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## 斑石鲷低氧耐受能力及血液 生理生化指标变化研究\*

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**摘要** 为阐明斑石鲷(*Oplegnathus punctatus*)低氧耐受能力及低氧胁迫过程中血液生理生化指标的变化规律,本研究通过生理学和生态学方法,查明2种规格斑石鲷在临界氧分压(critical oxygen tension,  $P_{crit}$ )和失去平衡点(loss equilibrium, LOE)时的溶解氧浓度,观察自然耗氧条件下其呼吸行为变化,分析低氧胁迫和恢复溶解氧过程中血液皮质醇和葡萄糖含量、血红蛋白(hemoglobin, Hb)浓度、红细胞(red blood cell, RBC)和白细胞(white blood cell, WBC)数目、红细胞积压(hematocrit, HCT)变化。结果显示,在水温(23.0±0.5)°C、氨氮浓度<0.5 mg/L、亚硝酸盐浓度 0~0.05 mg/L、盐度为30、pH为7.80的条件下,200和50 g斑石鲷的 $P_{crit}$ 值分别为(4.05±0.09)和(3.15±0.12) mg/L, LOE值分别为(1.16±0.08)和(0.93±0.11) mg/L,且50 g斑石鲷到达 LOE 值的时间( $t=480$  min)比200 g斑石鲷( $t=110$  min)更长;自然耗氧过程中,2种规格斑石鲷呼吸频率均呈先升高后降低的变化趋势,且均在 $P_{crit}$ 值处显著升高,达到最大值( $P<0.05$ )。低氧胁迫导致血浆葡萄糖和皮质醇含量显著升高,在 LOE 值处达到最大值( $P<0.05$ ),但50 g斑石鲷的葡萄糖和皮质醇含量上升幅度显著低于200 g斑石鲷( $P<0.05$ )。同时,低氧引起斑石鲷的WBC和RBC数目、Hb浓度和HCT显著升高,50和200 g斑石鲷分别在 $P_{crit}$ 和 LOE 值处达到最大值( $P<0.05$ )。恢复正常溶解氧24 h后,2种规格斑石鲷均恢复正常呼吸运动,上述生理生化指标均与对照组无显著差异( $P>0.05$ )。综上所述,2种规格的斑石鲷相比较,50 g斑石鲷的低氧耐受能力更强,200 g斑石鲷对低氧胁迫的应激反应更敏感,2种规格斑石鲷均可通过增加呼吸频率、提高血液WBC和RBC数、皮质醇、葡萄糖和Hb浓度,增强对溶解氧的吸收利用,应对低氧胁迫导致的生理状态改变,相关结果为斑石鲷高效养殖提供了基础数据。

**关键词** 斑石鲷; 低氧耐受; 呼吸频率; 血液学; 生理生化

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溶解氧是影响鱼类等水生生物生长和存活的重要水环境因子之一,物理、化学或生物等因素的季节性或突发性改变,均可引起水体中溶解氧降低甚至缺氧,导致鱼类产生强烈的应激反应(Abdel-Tawwab *et al.*, 2019; 吴碧银等, 2021)。为适应水环境溶解氧变化,鱼类在进化和繁衍过程中形成了不同应答模式和适应性调控机制,并通过一系列行为、生理和组织结构变化,如改变呼吸模式、增强血氧亲和力等,获取充足的氧气或者限制组织对氧气的需求,降低能量消耗,从而维持正常生理功能(张安杰, 2014; 钱辰颖, 2020; 王盼等, 2021)。临界氧分压(critical oxygen tension,  $P_{crit}$ )和失去平衡点(loss of equilibrium, LOE)是被广泛应用于评价鱼类低氧耐受能力的有效指标(宋银都等, 2019)。 $P_{crit}$ 和 LOE 越低,则表明鱼类低氧耐受能力越强,反之则越弱。明确鱼类  $P_{crit}$ 和 LOE,可为人工养殖条件下的水体溶解氧调控提供科学依据,从而减少损失。研究发现,低氧环境可导致鱼类的正常呼吸和生理代谢发生紊乱,从而影响鱼类的行为和生理生化等指标(Buentello *et al.*, 2000; Thurston *et al.*, 2011)。熊向英等(2016)研究发现,水中溶解氧含量降低,鲮鱼(*Mugil cephalus*)幼鱼生长缓慢,行动能力下降。低氧环境下鲤鱼(*Cyprinus carpio*)则可通过增加呼吸频率,获取更多氧气供应,适应低氧环境(Glass *et al.*, 1990)。目前,国内外对鱼类低氧研究主要集中在不同种属鱼类的低氧耐受,而对不同规格的同一种属鱼类低氧耐受性报道不多。麻艳群等(2021)研究发现,体质量对斑点叉尾鲷(*Ictalurus punctatus*)幼鱼耗氧率有显著影响,但未明确不同规格斑点叉尾鲷的  $P_{crit}$ 和 LOE 点,也未从相关敏感指标角度阐述不同体质量实验鱼之间的差异。刘金兰等(2012)分析了大、中、小 3 种规格乌苏里拟鲿(*Pseudobagrus ussuriensis*)的耗氧率和窒息点,但未从生理生化角度解析 3 种规格乌苏里拟鲿应对低氧胁迫的反应变化。因此,对不同规格同一鱼种低氧耐受性研究有待深入。

斑石鲷(*Oplegnathus punctatus*)俗称“黑金鼓”、“花金鼓”,在中国东南沿海、朝鲜半岛和日本等海域均有分布,其肉质鲜美、营养丰富,深受广大消费者的喜爱和青睐。我国北方冬季严寒,斑石鲷不能在外海越冬,目前主要采用“陆海接力养殖”模式,即在工厂化车间完成大规模苗种培育工作,待到海水温度升高至适宜生长水温,将斑石鲷转运至大型工程化围栏进行接力养殖。研究表明,大型工程化围栏远离岸线,圈围水体面积大,水流交换充分,自然禀赋优良,斑石鲷生长速度快,同时经过自然环境下野化驯养,其生理代谢功能得到显著提升(Jia *et al.*, 2021a)。围栏圈

养水体溶解氧是影响斑石鲷养殖生产的重要环境因子之一,其含量伴随着水体温度变化产生剧烈波动,呈现显著的季节性变化,水温越高,水分子之间的间隙越小,水的溶解氧能力就越差(饶胡敏等, 2017)。此外,水体中营养盐含量(薛彬等, 2016)、自然气候改变(胡蓉等, 2014)、藻类和光合细菌的分布也显著影响水体中的溶解氧浓度。目前,国内外对斑石鲷的研究主要集中在饲料营养(Wang *et al.*, 2021)、循环水养殖(张皓天等, 2020; 宋协法等, 2017)、运输应激(张宇雷等, 2017)、疾病诊断监测(Huang *et al.*, 2021)和免疫调控(Liu *et al.*, 2019)等方面。溶解氧作为重要环境因子,显著影响养殖鱼类的生长存活,而目前对斑石鲷低氧耐受的研究尚未有系统报道,同时,在低氧胁迫过程中相应生理生化反应尚不明确,基于此,本研究通过对比分析 2 种规格斑石鲷的  $P_{crit}$ 和 LOE,观察其呼吸行为的变化,分析低氧胁迫和恢复溶解氧过程中的血液生理生化指标[皮质醇、葡萄糖含量、血红蛋白(Hb)浓度、红细胞(red blood cell, RBC)和白细胞(white blood cell, WBC)数,红细胞积压(hematocrit, HCT)]的变化,阐明斑石鲷低氧耐受能力和生理反应,相关结果可为斑石鲷陆海接力养殖提供基础性数据。

## 1 材料与方法

### 1.1 实验材料

实验在山东莱州明波水产有限公司开展,实验材料均由该公司培育,斑石鲷体格规整、活力健康,平均体长分别为(9.80±1.10)和(20.00±4.20) cm,平均体质量分别为(50.10±0.70)和(200.30±4.20) g。实验开始前,将 2 种规格的斑石鲷置于 12 个实验桶(60 L)中暂养 2 周,每个桶放置 15 尾实验鱼,每天 08:00 和 16:00 各投喂 1 次(海童,中国潍坊三通生物工程有限公司),每次投喂量为鱼体体重的 3%。保持水体中溶解氧浓度高于 7.00 mg/L,水温为(23.0±0.5)℃,氨氮浓度<0.1 mg/L,亚硝酸盐浓度为 0~0.05 mg/L,正常光照。

### 1.2 实验设计

**1.2.1 低氧耐受能力实验** 实验参考封闭静水式方法(张安杰, 2014)并做改进。取 6 个实验桶,用塑料布将实验桶密封,实验开始时关闭循环水系统,做隔绝空气处理。通过溶氧仪(AZ86031 型,中国台湾鑫恒)自动监测和记录水体中溶解氧浓度变化,根据体重和溶解氧变化计算实验鱼代谢率  $MO_2$  [mg/(kg·h)],公式如下:

$$MO_2 = ([O_2]_{t_1} - [O_2]_{t_2}) \times \frac{V}{t} \times \frac{1}{BW}$$

式中,  $[O_2]_{t_1}$ 和 $[O_2]_{t_2}$  (mg/L)分别为监测时间点  $t_1$  和  $t_2$  时的溶解氧浓度;  $V$  为实验桶的总体积减去鱼的体积;  $t(h)$ 为时间点  $t_1$  和  $t_2$  间的间隔;  $BW(kg)$ 为实验鱼的体质量。

$P_{crit}$  值表示随溶氧水平下降, 鱼类不能维持其代谢率时的水体溶氧水平, 该值与鱼类低氧耐受能力呈反比, 即  $P_{crit}$  值越大, 说明鱼类耐低氧能力越弱, 反之则越强。将代谢率和溶氧水平作图, 采用双线法求出  $MO_2$  随溶解氧下降而出现显著下降拐点, 即得到  $P_{crit}$  值。

在监测记录水体溶解氧溶度变化的同时, 参照 Wu 等(2016)的方法, 观察实验鱼游泳和呼吸行为, 随机挑选 3 尾实验鱼, 记录其每分钟鳃盖闭合次数, 统计呼吸频率(次/min), 50 g 斑石鲷每隔 1 h 统计 1 次, 200 g 斑石鲷每隔 10 min 统计 1 次。当实验鱼失去平衡时(LOE; 侧翻, 腹部朝上), 记录此时溶解氧溶度并统计呼吸频率。

**1.2.2 低氧胁迫和恢复实验** 明确了 2 种规格斑石鲷的  $P_{crit}$  值和 LOE 值之后, 每个规格斑石鲷分别设置 1 个对照组和 3 个处理组, 对照组和处理组均为 3 个平行。实验开始时, 关闭处理组循环水系统, 用塑料布将实验桶密封, 至水中溶解氧浓度到达  $P_{crit}$  值和出现 LOE 现象[鱼体失去平衡、侧翻; 50 g 斑石鲷溶解氧为(0.93±0.11) mg/L, 200 g 斑石鲷溶解氧为(1.16±0.08) mg/L]时取样, 每桶随机取 3 尾鱼。取完 LOE 点样品, 立即打开循环水系统, 去掉塑料布进行恢复溶解氧处理(20 min 后溶解氧恢复至正常水平; 7.00 mg/L), 并在恢复 24 h (R 24)后取样。实验期间, 观察 2 种规格斑石鲷在低氧胁迫和恢复溶解氧过程中游泳和呼吸行为的变化, 统计斑石鲷的呼吸频率, 呼吸次数以鳃盖一张一合记为 1 次。

### 1.3 血液样本采集与处理

从每个平行取 3 尾鱼(共 9 尾), 迅速捞出置于 MS-222 海水溶液(200 mg/L)中。深度麻醉后, 使用 1 mL 注射器从尾静脉取血, 分为 2 份。1 份直接用于检测血液红细胞(RBC)数、白细胞(WBC)数、血红蛋白(Hb)含量和红细胞积压(HCT); 1 份放于含有 EDTAK<sub>2</sub> 抗凝剂的抗凝管中, 4℃ 静置 1.5 h, 4000 r/min 条件下离心 10 min 制备血浆, 存于-80℃ 冰箱中, 检测葡萄糖和皮质醇含量。

### 1.4 血液样本分析

采用迈瑞全自动血液细胞分析仪(BC-2800 vet)检测血液红细胞数、白细胞数、血红蛋白含量、平均红细胞积压。采用南京建成公司试剂盒检测葡萄糖含量, 所用波长为 563 nm。采用放射性免疫法检测血清中皮质醇含量, 所用仪器为放射免疫  $\gamma$  计数器, 详细步骤参见说明书。

### 1.5 数据分析

实验数据用平均值±标准差(Mean±SD)表示, 采用 SPSS 16.0 软件进行单因素方差分析(one-way ANOVA), 并采用 Tukey's 多重比较分析 2 种规格斑石鲷重要指标的差异, 显著性差异用  $P<0.05$  表示。

## 2 结果

### 2.1 斑石鲷低氧耐受能力

如图 1 所示, 随着水体溶解氧浓度下降, 2 种规格斑石鲷的呼吸频率呈逐渐上升然后下降的变化趋势, 50 g 和 200 g 斑石鲷分别在溶解氧浓度下降至

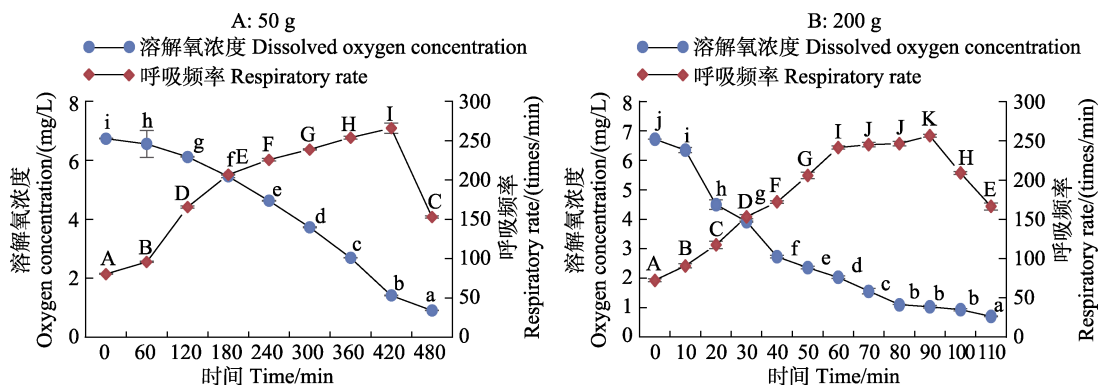


图 1 斑石鲷呼吸频率和溶解氧浓度

Fig.1 Dissolved oxygen consumption and respiratory rate of spotted knifejaw

不同字母表示显著性差异( $P<0.05$ )。下同。

The significant differences are shown in different letters ( $P<0.05$ ). The same as below.

1.27 mg/L (420 min)和 1.10 mg/L (90 min)时,呼吸频率达到最高( $P<0.05$ )。随后,随着溶解氧浓度的进一步降低,50 g 和 200 g 斑石鲷分别在实验后 480 min (1.16 mg/L)和 110 min (0.93 mg/L)出现 LOE 现象(腹部向上)。

根据“双线法”作图统计发现,2种规格斑石鲷的  $P_{crit}$  处水体溶解氧浓度分别为  $(3.15\pm 0.12)$  和  $(4.05\pm 0.09)$  mg/L (图 2)。当水体溶解氧浓度降至 2 种规格斑石鲷的  $P_{crit}$  值以下时,斑石鲷机体出现胁迫反应。

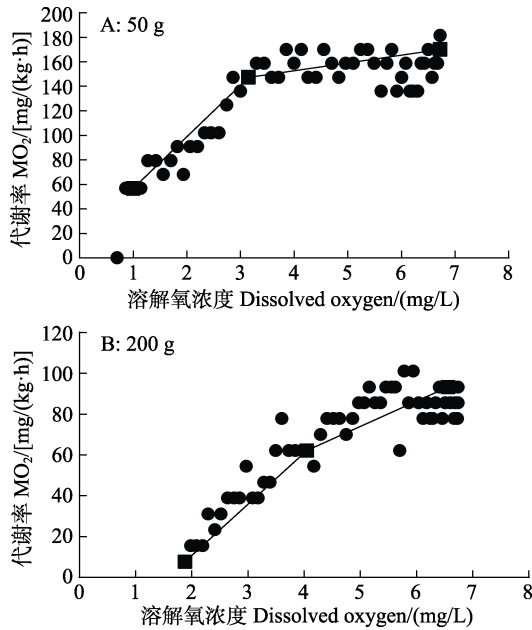


图 2 斑石鲷临界氧分压

Fig.2 Critical oxygen tension of spotted knifejaw

## 2.2 低氧胁迫对斑石鲷呼吸频率的影响

如图 3 所示,在低氧胁迫和恢复溶解氧过程中,2种规格斑石鲷的呼吸频率均呈先升高后降低的趋势,且 50 g 斑石鲷在  $P_{crit}$  处的呼吸频率显著高于 200 g 斑石鲷( $P<0.05$ ),LOE 点处 2 种规格斑石鲷的呼吸频率无显著差异( $P>0.05$ ),恢复正常溶解氧 24 h 后,2 种规格斑石鲷的呼吸频率均逐渐恢复,与对照组相比无显著差异( $P>0.05$ )。

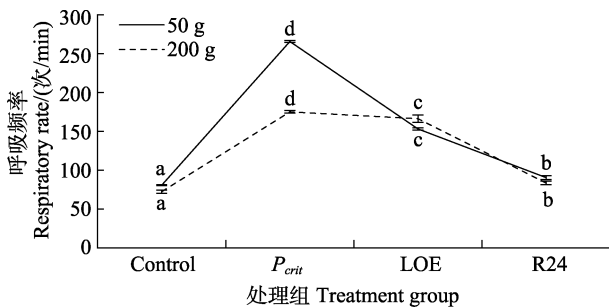


图 3 低氧胁迫和恢复溶氧过程中斑石鲷的呼吸频率

Fig.3 Respiratory rate of spotted knifejaw during hypoxia and re-oxygenation

## 2.3 低氧胁迫对斑石鲷血浆葡萄糖和皮质醇的影响

在低氧和恢复正常溶解氧过程中,2种规格斑石鲷的血浆皮质醇和葡萄糖浓度变化情况如图 4 所示,其血浆葡萄糖(图 4A)和皮质醇浓度(图 4B)均随养殖环境溶解氧浓度的降低而升高,50 g 斑石鲷的血浆葡萄糖和皮质醇浓度在  $P_{crit}$  值和 LOE 值处均较对照组有显著差异,且都在 LOE 值处达到最高值( $P<0.05$ );200 g 斑石鲷的血浆皮质醇浓度变化趋势与 50 g 斑石鲷相同。在恢复溶解氧阶段,血浆葡萄糖和皮质醇浓度逐渐降低,至恢复正常溶解氧 24 h 时,与对照组差异不显著( $P>0.05$ )。

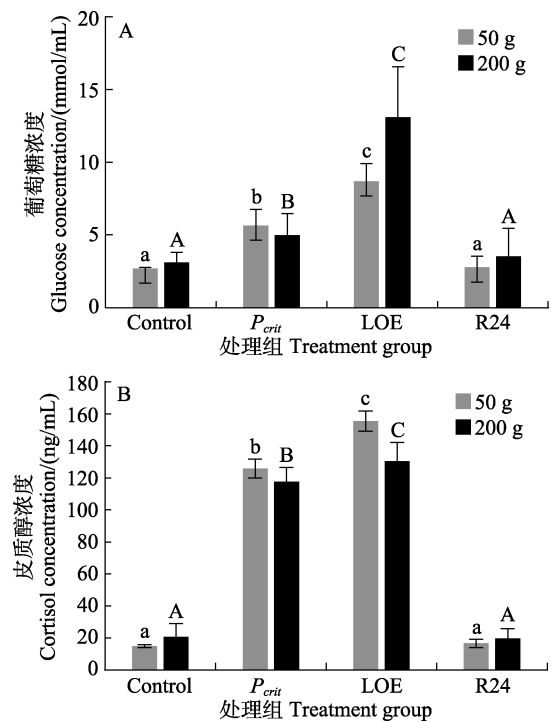


图 4 低氧胁迫和恢复溶氧过程中斑石鲷的血浆葡萄糖和皮质醇浓度

Fig.4 Changes of plasma glucose and cortisol content in spotted knifejaw during hypoxia and re-oxygenation

## 2.4 低氧胁迫对斑石鲷血液生理指标的影响

如表 1 所示,在  $P_{crit}$  值和 LOE 值处,50 g 斑石鲷的白细胞数、红细胞积压、血液红细胞数、血红蛋白浓度均显著高于对照组( $P<0.05$ ),且在  $P_{crit}$  值处达到最高值;而 200 g 斑石鲷的这 4 个指标均较对照组显著升高( $P<0.05$ ),其中,白细胞数、红细胞积压和血红蛋白浓度在 LOE 值时达到最大值,血液红细胞数在  $P_{crit}$  值时达到最大值。恢复正常溶氧 24 h 后,2 种规格斑石鲷的各项指标均与对照组无显著差异。

表 1 低氧和复氧过程中 2 种规格斑石鲷的血液生理指标变化  
Tab.1 Changes of blood physiological indexes of spotted knifejaw during hypoxia and re-oxygenation

| 血液生理指标<br>Blood<br>physiological<br>indexes           | Control                   |                           | $P_{crit}$               |                           | LOE                       |                          | R24                       |                          |
|---|---------------------------|---------------------------|--------------------------|---------------------------|---------------------------|--------------------------|---------------------------|--------------------------|
|   | 50 g                      | 200 g                     | 50 g                     | 200 g                     | 50 g                      | 200 g                    | 50 g                      | 200 g                    |
| 白细胞数<br>Number of<br>leukocytes<br>( $10^9$ /mL)      | 164.10±15.59 <sup>a</sup> | 156.25±22.12 <sup>a</sup> | 197.43±3.79 <sup>b</sup> | 189.45±8.07 <sup>b</sup>  | 186.85±5.83 <sup>b</sup>  | 196.78±8.37 <sup>b</sup> | 168.28±16.62 <sup>a</sup> | 159.35±4.61 <sup>a</sup> |
| 红细胞数<br>Number of<br>erythrocytes<br>( $10^{12}$ /mL) | 2.52±0.16 <sup>a</sup>    | 2.58±0.43 <sup>a</sup>    | 3.48±0.14 <sup>c</sup>   | 3.56±0.11 <sup>b</sup>    | 3.04±0.28 <sup>b</sup>    | 3.40±0.23 <sup>b</sup>   | 2.68±0.17 <sup>a</sup>    | 2.72±0.39 <sup>a</sup>   |
| 血红蛋白浓度<br>Concentration<br>of hemoglobin<br>(g/L)     | 98.00±11.33 <sup>a</sup>  | 111.50±8.41 <sup>a</sup>  | 138.25±1.79 <sup>c</sup> | 126.75±14.75 <sup>b</sup> | 126.00±10.04 <sup>b</sup> | 131.75±6.72 <sup>b</sup> | 100.00±4.85 <sup>a</sup>  | 115.00±8.40 <sup>a</sup> |
| 红细胞积压<br>Erythrocyte<br>backlog/%                     | 35.60±5.37 <sup>a</sup>   | 49.58±2.07 <sup>a</sup>   | 59.70±2.18 <sup>b</sup>  | 54.53±1.56 <sup>b</sup>   | 55.52±4.32 <sup>b</sup>   | 57.43±2.35 <sup>b</sup>  | 38.48±3.62 <sup>a</sup>   | 50.46±2.87 <sup>a</sup>  |

### 3 讨论

鱼类低氧耐受能力决定了其对低氧环境的适应性,  $P_{crit}$  和 LOE 值是反映低氧耐受能力的重要指标,  $P_{crit}$  值越低, 表明鱼体在低溶氧水平下维持稳定代谢的能力越强(Ultsch *et al*, 2019)。鱼类  $P_{crit}$  值存在种属间差异, 同时受体质量的影响。Candebat 等(2020)研究发现, 当水中溶解氧浓度降至(2.92±1.48) mg/L 时, 大规格黄条鲷(*Seriola aureovittata*)出现应激反应, 而 Robin 等(2011)研究发现, 当水体溶解氧下降到(3.39±0.26) mg/L 时, 小规格大西洋鲑(*Salmo salar*)开始产生应激, 表明鱼类的  $P_{crit}$  值受鱼类种属和鱼体规格影响。在同类鱼中, 不同体质量仍可影响其  $P_{crit}$  值。Pichavant 等(2002)研究发现, 当水中溶解氧值为 3.5~5 mg/L 时, 大菱鲆(*Scophthalmus maximus*)(120 g)在 7 d 内无明显反应, 而 Jia 等(2021b)研究发现, 当水中的溶解氧浓度下降到 5 mg/L 时, 170 g 大菱鲆游泳异常, 至 3.34 mg/L 时达到其  $P_{crit}$  值, 说明鱼类  $P_{crit}$  值受其体质量的影响。除  $P_{crit}$  之外, LOE 值同样受到鱼体质量的影响。崔宽宽等(2018)研究发现, 不同规格花鲈(*Lateolabrax maculatus*)的 LOE 点不同, 小规格花鲈(平均体质量为 1.6~2.3 g)LOE 点的溶解氧范围为 0.33~0.58 mg/L, 而体质量为 10.1~13.3 g 的花鲈的 LOE 值为 0.30~0.38 mg/L, 体质量在 45.10~59.30 g 范围内的花鲈的 LOE 点溶氧值区间为 0.15~0.23 mg/L, 表明花鲈的 LOE 值与其体质量呈负相关。本研究中, 50 g 和 200 g 斑石鲷的  $P_{crit}$  值分别为(3.15±0.12)和

(4.05±0.09) mg/L, LOE 值分别为(1.16±0.08)和(0.93±0.11) mg/L, 表明斑石鲷的低氧耐受能力与体质量密切相关, 小规格斑石鲷低氧耐受能力更强。针对中华倒刺鲃(*Spinibarbus sinensis*)的低氧研究中也报道了类似结果(赵文文等, 2013)。随着水环境中溶解氧浓度的下降, 鱼类会通过回避低氧区、水面呼吸、增加呼吸、不规律游动等行为获得更多溶解氧供应, 维持正常生理代谢(阮雯等, 2020)。Wannamaker 等(2000)研究发现, 不同热带鱼为适应低氧环境表现出不同行为, 当水中溶解氧浓度低于 2.5 mg/L 时, 大西洋油鲱(*Brevoortia*)幼鱼、底鳉(*Fundulus heteroclitus heteroclitus*)幼鱼等都能回避低氧水域且出现水面呼吸(浮头)等现象。李欣茹(2018)在暗纹东方鲀(*Takifugu obscurus*)低氧耐受的研究中也发现, 当水中的溶解氧浓度下降以后, 暗纹东方鲀呼吸频率增加, 同时伴随不规律游动; 当水中的溶解氧浓度低于 1.42 mg/L 时, 暗纹东方鲀开始抽搐、翻转, 最终昏迷。本研究中, 50 g 和 200 g 斑石鲷的游动随水中溶解氧浓度的下降逐渐加快, 呼吸频率逐渐升高, 且分别于水中溶解氧浓度降至 2 mg/L 以下(420 min)和 1.10 mg/L (90 min)时达到最高。研究表明, 50 g 斑石鲷  $P_{crit}$  值和 LOE 值较 200 g 斑石鲷低, 到达 LOE 值的时间更长, 因此, 其具有更强的耐低氧能力和环境适应性。

环境胁迫可导致鱼类血浆皮质醇浓度升高, 其可促进能量的重新分配, 以确保鱼类维持机体平衡(Flik *et al*, 2006), 低氧胁迫可以显著地影响血浆皮质醇浓度。王焱纬(2019)研究发现, 随着低氧持续时间的增



加,团头鲂血浆皮质醇浓度呈上升趋势,恢复正常溶解氧 24 h 后,血浆皮质醇浓度显著降低。本研究中,2 种规格斑石鲷血浆皮质醇浓度在低氧和恢复溶解氧过程中呈先上升后下降的变化趋势,在  $P_{crit}$  和 LOE 值处的血浆皮质醇浓度较对照组均升高,至 LOE 值处达到最高,说明斑石鲷的血浆皮质醇浓度显著受低氧影响(区又君等,2014)。除皮质醇外,血浆血糖作为鱼体重要能量来源同样在低氧过程中发挥重要作用(Li *et al.*, 2018)。研究表明,在鱼类正常代谢过程中,葡萄糖一部分进入肝脏中转化为糖原储存,一部分用于合成脂肪和氨基酸等,还有一部分进入血液成为血糖,为机体维持正常生理代谢供能(刘康,2017)。当鱼类经历低氧等外界环境突变时,会代偿性升高血液葡萄糖浓度,应对环境刺激诱导的能量供应,同时葡萄糖浓度升高变化幅度同鱼类规格密切相关。黄建盛等(2019)和王维政等(2021)分别研究发现,体质量为(240.78±30.24)和(50.44±2.78) g 的军曹鱼(*Rachycentron canadum*)低氧应激后血浆皮质醇浓度显著升高至 20 和 10 mmol/L,这表明规格越大对低氧环境反应越敏感。本研究中,低氧条件下 2 种规格斑石鲷的血浆葡萄糖浓度均增加,但小规格斑石鲷的血浆葡萄糖浓度较大规格斑石鲷上升幅度小,表现出更强的低氧耐受性。恢复正常溶解氧 24 h 后,2 种规格斑石鲷的血浆葡萄糖浓度均回落至正常值,说明恢复正常溶解氧 24 h 可显著缓解低氧胁迫对斑石鲷的影响(郭志雄,2020)。

RBC 是鱼类血液重要组成成分,其主要生理功能是携带和运输氧气,Hb 同样参与氧气运输,低氧条件可导致鱼类 RBC 和 Hb 显著增高(王晓雯等,2016)。研究发现,急性低氧条件下鲫鱼(*Carassius auratus*)幼鱼 RBC 和 Hb 显著升高,表明其可通过增加 RBC 和 Hb 增强自身血液载氧能力,从而适应低氧胁迫(张曦,2011)。本研究中,2 种规格斑石鲷的 RBC 和 Hb 变化趋势相同,均在  $P_{crit}$  和 LOE 值处升高,恢复 24 h 后回落至正常值。说明斑石鲷血液指标随水中溶解氧浓度的改变而变化。王志飞等(2019)研究同样发现,秀丽高原鳅(*Triplophysa venusta*)在低氧环境中血液 RBC 和 Hb 含量显著上调。此外,李欣茹(2018)研究发现,低氧胁迫环境可导致暗纹东方鲀血液 HCT 显著上升,在恢复正常溶解氧过程中该值回落,说明低氧条件下暗纹东方鲀可调节 HCT,增加自身对低氧的适应性。本研究中,2 种规格斑石鲷的血液 HCT 随水中溶解氧含量的下降显著上升,在恢复正常溶解氧 24 h 后恢复至正常值,表明低氧条件下,斑石鲷同样可以通过增加 HCT 降低自身对氧气

的需求,从而增加自身低氧环境适应性。鱼体 WBC 主要起免疫和防御作用,保护鱼体抵抗侵害。沈凡等(2010)研究表明,黄颡鱼(*Pelteobagrus fulvidraco*)血液 WBC 在低氧环境下显著增加,鱼体应对低氧环境开启免疫和防御屏障。本研究中,2 种规格斑石鲷的血液 WBC 随水中溶解氧浓度的下降均呈升高趋势,分别在  $P_{crit}$  和 LOE 值处达到最高值,至恢复正常溶解氧后 WBC 减少,说明斑石鲷在低氧环境下,提高机体免疫功能,应对低氧胁迫可能带来的损伤(常志成等,2018)。此外,50 g 斑石鲷的血液生理指标均在  $P_{crit}$  值处达到最大值,而 200 g 斑石鲷的血液 WBC、Hb 和 HCT 都在 LOE 值处达到最大值,表明不同规格斑石鲷在应对低氧应激时,对低氧耐受能力不同,导致其启动生理反应的进程存在差异(丁彦文等,2000;孙耀等,2001)。

综上,本研究发现,2 种规格斑石鲷的低氧耐受能力存在显著差异,小规格斑石鲷对低氧环境具有更强的适应性,在低氧应激胁迫过程中可通过增加呼吸频率、提高血液 RBC 和 Hb 含量增加机体对  $O_2$  的摄入,增加皮质醇和葡萄糖含量为机体提供能量,应对低氧应激诱导的生理变化。恢复正常溶解氧 24 h 后,2 种规格斑石鲷逐渐恢复到正常状态。相关结果可以为斑石鲷陆海接力高效养殖提供理论依据,减少因环境溶解氧改变带来的经济损失。

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## Hypoxia Tolerance and Alternation of Blood Physiological and Biochemical Indexes in Spotted Knifejaw *Oplegnathus punctatus*

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**Abstract** Dissolved oxygen is an important environmental factor affecting the growth and survival of aquatic organisms, including fish. Hypoxia has gradually become common in aquatic ecosystems and poses a significant challenge for fish farming. A decrease of dissolved oxygen levels in the body or a lack of oxygen will lead to a severe stress response for fish, hindering the development of the aquaculture economy. Dissolved oxygen is an important environmental factor that affects the aquaculture production of spotted knifejaw (*Oplegnathus punctatus*). The solubility of oxygen decreases with the increase of temperature, and the solubility of gaseous substances decreases with the increase of temperature and pressure. These show significant seasonal variation: The higher the temperature, the smaller the gap between the water molecules, and the lower the dissolved oxygen. Fish in a low-oxygen environment breathe normally and this can lead to physiological metabolic disorders, hence affecting fish behavior and physiological and biochemical indicators. However, in the process of evolution, fish have also developed different response modes and adaptive regulation mechanisms to maintain normal physiological functions. To clarify the changes in physiological and biochemical indices in blood during hypoxic tolerance and hypoxic stress, physiological and ecological methods were used in this study. The solubility of dissolved oxygen under two different specifications of critical oxygen tension ( $P_{crit}$ ) and loss equilibrium (LOE) were investigated, and the changes in respiration behavior under natural oxygen consumption were observed. Changes in cortisol and glucose, concentration of hemoglobin (Hb), number of red blood cells (RBC) and white blood cells (WBC), and hematocrit (HCT) during hypoxic stress and recovery were analyzed. Results showed that in a water temperature of  $(23.0 \pm 0.5)^\circ\text{C}$ , the ammonia nitrogen concentration was less than 0.5 mg/L, nitrite concentration 0~0.05 mg/L, salinity 30 and pH 7.80; the

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dissolved oxygen concentrations at  $P_{crit}$  were  $(4.05\pm 0.09)$  mg/L and  $(3.15\pm 0.12)$  mg/L for 200 g and 50 g *O. punctatus*, respectively. The concentrations of dissolved oxygen at LOE value were  $(1.16\pm 0.08)$  mg/L and  $(0.93\pm 0.11)$  mg/L. The time until the 50 g *O. punctatus* reached LOE ( $t=480$  min) was longer than that of the 200 g *O. punctatus* ( $t=110$  min). In addition, there were significant changes in respiration rate between hypoxia and re-oxygenation in both the 200 g and 50 g *O. punctatus*. In the process of natural oxygen consumption, the respiration rate of the two first increased and then decreased, and increased significantly at the  $P_{crit}$  value. When the dissolved oxygen concentration decreased to 1.27 mg/L (420 min) and 1.10 mg/L (90 min), respiration rates of the 50 g and the 200 g fish reached their maximum ( $P<0.05$ ). After recovery in normal dissolved oxygen for 24 h, the respiration rate did not differ significantly between the two sizes of *O. punctatus* nor with the control group. Plasma glucose and cortisol showed similar results to the respiratory rates; hypoxic stress led to significant increases and the highest values were obtained at LOE ( $P<0.05$ ). The plasma cortisol concentration was the same in the two sizes of fish. While the values for the 50 g *O. punctatus* were significantly lower than those of the 200 g fish during hypoxic stress and re-oxygenation ( $P<0.05$ ), the glucose and cortisol did not differ significantly from the control after recovery in normal dissolved oxygen for 24 h. Hypoxic stress had a significant effect on the blood biochemical indexes of *O. punctatus*. At  $P_{crit}$  and LOE values, the number of WBCs, RBCs, Hbs and HCT, at 50 g were significantly higher than those in the control group ( $P<0.05$ ), and the maximum values were at the  $P_{crit}$  value. The concentrations of the four indexes were significantly increased in the 200 g *O. punctatus* compared with the control group ( $P<0.05$ ). The maximum values of WBC, HCT, and Hb were reached at LOE, and the maximum values of RBC were reached at  $P_{crit}$ . After recovery in normal dissolved oxygen for 24 h, the two sizes of *O. punctatus* showed normal swimming behavior and respiratory activities, and the above physiological and biochemical indexes did not differ significantly from those of the control group ( $P<0.05$ ). Lastly, the body weight of *O. punctatus* differed during hypoxia-induced hematological and biochemical responses. In *O. punctatus*, the 50 g fish were more tolerant to hypoxic environments than the 200 g, while the 200 g fish were more sensitive to hypoxic stress. Both sizes of *O. punctatus* could enhance the absorption and utilization of dissolved oxygen by increasing their respiratory rate, increasing the number of WBCs and RBCs, and increasing the concentrations of cortisol, glucose, and Hb in response to hypoxia stress. In this study, the tolerance to hypoxia and the physiological responses of different sizes of *O. punctatus* were elucidated; their adaptability to low dissolved oxygen was explored, and their tolerance threshold to hypoxia was determined. The results provide data for land-sea relay breeding of *O. punctatus*, a theoretical basis for efficient land-sea relay, and an early warning range of hypoxia for breeding *O. punctatus*, to help reduce economic losses caused by decreased oxygen.

**Key words** Spotted knifejaw *Oplegnathus punctatus*; Hypoxia tolerance; Respiratory frequency; Hematology; Physiology and biochemistry