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低氧胁迫对鱼类生理功能影响及调控机制

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摘 要

氧气是动植物生存的必要条件之一。对于鱼类来说，溶解氧对其生命活动影响深远。溶解氧水平严重干扰了其生存、发育以及繁殖等生命过程。本文通过论述低氧对鱼类生理代谢、行为活动、组织形态和生殖发育等方面的影响，鱼类应对低氧胁迫的生理和分子调控机制，以及研究低氧胁迫分子调控机制的实验方法，为探究鱼类低氧胁迫分子调控机制提供参考借鉴，同时为培育耐缺氧型养殖鱼类提供理论支撑。

关键词

溶解氧，低氧应激，生理功能，分子调控机制

Effects of Hypoxia Stress on Physiological Function and Regulation Mechanism in Fish

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Abstract

Oxygen is one of the necessary conditions for the survival of animals and plants. For fish, dissolved oxygen has a profound impact on their life activities. Dissolved oxygen levels seriously interfere with life processes such as survival, development, and reproduction. In this paper, by discussing

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the effects of hypoxia on physiological metabolism, behavioral activities, tissue morphology, reproductive development, etc., of fish, the physiological and molecular regulation mechanisms of fish in response to hypoxia stress, and the experimental methods for studying the molecular regulation mechanism of hypoxia stress, so as to provide reference for exploring the molecular regulatory mechanism of hypoxia stress of fish, and at the same time provide theoretical support for cultivating hypoxia-tolerant aquaculture fish.

Keywords

Dissolved Oxygen, Hypoxia Stress, Physiological Function, Molecular Regulation Mechanism

1. 引言

溶解氧(Dissolved oxygen, DO)是鱼类整个生命周期过程中，影响其生长、繁殖和生存的重要因素之一[1]。随着温室效应累积，全球变暖加剧、养殖密度增加和水体污染等生态环境问题的日益凸显，全球低氧海域面积呈逐年增加趋势[2] [3] [4] [5]。低氧显著影响鱼类的生理代谢、行为活动、组织形态和生殖发育，严重制约了水产养殖业的健康发展。1992 年，Semenza and Wang 在哺乳动物 Hep3B 细胞系中最先发现了感受氧气的基因——低氧诱导因子[6]。在鱼类低氧方面，国内外学者进行了大量研究，Saha 等(2022)对低氧抑制硬骨鱼的生殖和发育进行了详细描述[7]。肖武汉(2014)从分子生物学水平论述了鱼类低氧适应策略和低氧信号途径网络调控[8]。低氧刺激鱼类产生氧化应激、血液生理生化和能量利用等生理应答反应[9] [10] [11]。这些研究有助于我们更好地了解鱼类在低氧状态下的组织形态学、生理学和分子生物学等方面变化，明确低氧应答策略，为培育耐低氧鱼类新品种提供理论支撑，对降低低氧引起的经济损失具有重要实践意义。

2. 低氧对鱼类的影响

2.1. 低氧对鱼类生理代谢影响

低氧会引起鱼类生理生化和代谢的变化，并通过改变自身代谢途径为机体提供能量[12] [13] [14] [15]。低氧胁迫下，西伯利亚鲟(*Acipenser baerii*)幼鱼血液红细胞数量增加，血红蛋白质量浓度降低，血氧亲和力降低，给组织提供更多氧气，肝脏代谢率降低，通过降低超氧化物歧化酶和过氧化氢酶活力来保护肝脏[16]。王维政等(2020)发现低氧致使军曹鱼(*Rachycentron canadum*)幼鱼肝脏过氧化物歧化酶、谷胱甘肽过氧化物酶活力和丙二醛含量呈先升高后下降趋势，乳酸脱氢酶活力先升高后降低，肝糖原含量先下降后恢复至正常水平，通过厌氧糖酵解分解糖原，维持正常的血糖浓度[17]。在对尼罗罗非鱼(*Oreochromis niloticus*)进行急性缺氧应激时，碳水化合物代谢尤其是无氧糖酵解在能量供应中起着重要作用，而在长期缺氧条件下，脂代谢会代替糖酵解来提供能量[15]。缺氧下的虹鳟(*Oncorhynchus mykiss*)会表现出异常游泳，血清皮质醇升高，通过增加血清血红蛋白来增加输氧能力[18]。

2.2. 低氧对鱼类行为的影响

在低溶解氧环境下，鱼类会通过改变行为方式来应对低氧。例如浮头、游泳速度减慢等。在严重缺

氧条件下，点带石斑鱼(*Epinephelus coioides*)鱼口持续处于张开状态[19]。徐贺等(2016)表明在水体低氧时，鱼类的行动变迟缓，呼吸频率下降，间接导致鱼类摄食活动减少[5]。当缺氧严重到一定程度时，鱼类停止进食，呼吸相关活动减少，休息时间增加，同时会抑制新陈代谢活动[20]。鱼类通过水面呼吸来抵抗急性缺氧环境[21]。与其他鱼不同，Urbina 等(2011)在研究南乳鱼(*Galaxias maculatus*)行为时发现，其更适合逃避缺氧(行为适应)，而不是忍受缺氧(生理适应) [22]。随着溶解氧浓度的降低，南乳鱼鳃盖开合频率增加，游泳速度加快，向水面移动并表现出水面呼吸，甚至跃出水面，与其他鱼相比表现出更高的耗氧率。

2.3. 低氧对鱼类组织形态的影响

鱼类在低氧下组织结构发生变化，通过影响离子代谢，代偿性调控低氧胁迫导致的生理损伤，保障正常生命活动，但严重低氧则会导致其死亡。大量的研究发现，低氧条件下，鱼类的肝脏、鳃以及心脏组织形态结构发生了显著改变。斑点叉尾鮰(*Ictalurus punctatus*)低氧条件下表现出肝坏死和出血[23]。急性低氧胁迫下，卵形鲳鲹(*Trachinotus ovatus*)肝组织间出现空泡、小叶结构破坏，细胞内线粒体数量减少，出现过氧化物酶体，肝细胞间血窦剧烈扩张[16]。低氧胁迫下大黄鱼(*Larimichthys crocea*)幼鱼、鲢(*Hypophthalmichthys molitrix*)、虹鳟、青海湖裸鲤(*Gymnocypris przewalskii*)和卵形鲳鲹等鱼类的鳃小片出血，基部出现空洞，上皮细胞出现角质化、脱落且与鳃小片分离，鳃小片呈“S”状扭曲等病理变化[16] [24] [25] [26] [27]。在许氏平鲉(*Sebastes schlegelii*)和欧洲鲈(*Dicentrarchus labrax*)的鳃低氧胁迫下会出现鳃小片增生、肥大、末端膨大、上皮细胞水肿等现象[28] [29]。低氧也会导致心脏结构的改变。在低氧胁迫下斑马鱼(*Danio rerio*)和纯黑朴丽鱼(*Haplochromis piceatus*)的心脏心室流出道变小，中央心室腔和小梁周围的腔隙减少，心肌细胞核数目增加[30]。鲑鱼心室致密层厚度增加[31]，致密心肌毛细血管密度增加[32]。

2.4. 低氧对鱼类生殖发育的影响

低氧对硬骨鱼的生殖发育的影响已被广泛报道，如致使内分泌紊乱，抑制卵巢和睾丸的生长，降低精子和卵子质量，抑制受精和孵化过程，从而损害鱼类的整个生长发育过程。低氧条件下大底鳉(*Fundulus grandis*)血清中性激素水平降低，产卵时间延后，产卵变少[33]。慈鲷(*Pseudocrenilabrus multicolor victoriae*)和短盖肥脂鲤(*Piaractus brachypomus*)的性激素水平在低氧下也会受到影响[34] [35]。鲤鱼(*Cyprinus carpio*)的性腺中的胆固醇生物合成受到抑制以及性激素的生成过程受到破坏，这将影响到性腺发育、产卵成功率、精子活力、受精、孵化率和幼鱼的存活率[36] [37]。低氧处理后，细须石首鱼(*Micropogonias undulatus*)两性性腺生长、配子发生以及生殖内分泌功能急剧下降[38]。在长时间低氧胁迫后，底鳉、斑马鱼和细须石首鱼生殖腺指数显著降低，产卵量减少，卵母细胞、精子发育受到严重阻碍甚至停滞，育苗存活率低[33] [38] [39] [40] [41]。缺氧还影响硬骨鱼的性别分化，高密度养殖的鱼类通常会分化为雄鱼[42]。Robertson 等(2014)发现缺氧条件下 71%的斑马鱼分化为雄性，比正常对照组多 30% [3]。低氧不仅影响了斑马鱼胚胎形态的发育和心脏功能，还对其红细胞的产生和成熟产生抑制作用[43]。

2.5. 鱼类对低氧的应对策略

低氧胁迫对鱼类的行为、生理代谢、组织形态和生殖发育等方面带来了不同程度的影响，鱼类可以通过反馈代偿调控机制来应对低氧环境的变化。例如通过提高超氧化物歧化酶、过氧化氢酶和谷胱甘肽过氧化物酶清除有害的超氧自由基和过氧化氢维持内稳态平衡[44]。增加谷丙转氨酶和谷草转氨酶含量来保护肝脏组织免受损伤及病变[45]。虾虎鱼(*Gillichthys mirabilis*)处于低氧环境时，其肝脏甘油三酯水解的

相关途径基因上调，甘油三酯合成途径相关基因下调[46]。在低氧胁迫过程中，青田鱼(*Cyprinus carpio var. qingtianensis*)增强糖酵解和磷酸戊糖途径为机体提供能量和还原力，而在复氧过程中糖异生途径加强，合成大量糖补充低氧消耗的糖量，另外还通过信号通路调节细胞增殖分化以及能量代谢过程[47]。尼罗罗非鱼也是通过提高无氧糖酵解代谢途径中的磷酸果糖激酶、丙酮酸激酶和乳酸脱氢酶来适应低氧环境[48]。许氏平鲉、斑石鲷(*Oplegnathus punctatus*)、鲫(*Carassius auratus*)和暗纹东方鲀(*Takifugu obscurus*)等的红细胞数目、血红蛋白含量增加，增强血液载氧能力[28] [49] [50] [51]。

3. 低氧诱导分子机制

1992 年 Semenza and Wang 在哺乳动物 Hep3B 细胞系中发现了 HIF，2019 年 William G. Kaelin Jr，Sir Peter J. Ratcliffe 和 Gregg L. Semenza 获得诺贝尔生理学或医学奖，获奖理由是“发现了细胞如何感知和适应氧气的可用性”，阐明了细胞的氧敏感机制，确认了 HIF 在细胞缺氧反应中的关键作用[52]。随着全球变暖、人类活动增加、水体富营养化等问题的日益严重，水体溶解氧含量不稳定。对于鱼类来说，水体缺氧会给其带来严重伤害，甚至死亡。探究其应答低氧胁迫的分子调控机制对鱼类抗低氧分子育种具有重要意义。

3.1. HIF

HIF (Hypoxia-inducible factor)是在哺乳动物 Hep3B 细胞系中调节促红细胞生成素(erythropoietin, EPO)表达研究中发现的[6]。HIF 是缺氧信号通路中的主要调控因子，在缺氧期间基因表达的调控中起着中心作用，广泛表达在动植物组织中[53]。HIF 是一种异源二聚体，由氧依赖型的 α 亚基和组成型表达的 β 亚基(aryl hydrocarbon nuclear translocator, ARNT)组成，两者都是 PER-ARNT-SIM (PAS) DNA 结合蛋白家族的 bHLH 蛋白的成员[54]。HIFα 有三种亚型：HIF1α、HIF2α 和 HIF3α，其中 HIF1α 在细胞和组织中广泛表达，而 HIF2α (或 EPAS1)具有组织特异性[55] [56]，HIF3α (或者 IPAS)是关系最远且研究最少的亚型，是一种存在于各种剪接变体中的组织特异性蛋白，其中大多数作为 HIF1α 和 HIF2α 活性的负调控因子[57] [58] [59] [60] [61]。在常氧状态下，HIF 结构不稳定易被分解[53]，HIFα 的氧依赖性降解结构域的特异脯氨酸残基被脯氨酸羟化酶共价修饰[8] [62]。当羟基化时，HIFα 被 von Hippel-Lindau 蛋白(pVHL)识别，并通过蛋白酶体泛素化途径进行降解。缺氧时，脯氨酸羟化酶活性降低，特异性脯氨酰残基不发生羟基化，HIFα 稳定表达并积累，进入细胞核中与 HIFβ 亚基结合成二聚体，并与下游基因启动子或增强子的缺氧应答元件(hypoxic response element, HRE)结合，诱导下游基因表达以应对低氧胁迫[53] [59] [63]。鱼类中，目前在许氏平鲉、南极冰鱼(*Chionodraco myersi*)、荫平鲉(*Sebastes umbrosus*)等中成功克隆出 HIF1α 和 HIF2α 两种亚型，斑马鱼、斑点叉尾鮰、鳙(*Hypophthalmichthys nobilis*)和纳木湖裸鲤(*Gymnocypris namensis*)等已发现三种 HIFα 亚型。

3.2. 信号通路验证方法

3.2.1. 转录组测序

转录组(Transcriptome)是一个细胞或一群细胞中所有 RNA 分子的集合，转录基因的类型和数量取决于细胞的类型和所处环境，并受到严格控制。这种转录过程的变化通常是由环境改变和疾病发生造成的。作为 DNA 和蛋白质组之间的中介，以及调控作用的分子，了解细胞或组织转录组变化对于了解细胞响应环境变化的相关调控机制至关重要。因此，转录组测序通常被用作细胞功能状态的代表[64]。转录组测序技术已广泛应用于鱼类生殖发育、环境胁迫、代谢调控和免疫疾病等方面。Mu 等(2020)利用转录组揭示了低氧胁迫下大黄鱼鳃和心的分子调控机制[65]。转录组已应用在研究许氏平鲉免疫调节、营养代谢、急

性温度应激等方面[66] [67] [68] [69]。转录组测序也在虹鳟、鲢、草鱼、鲫、罗非鱼和青田田鱼等鱼的生理免疫方面应用[18] [42] [70] [71] [72] [73]。

3.2.2. 双荧光素酶报告基因

双荧光素酶报告基因(Dual-Luciferase Reporter)系统通常是以萤火虫荧光素酶(Firefly luciferase)为报告基因，以海肾荧光素酶(Firefly luciferase)为内参基因，检测转录因子与目的基因启动子或增强子 DNA 相互作用的一种检测方法，具有灵敏度高、特异性好、动态范围广、检测时间短等特点，而且能保持细胞完整性[74] [75]。常用的两种不同类型的荧光素酶对底物的要求不同，产生不同波长的荧光，因此可以从单一样品中依次量化它们[76]。其原理是：将目的基因转录调控元件构建入带有荧光素酶的表达载体，构建成报告基因质粒，使这段序列调控 luciferase 的转录表达。然后将报告基因质粒转染细胞，给予其不同的处理后裂解细胞，并加入底物荧光素(luciferin)，luciferase 可催化 luciferin 发出荧光(最强波长在 560 nm 左右)。检测得到的荧光值高低可以判断不同处理组对该转录调控元件的影响。为避免由于质粒转染细胞时效率差异所造成的误差，通常会转入 Renilla luciferase 的报告基因质粒作为内参(最强波长在 465 nm 左右)，即双荧光报告系统。双荧光素酶报告基因检测已广泛应用于多个科学领域，多用于研究转录激活[77] [78] [79] [80]。王婷(2017)使用双荧光素酶报告基因实验检测长牡蛎(*Crassostrea gigas*)不同 HIFα 亚型与 HRE 结合活性[81]。董小敬(2015)使用此方法检测了虹鳟、花鲈(*Lateolabrax japonicus*)和大黄鱼中转录因子 SREBP-1 和 PPAR-α 基因对 Δ6 脂肪酸饱和酶的调控作用，实现对不饱和脂肪酸合成途径的验证[82]。Cai 等(2020)表明斑马鱼 *hif*3*α* 通过调控 *gata*1 调节红细胞生成来实现缺氧耐受[83]。Orlando 等(2020)用双荧光素酶报告基因方法验证了 EPO 不同位置不同长度 HRE 结合活性[84]。

3.2.3. 染色质免疫共沉淀

染色质免疫沉淀技术(Chromatin Immunoprecipitation assay, CHIP)是研究体内 DNA 与蛋白质相互作用的方法。它的基本原理是在活细胞状态下固定蛋白质－DNA 复合物，并将其随机切断为一定长度范围内的染色质小片段，然后通过免疫学方法沉淀此复合体，特异性地富集目的蛋白结合的 DNA 片段，通过对目的片断的纯化与检测，从而获得蛋白质与 DNA 相互作用的信息[85] [86] [87]。目前来说，CHIP 在研究人类疾病中广泛应用，例如转录因子 *sp*1 在心血管疾病中作用机制研究、G 蛋白偶联受体激酶 6 在肺腺癌的作用机制研究以及乳腺癌转移的分子机制等[88] [89] [90] [91]。在鱼类中，利用 CHIP 对尼罗罗非鱼性别分化、精子发生作用机制和斑马鱼低温驯化调控机制进行研究[92] [93] [94]。

4. 结论

鱼类依赖水环境生长、发育和繁殖，水体溶解氧是影响人工养殖过程中重要环境因素之一。不同程度的低氧对鱼类的生理代谢、行为活动、组织形态和生殖发育造成不同程度的损伤。因此，鱼类通过调节自身过氧化物酶含量和血红蛋白含量调控生理代谢；改变游泳速度、浮头、持续张开鱼口等行为缓解缺氧应激。低氧抑制鱼类的生殖器官发育、性别分化，致使鳃、肝和心脏组织形态发生改变。利用转录组测序、双荧光素酶报告基因检测和染色质免疫共沉淀等探究信号通路实验技术，系统研究鱼类在应对低氧胁迫时的生理学变化，探究其内在的分子调控机制，将为可提前预防低氧致死，减少经济损失提供重要技术支撑，同时也可为耐低氧鱼类新品种培育提供理论依据。

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