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厚壳贻贝中麻痹性贝毒的蓄积及其对滤食率的影响*

杨越聪[1,2] 郑关超[2] 赵辉辉[2] 张海涛[2] 谭志军[2,3] 吴海燕[2①]

(1. 上海海洋大学食品学院 上海 201306；2. 农业农村部水产品质量安全检测与评价重点实验室 中国水产科学研究院黄海水产研究所 山东 青岛 266071；3. 海水养殖生物育种与可持续产出全国重点实验室 中国水产科学研究院黄海水产研究所 山东 青岛 266071)

摘要 厚壳贻贝(*Mytilus unguiculatus*)是中国地理标志产品，具有重要的经济价值和产业地位。随着我国近海有害赤潮灾害的频发，厚壳贻贝中贝类毒素残留风险亟需关注。本研究通过室内暴露方式，评估了不同密度链状亚历山大藻(*Alexandrium catenella*)对厚壳贻贝滤食率的影响及其对麻痹性贝毒(paralytic shellfish toxins，PSTs)的蓄积代谢规律。结果表明，厚壳贻贝滤食率与产毒藻暴露密度、PSTs 含量呈显著负相关($P<0.05$)，最低降至初始值的 30.0%左右。厚壳贻贝对 PSTs 整体蓄积能力较弱，高密度组肝胰腺与软组织的日平均蓄积速率分别为 981.6 μg STXeq/kg/d 和 106.5 μg STXeq/kg/d。链状亚历山大藻与厚壳贻贝中 N-磺酰氨甲酰基类毒素-2 (N-sulfocarbamoylgonyautoxin toxins-2, C1)、N-磺酰氨甲酰基类毒素-3(C2)与膝沟藻毒素 5 (Gonyautoxin-5, GTX5) 3 种组分的初始含量最高，贻贝摄食产毒藻后肝胰腺中 C2 占比显著降低($P<0.05$)，由 74.1% (藻细胞)分别降低至 22.6% (高密度组)与 17.1%(低密度组)；C1 占比则由 10.6% (藻细胞)上升至 54.1% (高密度组)和 54.0% (低密度组)。厚壳贻贝的代谢速率最高可达到 1 860.3 μg STXeq/kg/d，显著高于其他双壳贝类。本研究表明，厚壳贻贝滤食率随 PSTs 产毒藻暴露时间呈下降趋势，且厚壳贻贝对 PSTs 的快速代谢与低转化、低残留等特点也表明其食用风险低于其他贻贝。本研究为评估厚壳贻贝中 PSTs 风险形成机理并为科学构建防控技术提供了研究基础。

关键词 厚壳贻贝；麻痹性贝毒；生物转化；滤食率

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麻痹性贝毒(paralytic shellfish toxins，PSTs)是一类具有神经毒性的生物毒素，海洋中主要由亚历山大藻属(*Alexandrium*)多个物种、链状裸甲藻(*Gymnodinium catenatum*)、巴哈马梨甲藻(*Pyrodinium bahamense*)产生(Rodríguez-Cabo *et al*, 2021)，在全球广泛分布并引发大量中毒事件(Bazzoni *et al*, 2020; Stüken *et al*, 2015)。PSTs 主要通过食物链传递蓄积到双壳贝类等海洋生物中并发生代谢转化(Reis Costa, 2016)，从而

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① 通信作者：吴海燕，副研究员，Email: wuhy@ysfri.ac.cn

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对消费者产生健康风险。在全球范围内年均造成 2 000 余人中毒，死亡率高达 15% (Wang *et al*, 2016)。因此，中国、美国、欧盟等国家或地区均对双壳贝类中 PSTs 设定了安全限量值(400 MU/100 g 或 800 μg STXeq/kg)(Visciano *et al*, 2016; Yoo *et al*, 2020; 梁玉波等, 2019)并定期监控(郑旭颖等, 2023)。近年来，以贻贝为代表的海洋贝类中屡次检出高含量的 PSTs，如 2016 年秦皇岛和 2017 年福建近海海域的贻贝中 PSTs 含量分别达到限量值的 50.7 倍和 42.7 倍(梁玉波等, 2019)。由此可见，贻贝已成为我国 PSTs 风险最为严峻的海洋贝类，亟需强化贻贝中 PSTs 蓄积转化研究，以期为我国贻贝产品中 PSTs 风险防控提出基础科学数据。

双壳贝类对 PSTs 的蓄积能力与其摄食行为高度相关，受摄食率、选择性摄食以及机体吸收产毒藻效率等因素影响(Fernández-Reiriz *et al*, 2008; Li *et al*, 2001)。Li 等(2001)对比了中国南海 2 种双壳贝类对毒素的蓄积与摄食能力差异，发现产毒藻塔玛亚历山大藻 (*A. tamarense*) 与非产毒藻伪矮海链藻 (*Thalassiosira pseudonana*) 暴露下华贵栉孔扇贝 (*Chlamys nobilis*)的 PSTs 含量远高于菲律宾蛤仔 (*Ruditapes philippinarum*)，同时，华贵栉孔扇贝对产毒藻的滤食率与吸收同化能力也强于菲律宾蛤仔。此外，PSTs 蓄积能力还与双壳贝类的神经敏感性有关，由于贻贝的神经元轴突对 PSTs 不敏感，导致贻贝对 PSTs 的蓄积能力高于其他贝类(颜天等, 2001)。此外，不同贻贝对 PSTs 的蓄积能力具有品种差异性 (Cadaillon *et al*, 2022; Goya *et al*, 2020)。Young 等 (2006)比较了 3 种贻贝的蓄积差异，发现厚壳贻贝 (*Mytilus unguiculatus*)毒素蓄积量分别为翡翠贻贝 (*Perna viridis*)及紫贻贝(*M. galloprovincialis*)的 1/2 和 1/3，这一结果同样在自然赤潮暴发期间得到证实。

厚壳贻贝是我国三大经济贻贝之一，主产地位于浙江舟山嵊泗地区，根据历史数据可见该地区为赤潮高发区(于仁成等, 2020)。其分布的 PSTs 产毒藻主要包括太平洋亚历山大藻(*A. pacificum*)、微小亚历山大藻(*A. minutum*)、链状亚历山大藻(*A. catenella*)(Gu *et al*, 2022)。以亚历山大藻属为主的 PSTs 产毒藻分布十分广泛(Paredes-Mella *et al*, 2021)，5—7 月是该地区 PSTs 风险较为严重的季节且多次检出 PSTs 残留的情况(彭志兰等, 2017)。嵊泗厚壳贻贝不仅是我国重要的地理标志产品(陈瑜等, 2020)，并且为首批入选《中欧地理标志协定》的贝类产品，具有重要的经济价值和社会影响。然而，目前针对厚壳贻贝中毒素蓄积代谢特征的研究严重不足，限制了这一重要经济品种安全防控技术的建立。本研究通过将厚壳贻贝暴露于不同密度链状亚历山大藻，揭示产毒藻暴露下厚壳贻贝对 PSTs 的蓄积代谢能力及滤食率的变化，为评估厚壳贻贝中 PSTs 风险形成机理并构建风险防控技术提供数据支撑。

1　材料与方法

1.1　实验材料

链状亚历山大藻(GY-H25 株)，购自上海光语生物科技有限公司。厚壳贻贝购自于浙江省舟山市嵊泗县，使用游标卡尺选择壳长(7.5±1.0) cm，壳宽(3.0±1.0) cm，且活性良好个体，在实验条件下净化 2 d 后用于暴露实验。饵料藻为小球藻 (*Chlorella vulgaris*)。石房蛤毒素(STX)，新石房蛤毒素(NEO)，膝沟藻毒素(GTX1、GTX2、GTX3 和 GTX4)，N-磺酰氨甲酰基类毒素(GTX5、C1 和 C2)，脱甲酰基类毒素(dcSTX、dcNEO、dcGTX2 和 dcGTX3)标准品购自加拿大国家海洋研究中心。

1.2　实验方法

1.2.1　*链状亚历山大藻培养*　　链状亚历山大藻接种于 f/2 培养基，初始接种密度为 6.5×10^5 cells/L，培养条件：温度 25 ℃，光照为 54 μmol/(m^2·s)，光暗时间比为 12 h∶12 h。每 3 天取样 10 mL 藻液加入 150 μL 鲁格试液(Lugol's)固定，充分摇匀，光学显微镜下进行观察和计数，取 3 次的平均值评估产毒藻的生长阶段。取 20 mL 生长平台期藻液 5 000 r/min 的条件下离心 15 min，取下层藻细胞用于 PSTs 分析。

1.2.2　*暴露实验*　　随机挑选 760 只厚壳贻贝，根据产毒藻的投喂量分为高密度组、低密度组和对照组，每个处理组设置 3 个平行，每个平行 84 只贻贝。实验共持续 30 d，其中，1~7 d 为蓄积阶段，8~30 d 为代谢阶段。蓄积阶段每天早晚 2 次对实验组贻贝投喂产毒藻，早晚投喂量为 1∶2。高密度组投喂量为 7.0×10^5 cells/d/只，低密度组投喂量为 2.8×10^5 cells/天/只。蓄积阶段结束后，实验组及对照组每组每天投喂 2 g 小球藻。每组分别于 0.5、1、2、4、6、7、8、10、12、14、18、22、26 和 30 d 各取 6 只贻贝清洗后切断闭壳肌，其中，2 只分离出软组织，其余 4 只分离出肝胰腺及可食组织(软组织中除肝胰腺部分)，每个组织分 3 个平行样品，筛网滤去表面水分后进行 PSTs 分析。

1.2.3　*PSTs 检测*　　产毒藻样品加入 5 mL 1%乙酸水溶液，冰浴超声破碎 5 min，单次破碎时间为 8 s

并停 2 s,4 ℃条件下 8 000 r/min 离心 10 min,取 1 mL 上清液过 0.22 μm Clarinert 针式过滤器(MCM 亲水)于进样小瓶待测。

贝类样品前处理参考 Wu 等(2022)与李瑾祯等(2023):5 g 样品加入 5 mL 1%的乙酸水提取毒素,沸水浴中煮沸 5 min,4 500 r/min 离心 10 min。取 1 mL 提取液,加入 5 μL 氨水过 ENVI-Carb 固相萃取柱净化,1 mL 75%乙腈水溶液(含 0.25%甲酸)洗脱,过 0.22 μm Clarinert 针式过滤器(Nylon 通用)后存于进样小瓶待上机检测。

液相色谱和质谱的条件参考张海涛等(2023)的方法,Sciex 5500 QTRAP 液相色谱–串联质谱,采用喷雾电离子源(ESI)多反应监测(MRM)正负离子切换模式。色谱柱 TSK-Amide-80 (3 μm, 2 mm×15 cm),柱温 40 ℃,流动相 A 为水(含 2 mmol/L 甲酸铵,50 mmol/L 甲酸),流动相 B 使用 95%乙腈水溶液(含 2 mmol/L 甲酸铵,50 mmol/L 甲酸)。梯度洗脱条件为 0~3.0 min,80% B;3.1~5.0 min,80%~40% B;5.1~10.0 min,40% B;10.1~11.0 min,40%~80% B;11.1~13.0 min,80% B;流速为 0.4 mL/min。离子源温度为 550 ℃,喷雾电压为 5 000 V–4 500 V,气帘气压力为 20 psi,雾化气压力为 30 psi,辅助加热器压力为 30 psi,碰撞气 Medium。

1.2.4 计算公式 (1)滤食率的计算方法为,在投喂产毒藻时与投喂产毒藻 1 h 后取 5 mL 水样加入鲁格试液用于固定藻细胞,显微镜下对水样中藻细胞计数。

$$I_R=[1-(C_0-C_{1h}/C_0)]\times 100 \quad (1)$$

式中,I_R 表示 1 h 厚壳贻贝的滤食率,C_0 与 C_{1h} 分别为投喂产毒藻时与投喂产毒藻 1 h 后 5 mL 水样中的藻细胞个数。

(2)日平均蓄积速率参考 Liu 等(2020)的计算方法:

$$\text{日平均蓄积速率}=(T_n-T_m)/(m-n) \quad (2)$$

式中,T_n 和 T_m 是蓄积阶段厚壳贻贝特定组织在第 n 天和第 m 天 PSTs 含量,单位为 μg STXeq/kg。

日平均代谢率计算公式为:

$$\text{日平均代积率}=(T_n-T_m)/(m-n)/T_n \quad (3)$$

式中,T_n 和 T_m 是代谢阶段厚壳贻贝特定组织在第 n 天和第 m 天 PSTs 含量,单位为 μg STXeq/kg。

1.2.5 数据处理 实验数据以平均值±标准差(Mean±SD)表示,利用 SPSS 25 和 Excel 2018 等软件进行单因素方差分析(one-way ANOVA),以 $P<0.05$ 为差异显著水平,结果利用 origin 2021 软件作图。

2 结果

2.1 链状亚历山大藻的生长与产毒特征

如图 1 所示,*A. catenella* 接种后细胞密度持续升高并于 24 d 达到平台期,藻密度为 1.5×10^7~1.6×10^7 cells/L,42 d 进入衰退期细胞密度持续降低。平台期产毒藻所产 PSTs 组分及含量为 C2:3.9~4.7 pg/cell,C1:0.8~3.1 pg/cell,GTX5:0.5~0.6 pg/cell,NEO:0.3~0.4 pg/cell,GTX4:0.1 pg/cell 和 GTX3:0.1 pg/cell (表 1),其中,C2、C1 和 GTX5 三种组分占比最高,平均单细胞产毒量为 2.0 pg STXeq/cell。因此,选用培养 24~30 d 的藻细胞进行暴露实验。

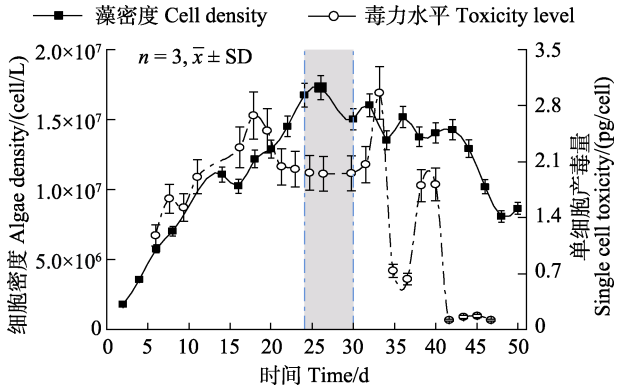

图 1 链状亚历山大藻生长曲线以及单细胞产毒量

Fig.1 Growth curve and single cell toxicity of *A. catenella*

2.2 厚壳贻贝 PSTs 蓄积对滤食率的影响

如图 2a 所示,厚壳贻贝滤食率随暴露时间呈下降趋势。暴露 7 d 时,滤食率分别降低为初始值的 26.7% (高密度组)和 37.1% (低密度组),高密度组显

表 1 链状亚历山大藻生长平台期产毒情况

Tab. 1 Toxicity production of *A. catenella* at stationary phase

接种时间 Inoculation time/d	PSTs 各组分含量 Content of each PSTs compoent/(pg/cell)					
	NEO	GTX4	GTX3	GTX5	C1	C2
24	0.3±0.1	0.1	0.1	0.5±0.1	0.8±0.3	4.7±1.2
26	0.4±0.1	0.1	0.1	0.6±0.1	2.2±0.3	4.5±0.8
30	0.4±0.1	0.1	0.1	0.6±0.2	3.1±0.1	3.9±0.1

著低于低密度组(P<0.05)。蓄积阶段不同组织中 PSTs 含量均表现为上升趋势，而蓄积含量具有显著差异。肝胰腺为毒素蓄积靶器官，PSTs 含量最高为 7 458.2 μg STXeq/kg (高密度组)和 2 555.9 μg STXeq/kg (低密度组)(图 2b)。贻贝肝胰腺和软组织日平均蓄积速率，高密度组分别为 981.6 μg STXeq/kg/d 和 106.5 μg STXeq/kg/d，低密度组分别为 365.1 μg STXeq/kg/d 和 40.8 μg STXeq/kg/d。整个实验阶段，仅高密度组 6 d 软组织中 PSTs 含量超过安全限量值(图 2c)。高低密度组厚壳贻贝可食组织中 PSTs 含量均小于 108.6 μg STXeq/kg (图 2d)。由表 2 可知，PSTs 含量与滤食率呈显著负相关。肝胰腺中 PSTs 含量与滤食率相关性最强，而可食组织的相关性最弱。

代谢阶段各组织 PSTs 代谢速率由高到低依次为肝胰腺、软组织、可食组织。其中，实验 7~9 d 为快速代谢期，高密度组肝胰腺日代谢速率为 1 860.3 μg STXeq/kg/d。高密度组贻贝各组织日平均代谢率分为 18.4% (肝胰腺)、18.1% (软组织)和 13.1% (可食组织)。暴露 30 d 时，各组织 PSTs 含量仅为最高值的 50.0%以下，低于安全限量值。高密度组肝胰腺 PSTs 含量维持在 1 400.0 μg STXeq/kg 左右，低密度组则维持在 600.0 μg STXeq/kg 左右。

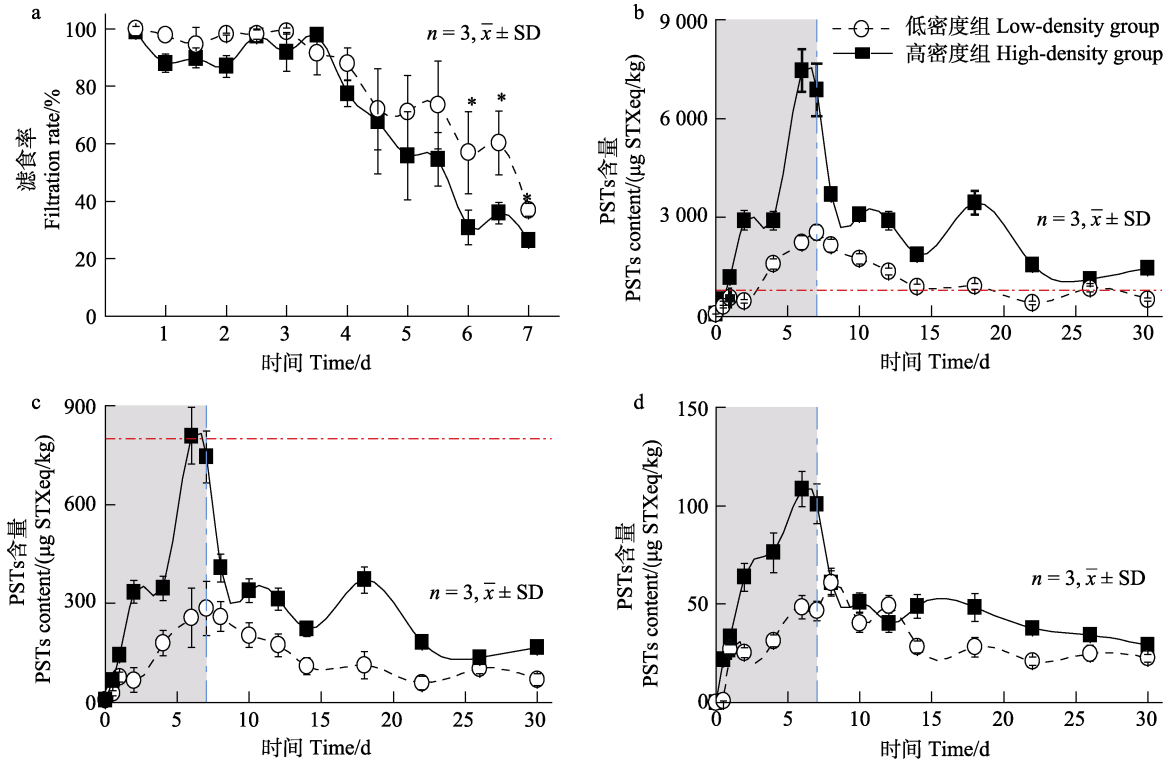

图 2　不同暴露密度下厚壳贻贝滤食率(a)及各组织(b：肝胰腺；c：软组织；d：可食组织)中 PSTs 含量变化

Fig.2　Changes of filtration rate (a) and PSTs content in different tissues (b: Hepatopancreas; c: Soft tissue; d: Edible tissue) of *M. unguiculatus* at different exposure densities

*表示组间有显著性差异(P<0.05)。

* denotes significant difference between groups (P<0.05).

表 2　不同暴露密度下厚壳贻贝各组织 PSTs 含量与其滤食率的相关性分析

Tab.2　Correlation analysis between PSTs content and filtration rate of *M. unguiculatus* at different exposure densities

因子 Factors		线性拟合方程 Linear fitting equation	判定系数 Coefficient of determination, R^2	皮尔森相关系数 Pearson's correlation
高密度组 High-density group	软组织 PSTs 含量	y=–3.160x+4.601	0.643	–0.881
	肝胰腺 PSTs 含量	y=–3.170x+4.527	0.644	–0.879
	可食组织 PSTs 含量	y=–2.755x+4.710	0.569	–0.864
低密度组 Low-density group	软组织 PSTs 含量	y=–6.684x+8.455	0.897	–0.963
	肝胰腺 PSTs 含量	y=–7.858x+9.788	0.924	–0.971
	可食组织 PSTs 含量	y=–2.541x+3.576	0.522	–0.811

2.3 厚壳贻贝中 PSTs 组成及转化

为评估厚壳贻贝对 PSTs 的代谢转化能力，本研究比较了产毒藻与肝胰腺中毒素组分及转化情况。在厚壳贻贝肝胰腺中，除 dcNEO 外 12 种混和标准品 PSTs 组分均有检出。不同暴露密度下厚壳贻贝肝胰腺中优势 PSTs 组分与含量由高到低排序均为 C1、C2、GTX5 (图 3)。主要的毒素转化发生在从产毒藻到内脏的转移过程，转化类型为差向异构化 C2→C1。

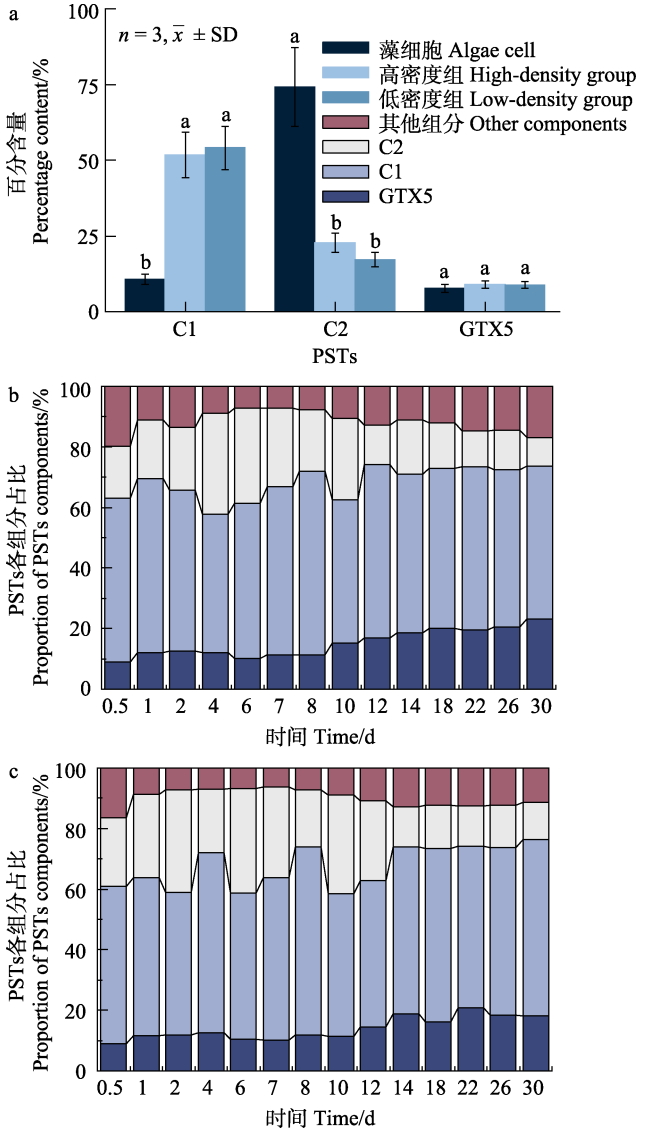

图 3 主要毒素组分在产毒藻和厚壳贻贝肝胰腺中百分含量(a)及暴露期间占比变化(b：高密度组、c：低密度组)

Fig.3 Content of the main toxin components in the hepatopancreas of *A. catenella* and *M. unguiculatus* (a) and changes in proportion during exposure (b: high-density group, c: low-density group)

柱形图上方字母不同表示差异显著(P<0.05)。

Different letters on the column indicate significant differences (P<0.05).

由图 3a 可知，在贻贝摄食产毒藻后 C2 百分含量显著降低(P<0.05)，由 74.1% (藻细胞)分别降低至 22.6% (高密度组)与 17.1% (低密度组)；C1 则由 10.6% (藻细胞)上升 54.1% (高密度组)和 54.0% (低密度组)。不同密度组贻贝与产毒藻中 GTX5 百分含量无显著差异。整个暴露实验中，C1 组分占比维持在 47.1%~62.0%，C2 组分含量占比维持在 13.1%~33.7%，GTX5 组分占比维持在 8.9%~20.8% (图 3b、c)。

3 讨论

3.1 厚壳贻贝 PSTs 蓄积对滤食率的影响

贝类在长期的进化过程中产生了独特的逆境适应机制，因此，在有害赤潮暴发期间维持较高的存活率(Lassudrie *et al*, 2014; Li *et al*, 2017; Turner *et al*, 2019)。有限的研究表明，自然赤潮条件下厚壳贻贝对 PSTs 蓄积能力弱于其他贻贝(Kim *et al*, 2006)。之前有诸多学者开展了紫贻贝在室内暴露条件下的 PSTs 蓄积代谢规律研究。邱江兵(2014)研究紫贻贝肝胰腺日平均蓄积速率可达到 1 736.0 μg STXeq/kg/d，最高 PSTs 含量为 868.0 μg STXeq/100g，为本研究 PSTs 最高含量的 1.2 倍。Sekiguchi 等(2001)将紫贻贝暴露于塔玛亚历山大藻中，其肝胰腺日平均蓄积速率为 1 370.0 μg STXeq/kg/d。本研究暴露组厚壳贻贝肝胰腺 PSTs 日平均蓄积速率均低于上述研究。此外，其他组织蓄积能力也相对较低，如厚壳贻贝软组织日平均蓄积速率显著低于翡翠贻贝(917.5 μg STXeq/kg/d)，厚壳贻贝最高 PSTs 含量也仅为翡翠贻贝的 22.0% (Andres *et al*, 2019)。张海涛等(2023)采用相同暴露条件下，紫贻贝肝胰腺 PSTs 含量与本研究相当，但可食组织中 PSTs 含量约为本研究中厚壳贻贝的 2 倍。本研究中，仅高密度暴露条件下厚壳贻贝第 6 天 PSTs 含量超过安全限量值。与之相比，Andres 等(2019)的研究中产毒藻暴露下翡翠贻贝仅 16 h 软组织 PSTs 含量就高于安全限量值。综上可见，厚壳贻贝对 PSTs 蓄积能力相对较弱。

双壳贝类对有害藻华的反应涉及复杂的生理和行为机制(Freitas *et al*, 2020; Lavaud *et al*, 2021)，短期影响主要包括摄食降低(Pousse *et al*, 2018)，闭壳肌关闭等摄食行为的影响(Comeau *et al*, 2019)。如 Bianchi 等(2019)的研究中利用链状亚历山大藻与塔玛亚历山大藻暴露紫贻贝，7 d 发现其滤食率降低 20.0%~30.0%。Fernandez-Reiriz 等(2008)的研究中，暴露于链状亚历山大藻的智利贻贝(*M. chilensis*) 1 d 后，其对产毒藻的滤食率与吸收消化降低约 67.0%。本研究

厚壳贻贝从蓄积阶段第 3 天开始滤食率持续下降，导致 PSTs 蓄积的降低，胡杨杨等(2019)研究发现，暴露于腹泻性贝类毒素的过程中厚壳贻贝也发现类似现象。这种摄食行为的变化为 PSTs 在双壳贝类体内蓄积的结果(Andres *et al*, 2019; Stüken *et al*, 2015)。狮爪扇贝(*Nodipecten subnodosus*)暴露于产 GTX 类毒素的链状裸甲藻，发现其出现滤食率降低后，依次出现壳瓣部分关闭、假粪增加、消化腺出现严重的氧化损伤等生理反应(Estrada *et al*, 2007)。综上所述，PSTs 暴露下的双壳贝类会产生摄食生理应激来减少其对产毒藻的摄食。

滤食率与产毒藻密度呈显著负相关(P<0.05)，这与相同暴露条件下紫贻贝滤食率随着毒素的蓄积呈正相关结论相反(张海涛等, 2023)。Tobke 等(2021)研究发现，智利贻贝对产毒藻中的有机物利用率远远低于非产毒藻。Lavaud 等(2021)研究发现，产毒藻暴露导致蓝贻贝(*M. edulis*)瓣膜开启幅度的降低，可显著减轻产毒藻对双壳贝类的生理影响。但其暴露实验中蓝贻贝的日平均蓄积速率为 516.0 μg STXeq/kg/d，高于本研究暴露组厚壳贻贝软组织中 PSTs 日平均蓄积速率，说明厚壳贻贝比蓝贻贝对 PSTs 的敏感性更高。此外，Lavaud 等(2021)研究表明，产毒藻的毒性大小、密度以及投喂方式都能够影响贻贝蓄积能力。因此，产毒藻差异会对厚壳贻贝中毒素蓄积产生影响，需要开展系统研究。

3.2　产毒藻暴露下厚壳贻贝中 PSTs 的代谢转化

双壳贝类肝胰腺为 PSTs 蓄积靶器官，通常该组织富集毒素后向其他组织转移完成代谢转化(Meng *et al*, 2023)。如扇贝的肝胰腺被证实具有 PSTs 解毒的功能(Wang *et al*, 2017)，毒素转移至代谢器官被转化降解，该过程可减轻组织损伤并促进机体恢复，对提升贝类的 PSTs 耐受能力具有重要影响。本研究厚壳贻贝中 PSTs 分布组织差异性显著，这与其他双壳贝类组织差异一致(Bianchi *et al*, 2019; 邴晓菲等, 2017)。高密度组厚壳贻贝各组织仅 2 d 代谢了约 50.0%的 PSTs，清除速率为 25.0%/d，为其他双壳贝类的 4.2~4.6 倍(邴晓菲等, 2017; 沈和定等, 2011)。厚壳贻贝低密度组各组织 PSTs 含量在代谢 7 d 后均低于欧盟安全限量标准，而暴露于相同时间和相同产毒藻密度的紫贻贝中 PSTs 含量远高于厚壳贻贝，高密度组紫贻贝肝胰腺维持在 2 000.0 μg STXeq/kg，低密度组紫贻贝肝胰腺维持在 1 000.0 μg STXeq/kg。这说明厚壳贻贝在具有低蓄积能力的同时，还具有快速代谢与低 PSTs 残留率等特点。

双壳类动物组织中最常见的生物转化为差向异构化，主要由藻类产生的 β-差向异构体(C2、GTX3 和 GTX4)在双壳类动物摄取后通常转化为热力学更稳定的 α-差向异构体(C1、GTX2 和 GTX1)(Sekiguchi *et al*, 2001)。暴露实验发现，产毒藻到内脏的转移过程中的毒素转化表现为 C2→C1 差向异构体转化。除此之外，在贻贝体内还检测出产毒藻中未检出的几种毒素组分(STX、dcSTX、GTX1、GTX2、dcGTX2 和 dcGTX3)，这是产毒藻中毒素组分转化的结果。例如，林卓如等(2022)将毛蚶(*Scapharca subcrenata*)暴露于太平洋亚历山大藻，发现毛蚶中出现了产毒藻中未检出的 GTX2 和 GTX3 两种组分，并推测是由 GTX1 与 GTX4 转化而来。本研究暴露实验中 3 种组分占比均无明显改变，这说明 3 种组分在贻贝中达到平衡比例后停止转化。而 Tang 等(2021)研究发现，厚壳贻贝在 PSTs 代谢阶段可以将高毒性的 GTX1/4 转化为低毒性的 C1/2。邱江兵(2014)报道紫贻贝体内出现了明显的 α、β 差向异构毒素的转化，并将塔玛亚历山大藻中的 GTX6 全部转化为 GTX5。Kim 等(2015)比较产毒藻和贻贝 PSTs 组分，发生的主要转化类型为 C2、GTX4 转化为 C1 和 GTX1。而 Wu 等(2022)研究表明，在自然条件下紫贻贝中毒素主要以原型组分直接代谢排出。本研究中，厚壳贻贝中 3 种毒素组分占比由高到低依次为 C1、C2、GTX5，且未发生转化现象。综上可见，不同贻贝对 PSTs 的转化能力存在差异。

4　结论

本研究探究了不同密度链状亚历山大藻暴露对厚壳贻贝 PSTs 蓄积、滤食率及代谢转化的影响。研究表明，厚壳贻贝对链状亚历山大藻的滤食率与产毒藻密度、PSTs 含量呈显著负相关，这是其对 PSTs 蓄积产生的应激反应。厚壳贻贝对 PSTs 的快速代谢与低转化、低残留等特点也表明其食用风险低于其他贻贝。研究结果为进一步探讨厚壳贻贝中 PSTs 风险形成规律、科学构建防控技术提供了研究基础。

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Accumulation of Paralytic Shellfish Toxins in *Mytilus unguiculatus* and Its Effect on Filtration Rate

YANG Yuecong[1,2], ZHENG Guanchao[2], ZHAO Huihui[2], ZHANG Haitao[2], TAN Zhijun[2,3], WU Haiyan[2①]

(1. *College of Food Science and Technology, Shanghai Ocean University, Shanghai* 201306, *China*; 2. *Key Laboratory of Testing and Evaluation for Aquatic Product Safety and Quality, Ministry of Agriculture and Rural Affairs, Yellow Sea Fisheries Research Institute, Chinese Academy of Fishery Sciences, Qingdao* 266071, *China*; 3. *State Key Laboratory of Mariculture Biobreeding and Sustainable Goods, Yellow Sea Fisheries Research Institute, Chinese Academy of Fishery Sciences, Qingdao* 266071, *China*)

Abstract Paralytic shellfish toxins (PSTs) are a class of neurotoxic marine biotoxins that are widely distributed and cause more than 2,000 poisoning events worldwide each year, with mortality rates of up to 15%. PSTs can accumulate through the food chain and are mainly distributed in marine organisms such as bivalve mollusks. Several countries and regions, including the European Union, China, and the United States, have established strict regulatory limits (400 MU/100 g or 800 μg STXeq/kg) for PSTs and implemented monitoring programs. As reported previously, factors such as filtration rate, selective feeding, and the efficiency of the organism in absorbing toxin-producing algae can significantly affect the accumulation of PSTs in bivalve mollusks. *Mytilus unguiculatus* is one of three major commercial mussel species in China, with important economic value and social impact. Due to its high nutritional value, it is extensively cultured as an important shellfish species in the Zhoushan archipelago of Zhejiang Province in China. *Alexandrium* spp. are the main toxin-producing algae in the area. PSTs have been detected in mussels between May and July after harmful algal blooms. Research into the elimination characteristics of PSTs accumulation in *M. unguiculatus* is urgently needed to establish a monitoring and control program.

In this study, 760 mussels were randomly selected and fed *A. catenella* at different cell densities, with a high-density group (7.00×10^5 cells/d), a low-density group (2.80×10^5 cells/d), and a control group. The experimental period lasted for 30 d, during which the accumulation period approximately represented days 1–7 and the elimination period days 8–30 d. A total of 14 sampling points were set up on days 0.5, 1, 2, 4, 6, and 7 of the accumulation period and days 1, 3, 5, 7, 11, 15, 19, and 23 of the elimination period. Six mussels were randomly collected at each sampling point and dissected into soft tissues, hepatopancreas, and edible tissues. Liquid chromatography-tandem mass spectrometry was used to determine the content of PSTs. During the accumulation period, 5 mL culturing seawater was collected during or 1 h after feeding, and Ruge's solution was added. The filtration rate of the mussels was determined by counting the quantity of *A. catenella* cells in the water. The results showed that the toxins in *M. unguiculatus* were not equally distributed. The highest PST content in hepatopancreas tissues was 7,458.2 μg STXeq/kg (high-density group) and 2,555.9 μg STXeq/kg (low-density group). The highest PST content in edible tissues was 108.6 μg STXeq/kg (high-density group). The hepatopancreas was identified as a target organ for toxin accumulation. From day 3 to day 7, the filtration rate of mussels decreased, eventually reaching 30% of its initial value. The filtration rate of *M. unguiculatus* in the high-density group was not significantly different from that of the low-density group during days 1–5 and was significantly lower during days 5–7. During the elimination phase, the PST elimination rate in mussels was 18.4% (hepatopancreas), 18.1% (soft tissues), and 13.1% (edible tissues). At day 30, the residual content of PSTs in the hepatopancreas of mussels was approximately 1 400 μg STXeq/kg in the

① Corresponding author: WU Haiyan, Email: wuhy@ysfri.ac.cn

high-density group and 600.0 μg STXeq/kg in the low-density group. Changes in the proportion of each PST component were transferred from *A. catenella* to *M. unguiculatus*. The proportion of C2 was significantly reduced from 74.1% (*A. catenella*) to 22.6% (high-density group) and 17.1% (low-density group) (P<0.05); the percentage of C1 increased from 10.6% (*A. catenella*) to 54.1% (high-density group) and 54.0% (low-density group) (P<0.05). No significant difference was observed in the percentage of GTX5 between *A. catenella* and mussels in the different density groups (P>0.05). No significant conversion was observed between the PST components in the hepatopancreas of mussels throughout the experiment.

Our data indicate that the daily accumulation rate of PSTs in *M. unguiculatus* was lower than that in other mussels. Moreover, the toxin elimination rate was higher than that of other mussels. A negative correlation was observed between the filtration rate of *M. unguiculatus* and the PST content of each tissue type. These results show that *M. unguiculatus* is more sensitive to PSTs than other mussels. During the stage of PST transfer from *A. catenella* to *M. unguiculatus*, a high proportion of C2 toxin was converted to C1 toxin. After accumulating in the hepatopancreas, the PST profile exhibited relatively stable performance. In summary, we conclude that, due to higher susceptibility to toxins and lower conversion rates, a lower risk is associated with the consumption of *M. unguiculatus* than with that of other mussels. Our findings will contribute to improving our understanding of the mechanisms underlying the PST accumulation risk in *M. unguiculatus* and provide valuable scientific insights for developing prevention and risk management strategies concerning PSTs.

Key words　*Mytilus unguiculatus*; Paralytic shellfish toxins; Biotransformation; Filtration rate