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溶解氧对三疣梭子蟹争斗行为和能量代谢的影响

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摘要: 为查明溶解氧浓度对三疣梭子蟹(*Portunus trituberculatus*)争斗行为和能量代谢的影响, 本研究利用室内构建的水生动物争斗行为观测系统, 量化了(2.5±0.5) mg/L、(4.5±0.5) mg/L 和(6.5±0.5) mg/L 溶解氧浓度下三疣梭子蟹的争斗行为, 测定了三疣梭子蟹肝胰脏中糖原以及血淋巴中葡萄糖和乳酸含量的变化, 主要结果如下: (1) 随着溶解氧浓度的降低, 三疣梭子蟹争斗持续时间显著减少, 在 2.5 mg/L 处理组达到最小值, 显著低于其他处理组($P<0.05$), 同时个体间的争斗强度也减弱。(2) 随着溶解氧浓度的降低, 三疣梭子蟹争斗行为表现次数显著减少, 且在争斗过程中, 3 个处理组胜利者的接触行为和非接触行为表现次数均显著高于失败者($P<0.05$)。(3) 随着溶解氧浓度的降低, 三疣梭子蟹血淋巴中葡萄糖和乳酸含量均显著增加, 肝糖原含量显著降低($P<0.05$)。(4) 在 3 个溶解氧浓度下, 与争斗前的个体相比, 争斗后三疣梭子蟹肝糖原的含量显著降低, 血淋巴中葡萄糖和乳酸含量均显著升高($P<0.05$)。研究结果初步表明, 低氧条件下三疣梭子蟹无氧呼吸强度增加, 能量供应效率降低且乳酸累积增加, 三疣梭子蟹个体的争斗意愿和争斗强度降低, 能量代谢的差异是导致其争斗行为表现差异的主要原因之一。

关键词: 三疣梭子蟹; 溶解氧; 争斗行为; 能量代谢
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“争斗行为”是同种个体间为了获取生长和繁殖等活动所需的资源而发生的打斗行为[1-4]。糖原和葡萄糖作为机体的能量库, 可为动物的争斗行为提供能量; 而机体能量代谢状况决定动物的争斗成本和争斗策略, 最终影响争斗结果[5-7]。研究发现, 动物能量供应与溶解氧浓度有关, 当处于低氧状态时, 水生动物主要依靠无氧呼吸提供所需能量, 其争斗强度和争斗时间随之降低[8-9]。且争斗引起的功能性缺氧还会导致机体无氧呼吸增强, 乳酸含量提高进而影响争斗策略和后续行为[10]。

在水产养殖活动中, 溶解氧是影响养殖动物存活和生长的重要因素之一[11-12]。受恶劣天气、气候变化以及温跃层的影响, 养殖水体会出现缺氧情况, 高密度养殖更容易导致水体溶解氧浓度下降[13-16]。溶解氧浓度下降时, 鱼虾等摄食减少, 病害增多, 生长缓慢甚至大量死亡[17-18], 同时摄食等行为发生变化[19]。

三疣梭子蟹(*Portunus trituberculatus*)是我国重要的海水池塘养殖蟹类, 2019 年养殖产量达 11.38 万 t[20], 争斗残食导致的低成活率是制约其养殖产量提高的重要因素之一[21]。研究发现, 三疣梭子蟹的争斗行为受温度和资源等外界条件的影响[6,22], 有关溶解氧浓度对其争斗行为和能量代谢影响的研究还未见报道。为探究不同溶解氧浓度下三疣梭子蟹争斗行为和能量代谢的变化, 本研究利用室内搭建的争斗行为观测系统, 采用视频拍摄分析方法, 量化分析了 3 种溶解氧浓度下三疣梭子蟹的争斗行为, 并结合糖原、葡萄糖

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以及乳酸含量的变化，探究能量代谢对争斗行为的响应，研究结果可为三疣梭子蟹池塘养殖管理提供行为学依据。

1 材料方法

1.1 实验材料

实验于 2018 年 8 月在中国海洋大学海水养殖教育部重点实验室进行。实验选用雄性三疣梭子蟹，来源于山东省胶南市琅琊镇养殖厂。在玻璃水族箱(40.5 L, 45 cm×30 cm×30 cm)暂养 7 d，暂养温度为(24±0.5) ℃，盐度为 30，光照周期为 12L : 12D，24 h 持续充气，保持溶解氧浓度为(5.5±0.5) mg/L。每天于 08:00 投喂适量的菲律宾蛤仔(*Ruditapes philippinarum*)，投喂 3 h 后将粪便及残饵清除，并换水 1/3。

1.2 实验设计与方法

暂养结束后，随机挑选附肢完整无损伤、处于蜕皮间期、体型相近的三疣梭子蟹[体长(100.0±5.0) mm, n=192]]开始实验。由于三疣梭子蟹在 2 mg/L 及以下的溶解氧浓度无法存活，溶解氧至少维持 4 mg/L 以上才能保证其正常的生长发育，其适宜生长的溶解氧浓度为 5~6 mg/L[23]，因此本实验设 3 个处理组，溶解氧浓度分别为(2.5±0.5) mg/L、(4.5±0.5) mg/L 和(6.5±0.5) mg/L。通过调节通入海水中的氮气量来控制低溶解氧浓度，调节通入氧气量控制中、高溶解氧浓度。三疣梭子蟹在各溶解氧浓度的海水中适应 15 min 后即开始争斗实验。每个处理组各 64 只蟹用于实验，其中，32 只两两配对用于争斗实验(使用白色丙烯颜料于背甲处标记加以区分)，32 只用作争斗前的样本分析。争斗行为观测系统由摄像头(Hikvision, DS-2CD864, China)、显示器(Philips, 233i, China)、观察水族箱(直径 60 cm，高 50 cm)和隔板(60 cm×50 cm)组成(图 1)。

实验前将三疣梭子蟹饥饿 24 h，然后将成对的三疣梭子蟹分别放入中央具隔板的观察箱两侧(水深 30 cm)适应 15 min。观察箱中加入等量的菲律宾蛤仔肌肉组织匀浆浸出液诱发争斗，然后取出隔板连续拍摄 45 min。拍摄期间的水温为(24±0.5) ℃，盐度为 30，拍摄过程中保证水体溶解氧水平稳定。拍摄结束后迅速将三疣梭子蟹置于冰盘上，以 1 mL 一次性注射器于三疣梭子蟹螯足基部抽取争斗后机体的血淋巴 500 μL，之后迅速解剖并取出肝胰脏组织，置于 1.5 mL 离心管中，迅速放入液氮中保存。血淋巴于 4 ℃静置 24 h 后 3000 r/min 离心 10 min 取上清液，转入–80 ℃冰箱中保存待测。将各组剩余的 32 只未进行争斗实验的个体分别放入相应的观察水族箱，静置 60 min 后以同样的方法采集和处理血淋巴和肝胰脏样品。

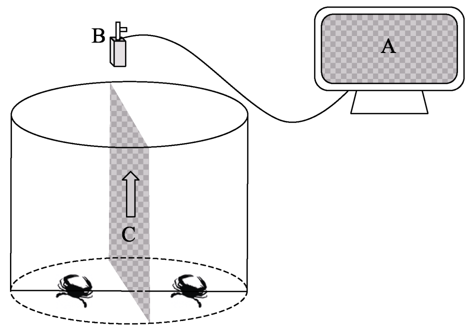

图 1 争斗行为观测系统

A. 监控器; B. 红外摄像机; C. 隔板 (20 cm×20 cm).

Fig. 1 Observation system used in the agonistic behaviors study

A. Monitor; B. Camera; C. Trapdoor (length×width: 20 cm×20 cm)

1.3 争斗行为量化

争斗的胜利者为导致对方连续撤退或者成功爬上对方的一方，另一方为失败者，争斗持续时间指从个体出现争斗行为到失败者出现连续撤退或表现出顺从行为的时间[24]。本实验参照 Matheson 等[24]和 Sneddon 等[25]的研究，定义了 4 种争斗强度：(1)很弱(very weak)：一只个体接近另一只个体并表现出攻击行为，另一个体表现出顺从且没有身体接触；(2)弱(weak)：两只个体都表现出攻击行为。肢体接触发生，直到获胜者成功爬上对方或失败者出现持续撤退；(3)中等(moderate)：两只个体的争斗升级到夹击和推搡，失败者出现撤退但展开螯足不断示威；(4)激烈(strong)：失败者虽出现撤退但不断的重新开始争斗过程。同时参照 Sneddon 等[25]的研究定义三疣梭子蟹的争斗行为(表 1)，并将争斗行为分为非接触性行为(进攻、撤退和螯足展示)和接触性行为

表 1　三疣梭子蟹争斗行为要素分类及描述
Tab. 1　The description of agonistic behaviors

行为要素 behavior	行为描述 description
进攻 move to	一只蟹接近另一只蟹 One crab approaches the other crab
撤退 move away	发生争斗行为后从另一只蟹身边撤离 One crab moves away directly from the crab in an aggressive interaction
螯足展示 chelipeds display	螯足张开进行展示 Chelipeds are held out in front
击打 strike	蟹使用一只或两只螯足击打对方 One crab hits out at the other with one or both chelipeds
夹取 grasp	蟹使用一只螯足夹取对方的背甲、螯足或其他附肢 One crab uses its chelipeds to pinch the carapace, chelipeds or legs of the other crab
推搡 push	蟹使用螯足将对方推开 One crab pushes its opponent using its chelipeds
爬上对方 climb on	一只蟹爬到另外一只蟹的背甲上方 One crab climbs on top of the other crab

(击打、夹取、推搡和爬上对方)两类。

1.4　生理指标测定

葡萄糖采用氧化酶法(glucose oxidase-peroxidase)测定，肝糖原采用蒽酮-硫酸比色法(anthrone-sulfuric acid colorimetry)测定，乳酸采用 NBT 比色法(NBT colorimetry)测定。血淋巴中葡萄糖和乳酸浓度单位为 mmol/L，肝糖原浓度单位为 mg/g。所有吸光度值由自动微量平板读取器读取(Synergy2，BioTek，美国)。均采用南京建成生物工程研究所的试剂盒进行测定。

1.5　数据分析

文中数据均使用平均值±标准差($\bar{x}$±SD)表示，对不同溶解氧浓度下三疣梭子蟹个体间争斗行为表现次数和争斗持续时间采用单因素方差分析(one-way ANOVA)，方差分析显示存在差异时，采用 Duncan 检验进行多重比较。方差齐性采用 Levene's 检验，若方差不齐，则对数据取平方根的反正弦或取对数处理。以溶解氧浓度和争斗为自变量，对能量代谢物质含量进行双因素方差分析(two-way ANOVAS)，多重比较采用 Bonferroni's 检验。采用配对样本 *t* 检验分析争斗过程中胜利者与失败者的行为表现次数的差异。上述分析以 P<0.05 作为差异显著水平，数据分析所使用的软件为 SPSS 21.0。

2　结果与分析

2.1　不同溶解氧浓度下三疣梭子蟹争斗持续时间和争斗强度

不同溶解氧浓度下三疣梭子蟹的争斗持续时间和争斗强度见图 2。从图中可以看出，随着溶解氧浓度的下降，三疣梭子蟹争斗持续时间和争斗强度均逐渐下降。2.5 mg/L 和 4.5 mg/L 溶氧组

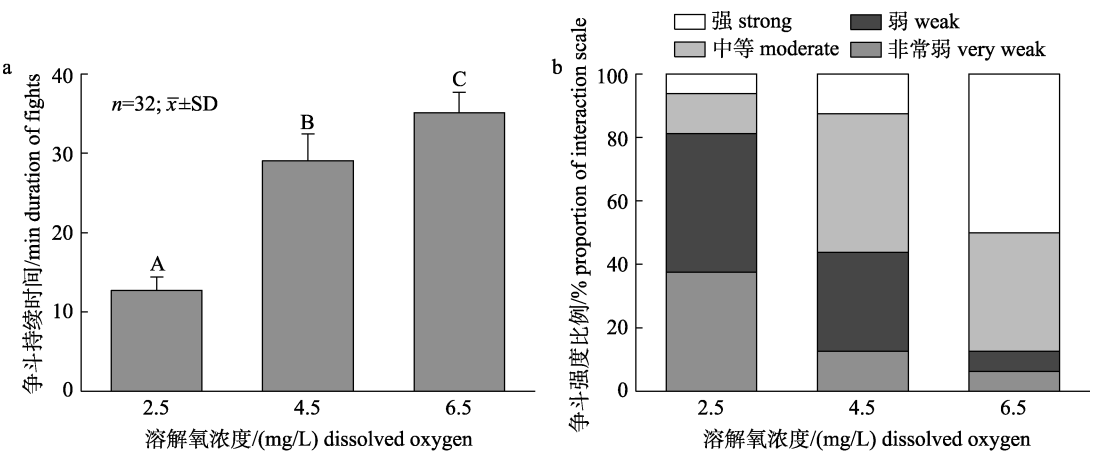

图 2　不同溶解氧浓度下的争斗持续时间(a)和争斗强度比例(b)
不同的字母表示争斗持续时间在不同处理组间差异显著(P<0.05).
Fig. 2　Duration of fights (a) and proportion of interaction scale (b) between different dissolved oxygen concentration
Different letters indicate significant difference among different treatment groups (P<0.05).

的争斗持续时间($\bar{x}_{2.5}$=12.71 min; $\bar{\chi}_{4.5}$=29.04 min)显著低于 6.5 mg/L 溶氧组($\bar{x}_{6.5}$=35.13 min, P<0.05)(图 2a)。在溶解氧为 2.5 mg/L 时, 争斗强度主要集中在"非常弱"、"弱"两种状态; 溶解氧为 4.5 mg/L 时, 争斗强度以"弱"和"中等"为主; 溶解氧为 6.5 mg/L 时, 争斗强度以"中等"和"强"为主(图 2b)。

2.2 不同溶解氧浓度下三疣梭子蟹的争斗行为

由图 3 可以看出, 随着溶解氧浓度的增加, 三疣梭子蟹活跃程度增加, 其争斗行为(接触性行为和非接触性)表现次数显著增加(P<0.05)。各溶解氧浓度下胜利者和失败者的非接触性行为和接触性行为差异显著(P<0.05), 其中, 胜利者的"进攻"、"螯足展示"行为表现次数随溶氧增加而增加且高于失败者, 随溶氧浓度增加梭子蟹更为活跃, "进攻"行为表现次数也随之增加(图 3a), 胜利者更具有攻击性, 其"击打"、"爬上对方"行为表现次数随之增加且远高于同组失败者(图 3b)。

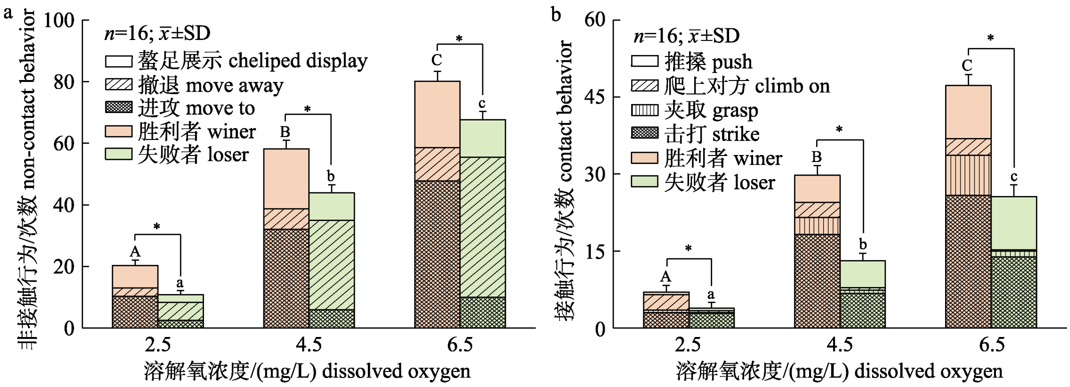

图 3 不同溶解氧浓度下胜利者和失败者争斗过程中非接触性行为(a)和接触性行为(b)表现次数
非接触性行为包括"进攻"、"撤退"和"螯足展示"; 接触性行为包括"击打"、"夹取"、"爬上对方"和"推搡".
不同大写字母表示不同溶解氧浓度下胜利者行为表现次数差异显著(P<0.05), 不同小写字母表示不同溶解氧浓度下失败者行为表现次数差异显著(P<0.05). *表示同一溶解氧浓度下胜利者和失败者之间行为表现次数差异显著(P<0.05).

Fig. 3 Mean times of non-contact behavior (a) and contact behavior (b) performed during fighting between winner and loser at different dissolved oxygen concentrations

Non-contact behaviors include "move to", "move away", "chelipeds display" and contact behavior consists of "strike", "grasp", "climb on" and "push". Different uppercase letters indicate that there is a significant difference in the number of performances of winners at different dissolved oxygen concentrations (P<0.05), different lowercase letters indicate that there is a significant difference in the number of performances of losers at different dissolved oxygen concentrations(P<0.05), and * denotes a significant difference that the number of behavioral performance between winners and losers at the same dissolved oxygen concentration (P<0.05).

2.3 不同溶解氧浓度下三疣梭子蟹肝糖原、葡萄糖和乳酸含量变化

从表 2 可以看出, 溶解氧浓度、争斗过程以及两者间的交互作用显著影响三疣梭子蟹肝糖原、血淋巴葡萄糖和乳酸含量(P<0.05)。由图 4 可知, 随着溶解氧浓度降低, 三疣梭子蟹血淋巴中葡萄糖和乳酸浓度均升高, 肝糖原浓度降低, 争斗前与争斗后机体肝糖原含量均显著降低(P<0.05)。同一溶解氧浓度下, 争斗前机体肝糖原含量均高于争斗后水平, 4.5 mg/L 和 6.5 mg/L 处理组肝糖原含量差异显著(P<0.05), 在 2.5 mg/L 处理组争斗前后肝糖原含量差异不显著(P>0.05)(图 4a)。争斗前 6.5 mg/L 处理组血淋巴葡萄糖含量显著低于其余两组, 2.5 mg/L 和 4.5 mg/L 处理组血淋巴中葡萄糖含量差异不显著(P>0.05); 争斗后的三疣梭子蟹血淋巴中葡萄糖含量随溶解氧浓度增加显著降低(P<0.05); 同一溶解氧浓度下, 争斗后血淋巴中葡萄糖的含量均高于争斗前含量, 葡萄糖含量各组间差异显著(P<0.05)(图 4b)。随着溶解氧浓度升高, 争斗前与争斗后三疣梭子蟹个体血淋巴中乳酸均显著降低, 同一溶解氧浓度下, 争斗后梭子蟹血淋巴中乳酸含量均显著高于争斗前水平(P<0.05)(图 4c)。

表 2 溶解氧(T)和争斗(Z)对三疣梭子蟹肝糖原、血淋巴中葡萄糖和乳酸含量的影响

Tab. 2　Effect of dissolved oxygen (T) and fight (Z) on the concentrations of glycogen in hepatopancreas, glucose and lactate in haemolymph

因变量 dependent variable	实验因素 source of variation	自由度 d*f*1, d*f*2	均方差 MS	*F*	*P*
肝糖原 glycogen in hepatopancreas	T	2, 31	6.516	60.328	**0.000**
	Z	1, 31	2.322	21.497	**0.000**
	T×Z	2, 31	0.388	3.592	**0.034**
血淋巴中的葡萄糖 glucose in haemolymph	T	2, 31	4.594	63.268	**0.000**
	Z	1, 31	11.765	162.026	**0.000**
	T×Z	2, 31	0.249	3.429	**0.039**
血淋巴中的乳酸 lactate in haemolymph	T	2, 31	57.610	176.992	**0.000**
	Z	1, 31	26.903	82.652	**0.000**
	T×Z	2, 31	1.397	4.291	**0.018**

注: T 表示不同的溶解氧处理; Z 表示争斗处理; T×Z 代表两者的交互作用, 加粗字体表示影响显著($P<0.05$).

Note: T denotes the dissolved oxygen and Z denotes the fight treatment as well as the T×Z denotes the interaction between dissolved oxygen and fight. *P*-values in bold indicate significant results ($P<0.05$).

3　讨论

在全球气候变化背景下, 伴随着水温上升和海水酸化, 环境变化影响着水生动物的生长、行为以及新陈代谢等生命活动[26-28]。在大量物种如克氏原螯虾(*Procambarus clarkii*)、美洲螯龙虾(*Homarus americanus*)、巨大拟滨蟹(*Pseudocarcinus gigas*)等的研究中表明, 环境条件是影响甲壳动物争斗行为的重要因素[23]。溶解氧是水域生态系统中最重要的生态因子, 其过低或过高都会影响水生生物的争斗行为表现[29]。如, 较低的溶氧浓度降低寄居蟹(*Pagurus bernhardus*)寻找庇护所以及发现捕食者和食物的能力, 进而降低其争斗能力[7]。本研究发现, 随着溶解氧浓度的降低, 三疣梭子蟹个体间争斗强度明显减弱, 争斗持续时间显著降低, 争斗行为表现次数也显著减少。在争斗中, 胜利者表现出了更多的“进攻”、“螯足展示”、“击打”、“爬上对方”行为, 表现更为主动和活跃, 更倾向于发起争斗, 并通过展示螯足向对手发起攻击[30]。这一结果与滨蟹(*Carcinus maenas*)[9]的研究结果相同。且随着溶氧浓度的降低, 胜利者和失败者之间接触性行为次数差异逐渐增加, 这一结果与寄居蟹(*P. bernhardus*)研究相似[31], 推测可能与争斗个体对低氧的耐受力不同有关, 需进一步验证。

甲壳动物的争斗行为需要能量支持, 其能量来源主要依赖有氧代谢和无氧代谢, 葡萄糖在溶氧充足时通过三羧酸循环途径产生大量能量, 低氧条件下则会导致无氧代谢强度增加[9]。糖原和葡萄糖作为水生动物机体的能量库, 代表个体的能量状态且与行为表现密切关联, 乳酸作为机体无氧呼吸的代谢产物之一, 可反映无氧呼吸供能的强度[5]。在本研究中, 随着溶解氧浓度降低, 三疣梭子蟹的肝糖原含量降低, 葡萄糖和乳酸含量增加, 这与滨蟹(*C. maenas*)的研究[10]相似, 即低氧环境中产生的乳酸抑制了蟹类的争斗活动。2.5 mg/L 处理组的争斗时间和强度低于 4.5 mg/L 处理组, 但其乳酸水平显著高于 4.5 mg/L 处理组, 这与中华绒螯蟹(*Eriocheir sinensis*)的研究相似[32], 推测可能是缺氧状态下机体无氧呼吸增强, 能量供应效率低且成本高, 长时间争斗所需的能量成本超出了争斗收益, 因此, 三疣梭子蟹通过降低争斗时间为其他活动节省能量, 而 6.5 mg/L 处理组, 机体乳酸含量最低, 说明在争斗过程三疣梭子蟹的能量供应主要以有氧代谢为主。

本研究中, 争斗后三疣梭子蟹血淋巴中葡萄糖的含量显著高于争斗前的水平, 4.5 mg/L 和 6.5 mg/L 处理组中肝糖原的含量均显著低于争斗前水平, 这与 Su 等[22]的研究结果相同, 表明在 4.5 mg/L、6.5 mg/L 溶解氧浓度下, 争斗过程中三疣梭子蟹利用糖原生成葡萄糖为其争斗提供能量。在溶氧浓度为 2.5 mg/L 时, 争斗前后三疣梭

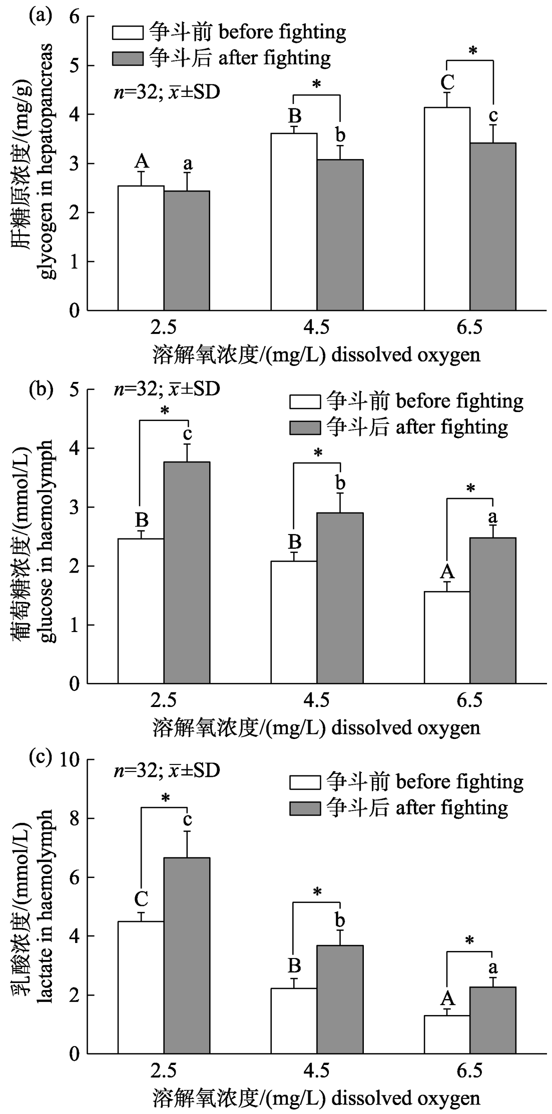

图 4 不同溶解氧浓度下三疣梭子蟹争斗前后肝糖原(a)、血淋巴中葡萄糖(b)和乳酸(c)的变化

大写字母表示不同溶解氧浓度下争斗前三疣梭子蟹个体间的差异, 小写字母表示不同溶解氧浓度下争斗后个体间的差异, *表示同一溶解氧浓度下争斗前后个体差异显著(P<0.05).

Fig. 4 The changes of glycogen in hepatopancreas (a), glucose (b) and lactate in haemolymph (c) before and after fighting of *Portunus trituberculatus* under different dissolved oxygen concentrations

Capital letters indicate the difference between individuals of swimming crabs before fighting in different dissolved oxygen concentrations. lowercase letters indicate the difference between individuals after fighting under different dissolved oxygen concentrations, and * indicates significant difference before and after fighting under the same dissolved oxygen concentration (P<0.05).

子蟹肝糖原差异不显著, 推测可能是低氧胁迫降低了其争斗强度和持续时间, 导致争斗过程中肝糖原消耗减少。本研究还发现, 随着争斗强度的提升以及争斗持续时间和行为表现次数的增加, 三疣梭子蟹血淋巴中葡萄糖的含量呈不断增加的趋势, 表明争斗强度和行为表现次数的增加会提升个体糖原降解的速率, 从而导致血淋巴中葡萄糖含量升高, 这与滨蟹(*C. maenas*)的研究结果相似[33]。无氧呼吸作为葡萄糖代谢的途径之一, 体现了机体的应激状态, 具有产能快却生成能量总量少的特点[34]。因此, 乳酸作为无氧呼吸的代谢产物, 可很好地反映个体在争斗过程中的代谢代价[35]。本研究中, 三疣梭子蟹争斗后血淋巴中乳酸的含量均显著高于争斗前水平, 且随着争斗持续时间和争斗行为表现次数的增加, 血淋巴中乳酸的含量显著增加, 表明随着争斗强度和行为表现次数的增加, 个体的能量代谢代价也不断增加。

综上所述, 随着溶解氧浓度的降低, 三疣梭子蟹无氧呼吸强度增加, 能量供应效率降低且机体乳酸累积增加, 导致个体的争斗意愿降低, 通过限制争斗强度和争斗时间保存能量。胁迫环境下个体能量代谢的差异是导致争斗行为改变的原因。

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Effect of dissolved oxygen on agonistic behavior and energy metabolism of the swimming crab (*Portunus trituberculatus*)

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Abstract: Fighting and cannibalism in crabs are key factors that limit their production and economic potential. Fighting between crabs is not only affected by biological factors, such as gender and size, but also closely associated with the environmental conditions in which they live. As a common environmental factor, dissolved oxygen significantly affects the physiological metabolism of animals, subsequently changing the fighting behavior of individuals. To study changes in fighting behavior and energy metabolism of the swimming crab (*Portunus trituberculatus*) under different dissolved oxygen conditions, we used an observation system for fighting behavior that was constructed in our laboratory. The fighting behavior of swimming crabs was quantified under different concentrations of dissolved oxygen [(2.5±0.5) mg/L, (4.5±0.5) mg/L, and (6.5±0.5) mg/L]. Changes in glycogen in the hepatopancreas, as well as changes in glucose and lactic acid in hemolymph, were measured. The main results revealed as following: (1) With a decrease in dissolved oxygen concentrations, the fighting duration of the swimming crabs was significantly reduced, with the minimum value observed in the 2.5 mg/L treatment group, which was significantly lower than that in the other treatment groups ($P<0.05$). Meanwhile, the fighting intensity between individuals also continuously decreased. (2) The frequency of fighting incidents for the swimming crabs significantly decreased with the decrease in dissolved oxygen, and the frequency of contact behavior and non-contact behavior among the winners in the three treatment groups were significantly higher than those of the losers in the fighting process ($P<0.05$). (3) With the decrease in dissolved oxygen concentration, the levels of glucose and lactic acid in the hemolymph of the swimming crabs were significantly increased, and the level of liver glycogen was significantly decreased ($P<0.05$). (4) After fighting, the liver glycogen level of the swimming crabs significantly decreased, whereas within the hemolymph, the glucose level significantly increased and the lactic acid level was significantly higher than what it was before fights ($P<0.05$). The experimental results showed that the intensity of anaerobic respiration, energy supply efficiency, and lactic acid accumulation of swimming crabs were increased under hypoxia stress, which resulted in the decrease in fighting willingness and fighting intensity of the swimming crabs. Therefore, differences in energy metabolism may be one of the physiological mechanisms leading to differences in fighting behaviors under stress.

Key words: *Portunus trituberculatus*; dissolved oxygen; agonistic behavior; energy metabolism

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