性氧的积累，增强抗氧化酶活性(Paul-Pont *et al*, 2016)。金头鲷鱼(*Sparus aurata*)在聚甲基丙烯酸甲酯(PMMA)纳米塑料暴露后，能够诱发机体产生抗氧化反应(Brandts *et al*, 2021)。本研究中，SPS-MPs 单独暴露能够显著增加长牡蛎血淋巴组织中 ROS 含量，这可能是由于 SPS-MPs 引起长牡蛎血淋巴组织发生氧化应激所导致，而 LPS-MPs 暴露对长牡蛎血淋巴细胞 ROS 含量无显著影响，说明微塑料尺寸越小，对长牡蛎血细胞 ROS 含量的影响越大。与之类似，SPS-MPs 暴露能够抑制长牡蛎血淋巴细胞的吞噬活性，影响其

图 4　升温和微塑料暴露对长牡蛎消化腺和鳃组织中免疫相关基因 mRNA 表达量的影响(n=6)

Fig.4　The mRNA expression of immune related genes in digestive glands and gills of *C. gigas* exposed to elevated temperature and MPs (n=6)

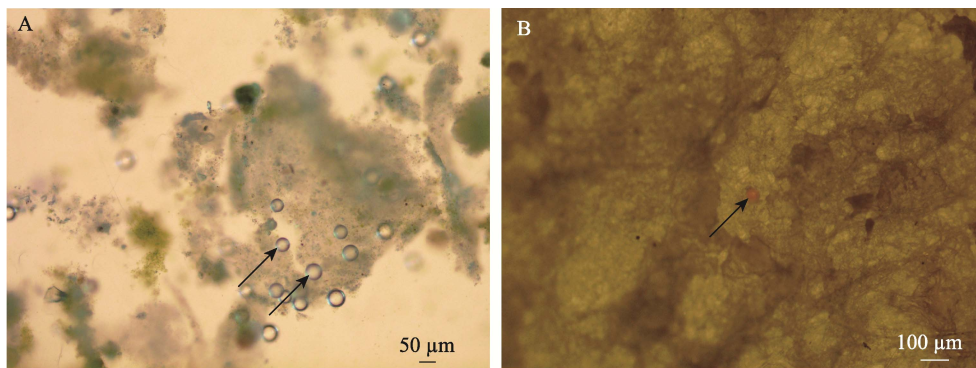

图 5　显微镜视野下长牡蛎粪便中(A)和组织消解后(B)的 LPS-MPs

Fig.5　Microscopic view of LPS-MPs in feces (A) and after tissue digestion (B) of *C. gigas*

细胞免疫功能，而 LPS-MPs 对长牡蛎血淋巴细胞的吞噬活性无影响。同样，厚壳贻贝(*Mytilus coruscus*)暴露于聚苯乙烯微塑料 21 d 后，其血淋巴细胞的吞噬活性受到抑制(Huang *et al*, 2022)。Pavičić-Hamer 等(2022)研究也表明，PMMA 微塑料暴露能够诱发紫贻贝血淋巴细胞的免疫反应，引起血细胞总数的增加，并抑制细胞活力。此外，Phothakwanpracha 等(2021)研究也表明，小粒径微塑料具有更强的毒性作用。

前期研究表明，生物在受到热应激胁迫时会产生 ROS，从而诱发氧化应激反应(Banh *et al*, 2016)。然而，本研究发现，升温对长牡蛎血淋巴细胞中 ROS 的产量无影响，但在 25 ℃条件下，升温组长牡蛎血淋巴细胞 ROS 整体有升高趋势，推测未发现显著性差异的原因可能与牡蛎的个体差异有关。与之类似，升温对大马蹄螺(*Trochus niloticus*)血淋巴细胞中 ROS 含量无显著性影响(Zhang *et al*, 2021)。本研究中，25 ℃+LPS-MPs 复合暴露组长牡蛎血淋巴细胞吞噬活性相较于 LPS-MPs 单独暴露组显著抑制，提示复合暴露组血淋巴组织免疫功能受到抑制。尽管升温组血淋巴的吞噬活性有升高趋势，但升温单独暴露组血淋巴细胞吞噬活性相较于对照组无显著性差异，这可能是由于牡蛎的个体差异所致。Rahman 等(2019)研究表明，升温(25 ℃)显著提高了长牡蛎、紫贻贝和蛤蜊(*Katelysia rhytiphora*)血淋巴细胞的吞噬活性。然而，Monari 等(2007)研究表明，升温(30 ℃)降低了蛤蜊(*Chamelea gallina*)血淋巴细胞的吞噬活性。

3.2　能量代谢

能量代谢相关标志物能够用于指示细胞能量水平的状态和环境压力的强度(Dong *et al*, 2016)。有研究发现，糖原在能量储备中发挥重要的作用(Smolders *et al*, 2003; Sokolova, 2013)。以往的研究表明，贝类体内糖原的储备情况能够直接反映贝类应对环境胁迫的能力，并且其含量受到自身的生理过程以及外界环境的影响(Cordeiro *et al*, 2016; 梅丽敏等, 2023)。本研究中，升温单独暴露对长牡蛎消化腺糖原含量无影响，可能是由于糖原被大量利用，因而没有表现出积累的趋势。与此相似，热应激对日本鼓虾(*Alpheus japonicus Miers*)肌肉组织中的糖原含量没有显著影响(李笑等, 2020)。然而，Zhang 等(2021)报道，海水升温能够导致大马蹄螺肌肉组织中糖原含量下降。这可能是由于长牡蛎与大马蹄螺具有不同的能量代谢机制。

升温和微塑料复合暴露对长牡蛎消化腺组织糖原含量的协同作用增加了糖原储备，可能是由于复合暴露组的长牡蛎具有更高的能量需求。这可能是由于海洋生物在复合压力条件下需要增加能量储备，其体内的氧化应激反应需要更高的能量来维持(Gagné *et al*, 2010)。

3.3　免疫相关基因表达

HSP90 基因是一种重要的分子伴侣蛋白基因，在生物体中能够被广泛诱导，在应对环境胁迫过程中起到重要的调节作用(Schopf *et al*, 2017)。*IκB* 基因是核因子 NF-κB 的抑制蛋白基因，NF-κB 是细胞免疫、促炎反应、凋亡和生长等基因转录激活的重要调节因子，*IκB* 基因 mRNA 的表达能够影响 NF-κB 等免疫炎症信号通路的调控作用，从而对环境胁迫产生免疫应答(Baeuerle, 1998; Jobin *et al*, 2000)。肿瘤抑制因子 *p53* 是一种重要的转录因子，在应对各种细胞应激(如 DNA 损伤)中发挥重要的作用(Lowe *et al*, 2013)。

在本研究中，升温和微塑料对长牡蛎消化腺组织 *HSP90* 和 *IκB* 基因 mRNA 表达的交互作用具有粒径依赖性：SPS-MPs 与升温表现为协同作用，mRNA 表达水平较高；LPS-MPs 与升温则表现为拮抗作用。这些结果提示，SPS-MPs 与升温复合暴露会引起长牡蛎消化腺组织较强的免疫反应，这可能是由于 SPS-MPs 相较于 LPS-MPs 对长牡蛎具有更强的毒性作用所致。与之相似，本研究发现，SPS-MPs 单独暴露相较于 LPS-MPs 单独暴露能够引起长牡蛎鳃组织 *HSP90* 基因表达量显著升高。柳佳佳等(2021)研究也表明，小粒径微塑料比大粒径微塑料对菲律宾蛤仔具有更强的毒性作用。在消化腺和鳃组织中，微塑料单独暴露能引起长牡蛎 *IκB* 基因表达量的上调，说明 *IκB* 基因在长牡蛎应对微塑料暴露的免疫应答中发挥重要的调控作用。同样，聚乙烯微塑料能够增加鲤鱼鳃组织中 NF-κB 通路的 IκB 激酶复合物(*IKKα* 和 *IKKβ*)基因和 NF-κB p65 基因的表达量(Cao *et al*, 2023)。升温和微塑料对长牡蛎鳃组织 *IκB* 基因的 mRNA 表达具有显著的拮抗作用，与消化腺组织表现出不同的调控模式，说明 *IκB* 基因的调控作用具有组织特异性，这可能是长牡蛎鳃和消化腺组织受到胁迫刺激后发挥免疫防御功能的调节机制不同所导致。此外，升温和微塑料对消化腺和鳃中 *p53* 基因表达均有拮抗作用，在 20 ℃，微塑料暴露能够降低 *p53* 基因的表达量，而在 25 ℃，微塑料暴露能够升高 *p53* 基因的表达量，说明复合暴露能够启动 *p53* 基因相关免疫信号通路，从而引起机体产生免疫应答。

4 结论

本研究以长牡蛎为研究对象，探究了升温与聚苯乙烯微塑料对长牡蛎免疫和能量代谢的复合毒性效应。结果发现，复合暴露会增强长牡蛎消化腺组织糖原储备；SPS-MPs 与升温复合暴露会引起长牡蛎消化腺组织 *IκB* 和 *HSP90* 基因表达上调，表明升温和 SPS-MPs 复合暴露会引起较强的免疫反应，且 SPS-MPs 相较于 LPS-MPs 毒性作用更强；升温和微塑料的拮抗作用导致消化腺和鳃组织中 *p53* 基因的表达量上调，说明 *p53* 基因参与了升温和微塑料复合暴露的免疫应答。此外，SPS-MPs 能够引起长牡蛎血淋巴细胞 ROS 积累，抑制吞噬活性。因此，升温与微塑料复合暴露能够诱导免疫反应，增加糖原储备，诱发血淋巴细胞产生氧化应激，提示 SPS-MPs 与升温长期复合暴露可能会对长牡蛎种群维持造成潜在威胁。

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Combined Effects of Elevated Temperature and Polystyrene Microplastics on Hemocyte Function, Immune-Related Gene Expression, and Energy Metabolism of *Crassostrea gigas*

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Abstract Bivalves are affected by various stressors, such as global warming and microplastics, in the marine environment. Microplastics are one of the most concerning pollutants worldwide, and high seawater temperatures caused by global warming influence the survival of marine organisms. However, little is known about the combined effects of elevated temperature and microplastics (MPs) on marine organisms, and most studies conducted in recent years have investigated the two factors, respectively. Thus, it is necessary to investigate the combined effects of elevated temperature and MP exposure on marine life. The Pacific oyster *Crassostrea gigas* is a widely distributed marine mollusk, and has very important economic value. The aim of the current study was to explore the toxic effects of elevated temperature and microplastic co-exposure on the hemocyte function, immune-related gene expression, and energy metabolism of *C. gigas*. In the current study, oysters were exposed to three levels of microplastics (no microplastics, 6 μm microplastics: SPS-MPs, and 50~60 μm microplastics: LPS-MPs) and two temperature levels (20 ℃ and 25 ℃) for 21 days, and the phagocytosis rate and reactive oxygen

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species (ROS) content of hemocytes, glycogen content in digestive glands, and immune-related gene expression in digestive glands and gills were examined on the 21st day. 2',7'-Dichlorodihydrofluorescein diacetate and fluorescent microspheres were used to measure the ROS content and phagocytosis ratein hemocytes of *C. gigas* by flow cytometry, respectively. The glycogen content was measured using detection kits. Total RNA was isolated by TRIzol reagent, and the concentration was measured by Nanodrop. M-MLV Reverse Transcriptase was used for cDNA synthesis. The expressions of immune-related genes [inhibitor of NF-κB (*IκB*), *p53*, and heat shock protein 90 (*HSP90*)] were examined by quantitative real-time PCR in the digestive glands and gills of oysters from each treatment group. Two-way ANOVA was used to analyze the interactive effects of elevated temperature and microplastics on tested parameters of oysters using SPSS software. The results showed that exposure to SPS-MPs could elevate ROS content and reduce phagocytosis in hemocytes, but no significant interaction was found between elevated temperature and microplastic effects on ROS content and phagocytosis rate in hemocytes (P>0.05). The 25 ℃+LPS-MPs exposure significantly decreased phagocytosis in hemocytes compared with single LPS-MPs and elevated temperature exposures (P<0.05). Single SPS-MPs exposure significantly decreased phagocytosis in hemocytes compared with single LPS-MPs exposure (P<0.05). In digestive glands, there was a significant interaction between elevated temperature and microplastics in glycogen content (P<0.05), and the combined exposure could increase the glycogen content compared with other treatments. In digestive glands, the 25 ℃+LPS-MPs exposure significantly increased glycogen content compared with single elevated temperature and single LPS-MPs exposure (P<0.05). In digestive glands and gills, there was a significant interaction between elevated temperature and microplastics in the expressions of *HSP90*, *IκB*, and *p53* genes (P<0.05). The 25 ℃+SPS-MPs exposure significantly upregulated the expression of *HSP90*, *IκB*, and *p53* genes in the digestive glands of oysters compared with single SPS-MPs and single elevated temperature exposures (P<0.05). The 25 ℃+SPS-MPs exposure significantly downregulated the expression of the *HSP90* gene in the gills of oysters compared with single SPS-MPs exposure (P<0.05). Single elevated temperature and single microplastics exposure significantly upregulated the expression of the *IκB* gene compared with the control in gills (P<0.05). The combined exposure of elevated temperature and microplastics showed a significant antagonistic effect on the expression of the *p53* gene in gills. Microplastics exposure downregulated *p53* gene expression compared with the control at 20 ℃, while it upregulated *p53* gene expression compared with single elevated temperature at 25 ℃. These results indicated that the *p53* gene plays an important role in regulating the immune response in both digestive glands and gills. The interaction between elevated temperature and microplastics on the mRNA expression of *HSP90* and *IκB* genes in digestive glands of *C. gigas* was size-dependent: A synergistic effect was found between SPS-MPs and elevated temperature, and an antagonistic effect was found between LPS-MPs and elevated temperature. A significant antagonistic effect was found between elevated temperature and microplastics on the mRNA expression of the *IκB* gene in gills, and the regulation pattern was different from that in the digestive glands, indicating that the regulation effect of the *IκB* gene was tissue-specific. In conclusion, the combined exposure of elevated temperature and microplastics can increase the glycogen content in the digestive glands of *C. gigas*, induce an immune response in digestive glands and gills, and trigger the oxidative stress response in hemocytes. Microplastics can cause stronger oxidative stress in hemocytes than elevated temperature. Moreover, a significant interactive effect was found between elevated temperature and microplastics on glycogen content in digestive glands and the expression of immune-related genes (*HSP90, p53*, and *IκB*) in digestive glands and gills. The results of this study provide valuable information for evaluating the toxic effects of microplastics on marine organisms under a global warming background.

Key words　*Crassostrea gigas*; Microplastics; Elevated temperature; Immune; Energy metabolism