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Cu^{2+}胁迫对魁蚶生理生化和组织结构的影响

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摘要: 采用生物毒性测试的方法, 研究了持续暴露96 h不同浓度Cu^{2+}胁迫对魁蚶生理代谢、组织结构及酶活性的影响。实验设置0.01、0.05、0.10、0.50和1.00 mg/L 组5个浓度梯度, 不加Cu^{2+}的正常海水作为对照。结果显示, Cu^{2+}胁迫对魁蚶耗氧率(OR)、排氨率(NR)、氧氮比(O∶N)均有显著影响, 并且与暴露时间和浓度有关。初次暴露时, OR, NR和O∶N都迅速下降, 最低值都出现在1.00 mg/L浓度组, 分别为(0.005±0.001) mg/(g·h)、(0.5±0.05) μmol/(g·h)、0.7±0.1, 仅为对照组的1%、29%和3%。暴露72 h内Cu^{2+}胁迫均使魁蚶代谢率不同程度下降, 之后不同处理组出现差异分化。暴露96 h时, 魁蚶对低浓度Cu^{2+}暴露表现出适应, 0.01 mg/L处理组呼吸代谢恢复至正常水平, O∶N与对照组无差异, 组织结构也未见明显损伤; 浓度超过0.05 mg/L处理组魁蚶生理代谢及组织结构受显著影响, O∶N大多降至9以下, 出现鳃丝受损和组织结构散乱等明显损伤; 0.10 mg/L处理组魁蚶体内ACP和ALP活性在鳃组织中增强, 肝脏中受到抑制, 而GPX和GST活性增强。研究表明持续暴露96 h Cu^{2+}浓度≥0.05 mg/L环境显著影响魁蚶生理代谢及组织结构, 0.10 mg/L Cu^{2+}浓度显著影响魁蚶组织中ACP、ALP、GPX和GST的活性。研究结果为认知魁蚶等滩涂贝类对Cu^{2+}胁迫的响应机制提供基础数据, 为预防滩涂潜在重金属污染风险及生物修复提供参考。

关键词: 魁蚶; Cu^{2+}胁迫; 生理生化; 组织结构
中图分类号: S 968.31 **文献标志码:** A

近年长江、闽江和珠江等主要水系表层沉积物中均有较高浓度Cu^{2+}积累[1], 莱州湾入海河水中Cu^{2+}浓度甚至高达2.76 mg/L[2]。滩涂是陆源工业污染与海源航运污染主要受纳场所, 重金属污染曾引起滩涂贝类大规模死亡[3]。“蓝牡蛎”和“绿牡蛎”现象就是滩涂贝类受重金属Cu^{2+}污染所致[4], 近年研究显示乐清湾、广东沿海等地表层沉积物中Cu^{2+}污染已存在生态风险[5-6], 滩涂是滩涂贝类赖以生存的环境, 滩涂养殖区有潜在Cu^{2+}污染风险。重金属对滩涂贝类影响的研究主要集中在生物毒性、富集和免疫响应等方面[7-11], 重金属污染尤其是Cu^{2+}胁迫对滩涂贝类生理生化[12]及组织损伤的研究较少, 持续胁迫对生理代谢的影响尚未涉及。

魁蚶(*Scapharca broughtonii*)隶属于软体动物门(Mollusca), 蚶属(*Arca*), 是亚洲重要滩涂增养殖经济贝类之一, 但由于环境污染、海洋生态系统恶化和过度捕捞等原因导致魁蚶资源减退[13],

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近年，魁蚶引起越来越多的关注与研究[14]，而有研究发现其软体部Cu^{2+}含量低于其他双壳贝类[15]，但对其组织结构和免疫响应尚不清晰。针对沿海滩涂潜在Cu^{2+}污染风险，选取魁蚶作为生物毒性测试的对象，并基于现有研究推测魁蚶生理可能对Cu^{2+}污染较为敏感。

本研究从魁蚶生理代谢、组织结构和组织内酶活性等不同层次入手，研究了不同浓度和持续暴露Cu^{2+}胁迫对魁蚶生理代谢、组织内酶活性的影响规律，以期为揭示魁蚶等滩涂养殖贝类对Cu^{2+}胁迫的响应机制提供基础数据，为滩涂潜在重金属污染风险评估预防及生物修复提供参考依据。

1 材料与方法

1.1 实验材料

实验在中国水产科学研究院黄海水产研究所海洋生物碳汇功能实验室进行。将魁蚶置于体积为60 L的聚乙烯箱暂养7 d，暂养密度为1个/L。期间连续充气适量投喂球等鞭金藻(*Isochrysis galbans*)3 011，每日换水1次，暂养水温为(20±1) ℃，pH为8.1±0.1，溶解氧为(6.5±0.3) mg/L，盐度为32.2±0.3。实验用海水Cu^{2+}背景值<0.003 mg/L，经过滤和充分曝气。实验前24 h停止投喂，选取健康、反应灵敏的个体随机分组。实验环境条件和实验密度等同暂养条件。实验魁蚶壳长为(37.7±1.3) mm，壳宽为(21.7±1.1) mm，壳高为(28.3±1.4) mm，湿重为(13.2±1.7) g。$CuSO_4·5H_2O$(分析纯)购自国药集团，用超纯水配制成1 g/L Cu^{2+}母液。

1.2 实验方法

基于96 h生物毒性测试实验结果，每24 h测定生物生理代谢变化作为反映环境污染程度的指标[16]。根据预实验结果(耗氧率显著变化)和国家海水水质标准[17]，设定5个处理组，Cu^{2+}浓度分别为0.01(国家二类水质标准)、0.05(国家三类和四类水质标准)、0.10、0.50和1.00 mg/L，分别简记为处理1、处理2、处理3、处理4和处理5组，不加Cu^{2+}的正常海水作为对照组；暂养后的魁蚶直接放入不同浓度Cu^{2+}溶液中进行代谢率等指标测定，作为初次暴露，记为T_i，以后每暴露24 h测定相应指标，分别记做T_{24}、T_{48}、T_{72}和T_{96}。实验容器与暂养容器相同，实验前所有容器用相同Cu^{2+}浓度海水浸泡24 h，避免容器吸附。每组3个平行，期间每暴露24 h取样后换水1次，适量充气。呼吸瓶为3 L聚乙烯塑料瓶，每瓶放4个魁蚶，加入不同Cu^{2+}浓度海水密封4 h。溶解氧和氨氮分别采用Winker碘量法与次溴酸钠氧化法测定[18]，根据空白瓶和实验瓶溶解氧(DO_0、DO_t，mg/L)与氨氮(N_0、N_t，μmol/L)含量，呼吸瓶体积V (L)、密封时间t (h)及魁蚶软体部干重W (65 ℃烘干至恒重，g) 计算耗氧率(OR) [mg/(g·h)]=$[(DO_0-DO_t)\times V]/(W\cdot t)$和排氨率(NR) [μmol/(g·h)]=$[(N_t-N_0)\times V]/(W\cdot t)$；氧氮比(O∶N)用魁蚶呼吸氧原子数与排出氨态氮原子数之比[19]。实验所有废水经沉淀、藻类吸附处理后排放。

选取处理1、3和5组，Cu^{2+}胁迫96 h进行组织学观察，并与对照组比较。每个处理组魁蚶取鳃、外套膜、斧足和肝脏组织，用Bouin氏液固定，经酒精梯度脱水、二甲苯透明与石蜡透明、石蜡包埋与修块，用切片机5 μm切片和苏木精—伊红染色(H.E)后，用Nikon 80i显微镜观察并拍照。

采集对照组和处理3组暴露24和96 h魁蚶鳃和肝脏组织样品测定酶活性。每组4个平行，用于酸性磷酸酶(ACP)、碱性磷酸酶(ALP)、谷胱甘肽过氧化物酶(GPX)、谷胱甘肽转移酶(GST)活性以及蛋白质含量测定，均采用试剂盒(南京建成生物工程研究所)进行测定。

1.3 数据处理

采用SPSS 19.0对实验数据分别进行Cu^{2+}浓度、处理时间及组织内酶活性的单因素方差分析(One-Way ANOVA)，比较相同浓度或时间下，魁蚶代谢速率变化规律和组织内酶活性差异；同时进行Cu^{2+}浓度与处理时间的双因素方差分析(Two-Way ANOVA)，比较两种因素的影响程度。显著性水平均为0.05。

2 结果

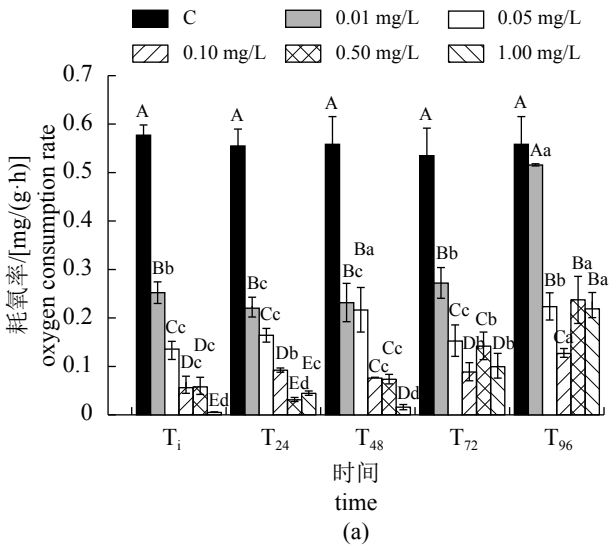
2.1 Cu^{2+}对魁蚶生理代谢的影响

实验期间对照组魁蚶OR为(0.6±0.04) mg/(g·h)，差异不显著($P>0.05$)。Cu^{2+}浓度和暴露时间对魁蚶OR均有显著影响($P<0.05$)，且存在交互作用。与对照组相比，T_{24}、T_{48}和T_{72}内Cu^{2+}胁迫

均使魁蚶耗氧率下降，T_i时处理组魁蚶OR仅为对照组的44%~1%，处理5组OR最低，为(0.005±0.001) mg/(g·h)；T_{96}时，处理1组OR升高，与对照组无显著差异(P>0.05)，而处理4组和5组则下降为对照组的58%~78%。相同处理组随着暴露时间的延长，OR总体呈上升趋势(图1-a)。

对照组魁蚶排氨率NR为(1.7±0.08) μmol/(g·h)，差异不显著(P>0.05)。Cu^{2+}胁迫浓度、暴露时间对魁蚶NR均有显著的影响(P<0.05)，且存在交互作用。T_i时，处理组魁蚶NR为对照组的71%~29%，其中处理5组NR最低，为(0.5±0.05) μmol/(g·h)。T_{24}、T_{48}、T_{72}内魁蚶排氨率不同程度下降，之后出现明显分化。T_{96}时，处理1组NR接近对照组，处理2组和3组下降为对照组的73%~68%，处理4组和5组出现升高现象，是对照组的1.18和1.22倍。随着暴露时间的增加，相同Cu^{2+}浓度处理组魁蚶NR呈现波动上升趋势(图1-b)。

对照组魁蚶氧氮比(O：N)为20.7±2.4，差异不显著(P>0.05)。与OR和NR相同的是，Cu^{2+}胁迫浓度和暴露时间对魁蚶O：N也有显著的影响(P<0.05)，且存在交互作用。T_i时，魁蚶O：N随Cu^{2+}浓度升高而下降，处理1组最高，为13.2±1.5，处理5组最低，为0.7±0.1；与对照组相比，Cu^{2+}胁迫在T_{24}、T_{48}和T_{72}均使魁蚶O：N下降；处理1组O：N在T_{96}时与对照组相比无显著差异(P>0.05)；而处理2、3、4和5组O：N分别为11.2±0.3、6.8±0.3、7.3±0.4和6.6±0.3。相同Cu^{2+}浓度处理组随着暴露时间的增加，魁蚶O：N总体呈波动上升趋势(图1-c)。



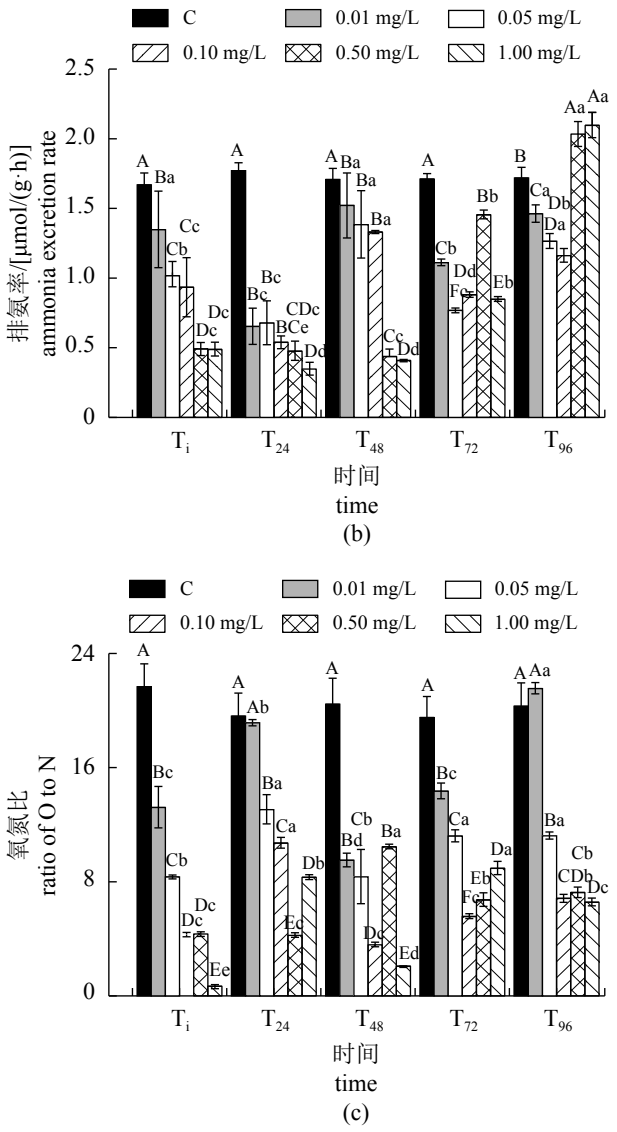


图 1　Cu^{2+}胁迫对魁蚶耗氧率(a)、排氨率(b)和氧氮比(c)的影响

C.对照组；不同大写字母表示相同暴露时间不同处理之间差异显著(P<0.05)；不同小写字母表示相同浓度不同暴露时间之间差异显著(P<0.05)

Fig. 1　Effect of Cu^{2+}stress on oxygen consumption rates (a), ammonia excretion rates (b) and ratios of O to N (c) of *S. broughtonii*

C. control groups; different upper letters on top of the bars at the same exposure duration indicate significant differences among groups (P<0.05); values with different lower case letter for the same concentration of copper treatment indicate significant differences among groups (P<0.05)

2.2　Cu^{2+}胁迫对魁蚶组织结构的影响

对照组魁蚶鳃丝排列整齐平滑，外套膜黏膜均匀无充血，外套膜边缘平整无充血(图2-a)；暴露96 h处理5组中魁蚶鳃丝排列散乱，收缩为

一团，鳃丝部分缺失、颜色呈灰白或者褐色，较少数鳃丝伴有血丝，外套膜黏膜大面积充血，外套膜边缘充血并向内翻卷(图2-b)。

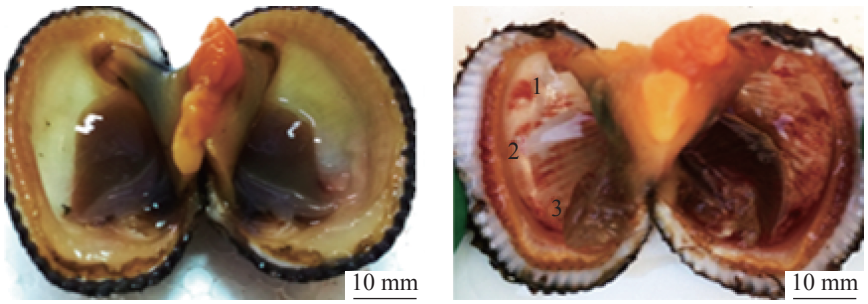
与对照组相比，暴露96 h处理1组对魁蚶鳃组织显微结构无明显影响；处理3组和5组鳃丝尖端出现膨大、细胞融合甚至细胞溶出，鳃丝排列散乱甚至无法保持正常状态，鳃丝间连接组织散乱甚至破碎以至较多部分脱离(图版Ⅰ)。暴露96 h处理1组和3组的魁蚶斧足显微结构未有显著改变，而处理5组肌纤维组织变的疏松散乱，部分肌肉组织蜷缩一团(图版Ⅱ)。暴露96 h处理1组和3组未有显著改变；处理5组外套膜显微结构中肌纤维疏松散乱，结缔组织出现细胞融合堆积(图版Ⅲ)。暴露96 h处理1组未有显著改变；处理3组和5组魁蚶肝脏组织连接处出现断裂，无法保持原有形态(图版Ⅳ)。

2.3 Cu^{2+}胁迫对魁蚶组织内酶活性的影响

对照组魁蚶鳃和肝脏组织中ACP、ALP活性分别为(76.4±7.0) U/(g prot)、(111.6±17.0) U/(g prot)和(91.1±7.8) U/(g prot)、(45.6±6.0) U/(g prot)，GPX、GST活性分别为(46.0±1.4) U/(mg prot)、(31.1±1.4) U/(mg prot)和(36.2±2.2) U/(mg prot)、(39.1±6.5) U/(mg prot)。暴露96 h处理3组对魁蚶鳃、肝脏组织中ACP、ALP、GPX和GST活性均有显著影响(P<0.05)。其中，暴露96 h魁蚶鳃组织中ACP和ALP活性显著高于对照组和暴露24 h组 (P<0.05)，其值分别为(172.6±9.3) U/(g prot)、(175.1±6.7) U/(g prot)，而肝脏中ACP和ALP均显著低于对照组(P<0.05)(图3-a)；对照组魁蚶鳃组织及肝脏中GPX和GST均显著低于暴露24 h和96 h组(图3-b)。

3 讨论

生物毒性测试是研究有毒有害污染物对生态系统中生物所引发的分子、细胞、器官、个体、群落等不同水平的损害[16]。Cu^{2+}作为生物体必需的一种微量元素，只要浓度不超过机体代谢需求就不具有毒性[20-21]。对照组魁蚶代谢率与现有报道中魁蚶耗氧率为0.62~0.66 mg/(g·h)，排氨率为1.66~2.22 μmol/(g·h)，氧氮比为19.1~23.8的研究结果相接近[22-23]；0.01 mg/L Cu^{2+}浓度暴露组代谢率先下降，而在96 h都恢复至与对照组相近水平，可见该浓度在魁蚶生理代谢可调节范围内，一旦Cu^{2+}浓度超过0.05 mg/L，对魁蚶代谢率均产生显著影响。但也有研究认为Cu^{2+}浓度达到更高值时才会对贝类代谢产生显著影响，超过0.20 mg/L时，显著抑制河蚬(*Corbicula fluminea*)的呼吸代谢[24]；高于0.33 mg/L时，对菲律宾蛤仔(*Ruditapes philippinarum*)呼吸代谢产生显著影响[25]；大于0.40 mg/L时，对泥蚶(*Tegillarca granosa*)的代谢产生显著影响[12]。本研究中魁蚶对Cu^{2+}的耐受性低于上述研究结果，可能是魁蚶对Cu^{2+}胁迫更为敏感。有研究认为当存在重金属污染时可降低或提高海洋双壳贝类的耗氧率，而耗氧率降低说明贝类可能进行了部分无氧代谢[26]；初次胁迫时，不同Cu^{2+}浓度胁迫导致魁蚶的OR、NR显著下降，可能因为魁蚶对Cu^{2+}暴露进行主动防御，降低了活动，减少了对能量的需求；随着暴露时间的延长，机体逐渐适应了Cu^{2+}持续胁



(a) (b)

图 2 Cu^{2+}胁迫对魁蚶组织结构的影响

(a)对照组，(b)1.00 mg/L组(96 h)；1. 外套膜黏膜充血；2. 外套膜边缘充血和翻卷；3. 鳃组织散乱和缺失

Fig. 2 Effect of Cu^{2+} stress on the tissue structure of *S. broughtonii*

(a)control group, (b)1.00 mg/L group (96 h); 1.the mucous membrane of mantle with extensive congestion; 2.the edge of mantle with congestion and turnover; 3.the messy and missing gill

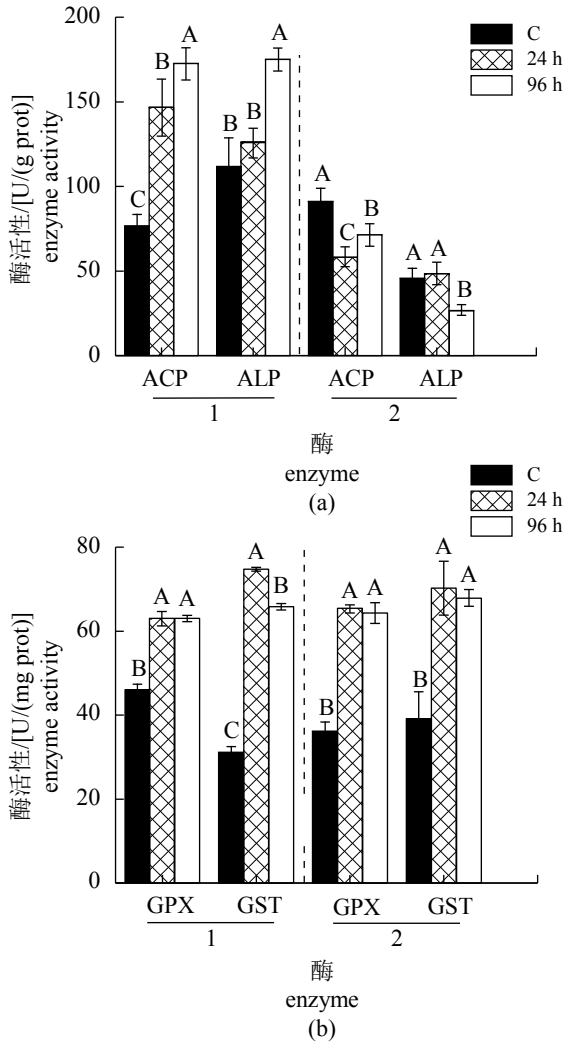


图 3　Cu^{2+}胁迫对魁蚶ACP、ALP(a)和GPX、GST(b)酶活性的影响

Cu^{2+}浓度为0.10 mg/L；1.鳃，2.肝脏；大写字母表示魁蚶酶活性差异显著(P<0.05)

Fig. 3　Effect of Cu^{2+} stress on the enzyme activities in *S. broughtonii*

Cu^{2+} concentration was 0.10 mg/L; 1.gill, 2.hepatopancreas; date with different upper case letters indicate significant difference among groups (P<0.05)

迫，OR和NR呈上升趋势，这与Moore等[27]的研究结果一致，他们认为耗氧增加表明机体对能量的需求增加，而排氨增加表明蛋白质分解代谢的加快。在高浓度和持续暴露下，魁蚶生理代谢又出现了OR和NR大幅度增加的现象，可能是Cu^{2+}胁迫导致组织受损和代谢紊乱所致，组织切片也反映高浓度Cu^{2+}胁迫导致魁蚶在96 h组织溃散。

O：N反映生物体内代谢情况，可推测特定环境条件下生物的供能方式，进而可以作为评估生理代谢的生物标志物[28-29]；同时O：N作为一种在实验条件下对胁迫敏感的指标，可以评估环境胁迫对贝类的毒性大小[30]。一般认为，O：N在7~9，则由蛋白质代谢供能占主导；O：N高于24，则由碳水化合物代谢供能占主导[31-33]；O：N低于15，则可能代谢大量蛋白质以满足机体抵御外界环境变化所需要的能量[34]。本研究中对照组O：N比为20.7±2.4，以消耗碳水化合物为主；Cu^{2+}浓度≥0.05 mg/L，O：N大多在9以下，说明魁蚶消耗大量蛋白质以抵御Cu^{2+}胁迫带来的影响，不利于魁蚶个体正常生长发育。因此，O：N可作为评估Cu^{2+}胁迫对魁蚶毒性大小的生理指标之一。

组织切片是一种经典而直接观察贝类组织结构受环境影响的手段，能有效反映贝类生理代谢状况。Bignell等[35]研究不同季节、疾病等因素对贝类组织结构的影响；有研究认为重金属主要积累在长牡蛎(*Ostrea gigas*)鳃血细胞、消化腺周围的结缔组织中，还有部分积累在鳃丝尖端[7]；Cd^{2+}和Pb^{2+}对泥蚶的鳃和肝胰腺组织也有类似的影响[36]。因此，本研究中通过对魁蚶的鳃、斧足、外套膜和肝脏等进行组织学观察，发现Cu^{2+}胁迫主要对魁蚶的鳃、肝脏、外套膜组织造成损伤，与解剖观察结果一致，其中0.01 mg/L组暴露96 h魁蚶组织结构未受明显影响，而0.10 mg/L组部分组织受到损伤，1.00 mg/L组出现大部分鳃丝受损严重、肝脏组织间连接受到破坏、斧足肌肉组织受损和外套膜组织出现细胞堆积等现象，与上述研究结果类似。魁蚶鳃、肝脏和外套膜等组织对Cu^{2+}胁迫较为敏感，反映出Cu^{2+}胁迫对魁蚶的生物毒性。

ACP作为典型的溶酶体酶参与外源蛋白质等物质的清除和分解的免疫过程，ALP作为多功能酶在贝类免疫系统中发挥重要作用[37]。本研究中暴露96 h的魁蚶鳃组织中，ACP和ALP活性显著受到诱导，表明魁蚶增强了免疫系统以应对持续的Cu^{2+}胁迫，而这可能与“毒物兴奋效应”有关，即为毒物作用下生物体内酶活性升高的应激反应，反之则称为“毒物抑制效应”[38]。实际上“毒物兴奋/抑制效应”受多重因素影响，即使同一物种也会因组织不同而异，暴露96 h魁蚶肝脏中ACP和ALP活性受到显著抑制则可能与“毒物抑制效应”有关，这表明魁蚶免疫系统遭到一定程度的损伤，并在组织结构上反映出，而组织

结构的损伤可能由氧化损伤引起。一般认为重金属污染物会引起生物体内的活性氧(ROS)自由基增加，对生物体造成活性氧损伤甚至死亡，ROS进一步分解产生H_2O_2，而GPX和GST则在转运与分解H_2O_2中发挥重要作用[39]。暴露24和96 h的魁蚶鳃与肝脏组织中GPX、GST活性显著升高，可能与上述“毒物兴奋效应”有关，趋势与Won等[40]对菲律宾蛤仔的研究结果相同，本研究表明，持续的Cu^{2+}胁迫导致魁蚶体内产生过量的H_2O_2，体内抗氧化防御系统增强以减少过量的H_2O_2带来的氧化损伤对组织结构的破坏。

魁蚶生理代谢对Cu^{2+}污染较为敏感，Cu^{2+}浓度≥0.05 mg/L时，魁蚶就出现不同程度的代谢紊乱，Cu^{2+}胁迫对魁蚶的生物毒性主要表现在组织结构损伤和酶活性的变化等方面。0.50和1.00 mg/L组在暴露96 h后，NR异常升高可能与部分组织溃散有关，具体机制尚不明确；魁蚶较其他滩涂贝类对Cu^{2+}胁迫可能更为敏感，而滩涂养殖区的环境因素较为复杂，未来可结合室内模拟实验与现场实验作进一步探究。

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Effect of Cu^{2+} stress on physiology biochemistry and histopathological structure of *Scapharca broughtonii*

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Abstract: The stressful effect of Cu^{2+} with exposure duration for 96 hours on physiology metabolism, histopathological structure and enzyme activity of ark shell *Scapharca broughtonii* was studied by the methods of biological toxicity test. The incubating concentrations of Cu^{2+} were set at (0.01, 0.05, 0.10, 0.50, 1.00) mg/L and a control group was set with no Cu^{2+} stress (normal seawater). Results showed that incubating concentration and exposure duration both had significant influence on physiological indices of *S. broughtonii*, including oxygen consumption rates (OR), ammonia excretion rates (NR) and ratios of O to N (ON). The OR, NR and ON all decreased sharply after initial exposure to the Cu^{2+} stress. The minimum values for OR, NR and ON were found to be (0.005±0.001) mg/(g·h), (0.5±0.05) μmol/(g·h), 0.7±0.1 with the 1.00 mg/L treatment at the end of the experiment, which equaled 1%, 29%, 3% of control groups, respectively. Generally, apparently reduced metabolic rate of *S. broughtonii* individuals exposed to Cu^{2+} stress was observed within 72 h. After 72 h, each group with different Cu^{2+} concentration behaves differently. After being incubated at 0.01 mg/L for 96 h, the *S. broughtonii* seemed to be acclimated to Cu^{2+} stress. Metabolic rates of the animals were restored to the control level, while ON was not different from the control groups and no detectable histopathologic damage was found. The physiological metabolism and histopathological structure of individuals under concentrations that higher than 0.05 mg/L Cu^{2+} were significantly affected after 96 h, while most of the ON in all the experimental groups were below 9 and histopathologic damage such as gills damage and tissues messy structure were found. The ACP and ALP activities of individuals exposed to 0.10 mg/L Cu^{2+} increased in gill and decreased in hepatopancreas, while GPX and GST increased in both tissues. Our results demonstrated that incubating concentration ($\geqslant$0.05 mg/L) after 96 h exposure significantly affect physiological metabolism and histopathological structure of the ark shell. ACP, ALP, GPX and GST activities of individuals incubated at 0.10 mg/L were significantly affected. Our results provide the basic data for studying the response mechanism of *S. broughtonii* and other coastal shellfishes to Cu^{2+} stress, and referential data for the prevention and biological reparation of potential heavy metal pollution risk in coast area.

Key words: *Scapharca broughtonii*; Cu^{2+} stress; physiological metabolism; histopathological structure

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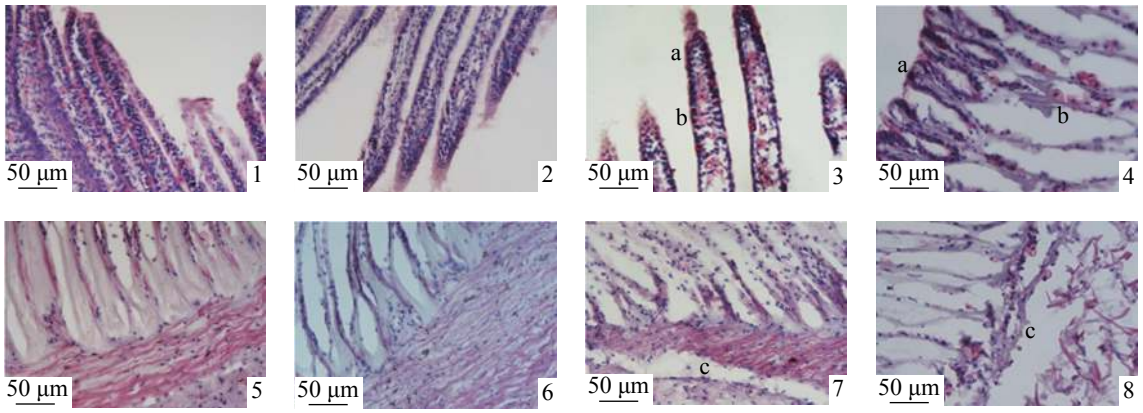
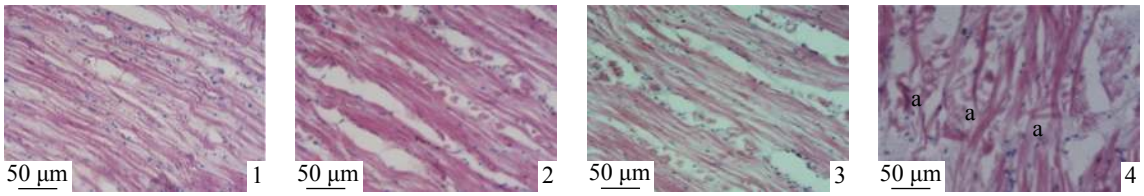


图版 Ⅰ　Cu^{2+}胁迫对魁蚶鳃丝显微结构的影响

1~4.分别为对照组、0.01、0.10和1.00 mg/L 组(96 h)，下同；5~8.分别为上述处理组鳃丝连接；a.鳃丝尖端膨大，b.细胞融合溶出和形态散乱，c.鳃连接组织脱离和破碎

Plate Ⅰ　Effect of Cu^{2+} stress on gill microstructure in *S. broughtonii*

1-4. the gill filaments of the control group, 0.01, 0.10, 1.00 mg/L group (96 h), respectively, the same below; 5-8. the gill connection of those groups; a. inflated top end of gill filaments, b. cell fusion and messy structure, c. diastasis and crush of gill connection

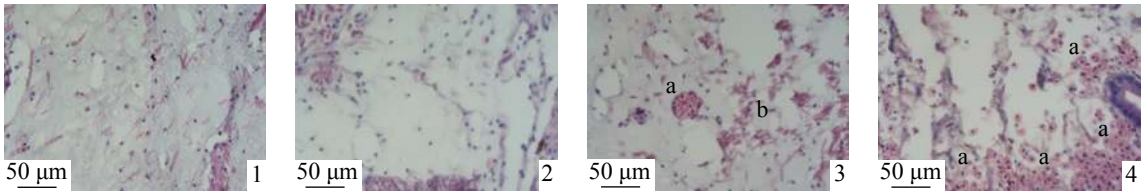


图版 Ⅱ　Cu^{2+}胁迫对魁蚶斧足肌肉显微结构的影响

a.斧足肌肉结构散乱和蜷缩

Plate Ⅱ　Effect of Cu^{2+} stress on foot muscles microstructure in *S. broughtonii*

a. messy and turnover on foot

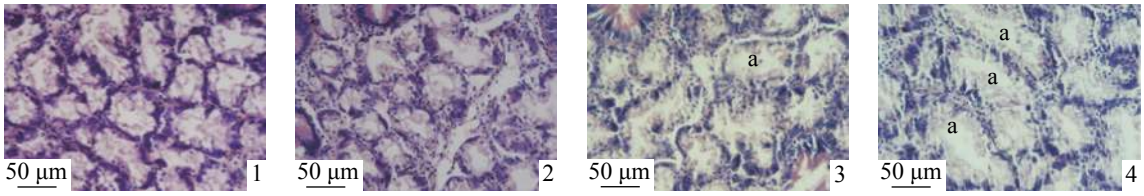


图版 Ⅲ　Cu^{2+}胁迫对魁蚶外套膜显微结构的影响

a.结缔组织细胞融合，b.肌纤维散乱

Plate Ⅲ　Effect of Cu^{2+} stress on mantle microstructure in *S. broughtonii*

a. cell fusion of connective tissue, b. messy structure of muscle fiber



图版 Ⅳ　Cu^{2+}胁迫对魁蚶肝脏显微结构的影响

a.肝脏组织连接处断裂

Plate Ⅳ　Effect of Cu^{2+} stress on hepatopancreas microstructure in *S. broughtonii*

a. the breakage in the connection of hepatopancreas tissue