doi: 10.7541/2020.088

不同$NaHCO_3$碱度对瓦氏雅罗鱼鳃组织结构的影响

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摘要: 研究采用组织学方法观察和比较了达里诺尔湖瓦氏雅罗鱼(*Leuciscus waleckii*, 碱水种)和松花江瓦氏雅罗鱼(淡水种)在相同碳酸盐碱度胁迫下(C_A0、C_A30和C_A50)鳃组织结构的差异, 探究瓦氏雅罗鱼碱水种耐高碱特性与鳃组织结构微观调整的适应性关系。结果显示, 随着碱度增加, 碱水种鳃丝变宽、鳃小片变长、鳃小片间距变大($P<0.05$); 淡水种鳃丝变宽、鳃小片间距变大($P<0.05$), 鳃小片长度在C_A30时显著变长($P<0.05$), 而在C_A50时与对照组无明显差异($P>0.05$)。碱水种在C_A30和C_A50的氯细胞数量与对照组相比明显增加, C_A50的氯细胞排列更加紧密并且有叠加现象, 扁平上皮细胞变大, 细胞表面增厚; 淡水种在C_A30时的氯细胞数量明显多于C_A50, 但在碱度胁迫下, 鳃小片出现破损, 扁平上皮细胞、柱细胞和血细胞融合、脱落现象严重。另外, 在碱水种和淡水种鳃耙上皮细胞中发现了大量黏液细胞分泌, 随着碱度增加, 黏液细胞由大而稀疏变为小而密集, 其中碱水种的黏液细胞数量较淡水种多, 而且排列更为整齐、密集。鳃组织学研究结果表明, 瓦氏雅罗鱼碱水种通过保持鳃组织结构和生理功能的完整性达到对高碱环境的长期适应, 而淡水种则因鳃细胞融合、脱落造成生理功能丧失, 不能长期适应高碱环境。研究结果可为淡水鱼类在盐碱水的移植驯化提供依据和指导。

关键词: 瓦氏雅罗鱼; 碳酸盐碱度; 鳃; 组织学

中图分类号: Q171.8; Q256 **文献标识码:** A **文章编号:** 1000-3207(2020)04-0736-08

我国有4.6×10^5 km^2低洼盐碱水域, 具有高碱度、高pH、水质类型复杂等特点, 其中碱度常先于盐度限制鱼类的生存和繁衍, 现绝大部分处于荒置状态[1, 2]。瓦氏雅罗鱼(*Leuciscus waleckii*), 俗称东北雅罗鱼、华子鱼、白鱼, 隶属于鲤形目、鲤科、雅罗鱼亚科、雅罗鱼属, 在我国黑龙江、辽河、黄河流域及内陆盐碱湖泊均有分布, 具有极强的耐高碱特性, 能够耐受内蒙古达里诺尔湖碳酸盐碱度(Carbonate alkalinity, C_A)高达54 mmol/L, pH9.6的极端恶劣环境[3]。解析瓦氏雅罗鱼耐高碱的生理和分子机制, 培育耐高碱新品种, 对推进盐碱水域的开发利用进程具有重要的战略意义。

鳃是鱼类气体交换、渗透压调节、离子转运、氨氮排泄等重要生理过程发生的主要场所, 其形态结构发生变化直接影响鱼类存活[4]。鳃是研究环境变化对鱼类影响的代表性器官, 其形态结构及功能随着水体盐度[5, 6]、碱度[7]、pH[8]、温度[9]等环境因子的变化而发生变化。因此, 开展鳃的组织学调查是评估环境污染、鱼病发生程度的重要研究手段[10, 11]。

瓦氏雅罗鱼作为耐碱鱼类研究的模式生物, 前期围绕其耐高碱特性, 已从生理生化、分子生物学、基因组学等方面开展了一系列研究, 尤其借助于高通量测序技术, 发现很多基因及通路在离子交换、渗透压调节、酸碱平衡等生理过程发挥重要作用[12—15]。这些重要的生理活动与鳃的结构与功

收稿日期: 2019-10-21; **修订日期:** 2020-05-14

基金项目: 国家自然科学基金(31602136); 黑龙江省自然基金(C2016070); 中国水产科学研究院基本科研业务费(2019ZD0601)资助 [Supported by the National Natural Science Foundation of China (31602136); Natural Science Foundation of Heilongjiang Province of China (C2016070); Central Publicinterest Scientific Institution Basal Reserch Fund, CAFS (2019ZD0601)]

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能变化密不可分。本研究拟通过比较不同碳酸盐碱度胁迫下, 达里诺尔湖瓦氏雅罗鱼(碱水种)和松花江瓦氏雅罗鱼(淡水种)鳃组织结构的微观变化, 探究达里诺尔湖瓦氏雅罗鱼耐高碱特性与鳃组织结构微观调整的适应性关系, 为鱼类耐盐碱的生理和分子机制研究提供科学依据。

1 材料与方法

1.1 实验鱼

实验鱼为内蒙古达里诺尔湖瓦氏雅罗鱼自交F_2和松花江瓦氏雅罗鱼自交F_2, 饲养于黑龙江水产研究所呼兰实验站。选择大小一致, 体质量为(22.24±4.68) g的个体45尾, 运回实验室, 在循环可控水族箱(42.6 cm×28.4 cm×29.3 cm)暂养7d。实验用水为过滤、曝气24h的自来水, 水质检测指标见表 1。

1.2 不同$NaHCO_3$碱度胁迫实验

实验设置对照组(C_A0)、$NaHCO_3$碱度30 mmol/L(C_A30)、$NaHCO_3$碱度50 mmol/L(C_A50) 3组, 每组3个重复, 每个重复5尾实验鱼。采用缓慢提升碱度的方法开展胁迫实验。以5 mmol/(L·d)的速度缓慢提升, 碱度至30和50 mmol/L 后维持该碱度22d, 每天换水1/2, 实验期间不投喂, 采用YSI多功能水质分析仪监测水质温度、pH、溶氧、盐度、氨氮等指标, 用酸碱滴定法测定水体碱度。不同碱度胁迫期间, 各实验组水体检测指标见表 1。

1.3 样品采集及固定

在碱度胁迫实验结束后, 对照及两个碱度处理组各取5尾实验鱼的鳃组织, 于Bouin’s液固定(苦味酸饱和溶液+福尔马林25 mL+冰醋酸5 mL) 24h后, 转移至70%酒精于4℃保存备用。

1.4 组织切片及拍照观察

首先从70%酒精中取出组织, 参考杨建等[16]的方法, 切成大小适中的组织块, 进行脱水、透明和包埋。具体步骤如下: 50%酒精(12h)→70%酒精(过夜)→80%酒精(1h)→90%酒精(30min)→95%酒精(30min)→100%酒精Ⅰ(15min)→100%酒精Ⅱ(5min)→酒精、二甲苯(1∶1, 30min)→二甲苯(15min)→二甲苯∶石蜡=1∶1(1h, 烘箱)→包埋机(Leica EG1150)包埋。包埋后使用切片机(Microm HM 200)切成5 μm的薄片, 在涂有蛋清甘油(1∶1)的载玻片上滴满蒸馏水, 并将切片展开。

然后进行脱蜡、染色。具体步骤如下: 二甲苯(10min)→二甲苯、酒精(1∶1, 5min)→100%酒精(5min)→85%酒精(5min)→80%酒精(5min)→蒸馏水冲洗(10min)→苏木子染色(10min)→流水冲洗(15min)→盐酸分化液(8s)→流水冲洗(15min)蒸馏水冲洗(1min)→伊红复染(2min)→流水冲洗(15min, 根据染色情况控制冲洗时间)→80%酒精(5min)→90%酒精(5min)→100%酒精Ⅰ(5min)→100%酒精Ⅱ(3min)→二甲苯、酒精(1∶1, 2min)→二甲苯Ⅰ(5min)→二甲苯Ⅱ(5min)→中性树胶封片、烘干。

最后采用光学显微镜观察、拍照(Olympus BX 53&DP74)。测微尺测定鳃呼吸面鳃丝宽度、鳃小片长度及鳃小片间距。

1.5 数据处理

实验所有数据均以平均值±标准差表示。采用SPSS13.0软件对鳃丝主干宽度、鳃小片长度及鳃小片间距进行单因素方差分析(One-way ANOVA)。采用Duncan法进行组内多重比较分析, 采用*t*检验进行组间差异比较分析。$P<0.05$具有显著差异, $P<0.01$具有极显著差异。

2 结果

2.1 不同$NaHCO_3$碱度对瓦氏雅罗鱼鳃组织结构的影响

组织切片结果显示, 在正常条件下(C_A0), 两种实验鱼的鳃丝和鳃小片完整, 鳃丝末端膨起呈棒状, 鳃小片垂直于鳃丝, 呈柳叶状向两侧伸展。细胞核小, 染成蓝紫色。在$NaHCO_3$碱度胁迫下, 两种实验鱼的鳃丝宽度、鳃小片长度和鳃小片间距都发生了显著变化(表 1)。雅罗鱼碱水种鳃小片无破损, 而淡水种在C_A30开始出现破损, C_A50时破损面积增大(图 1)。

随着碱度增加雅罗鱼碱水种鳃组织结构均发生了显著变化, 鳃丝变宽、鳃小片变长、鳃小片间距变大($P<0.05$)。同样地, 随着碱度增加, 雅罗鱼淡水种鳃组织结构也发生了显著变化, 鳃丝变宽、鳃

表 1 对照组和实验组水体检测指标

Tab. 1 Water index control group and experimental group

组别 Group	温度 Temperature (℃)	pH	溶解氧 DO (mg/L)	盐度 SAL (g/L)	离子态铵 NH_4^+ (mg/L)	气态氨 NH_3 (mg/L)	实测碱度Examined alkalinity (mmol/L)
C_A0	19.32±2.05	7.56±0.09	7.44±0.45	0.06±0.01	2.37±0.81	0.04±0.03	0.44±0.05
C_A30	19.22±1.29	9.44±0.08	7.58±0.42	1.39±0.06	1.40±0.40	1.47±0.49	29.70±0.81
C_A50	18.94±1.21	9.55±0.06	7.51±0.06	2.27±0.08	1.32±0.31	1.75±0.55	48.90±4.14

小片间距变大(P<0.05), 鳃小片长度在C_A30时显著变长(P<0.05), 但在C_A50时与对照组无明显差异(表 2)。

在相同碱度胁迫下, 与淡水种相比, 雅罗鱼碱水种鳃丝显著变宽(P<0.01), 鳃小片长度在C_A50时显著变长(P<0.01), 鳃小片间距在C_A30时显著变大

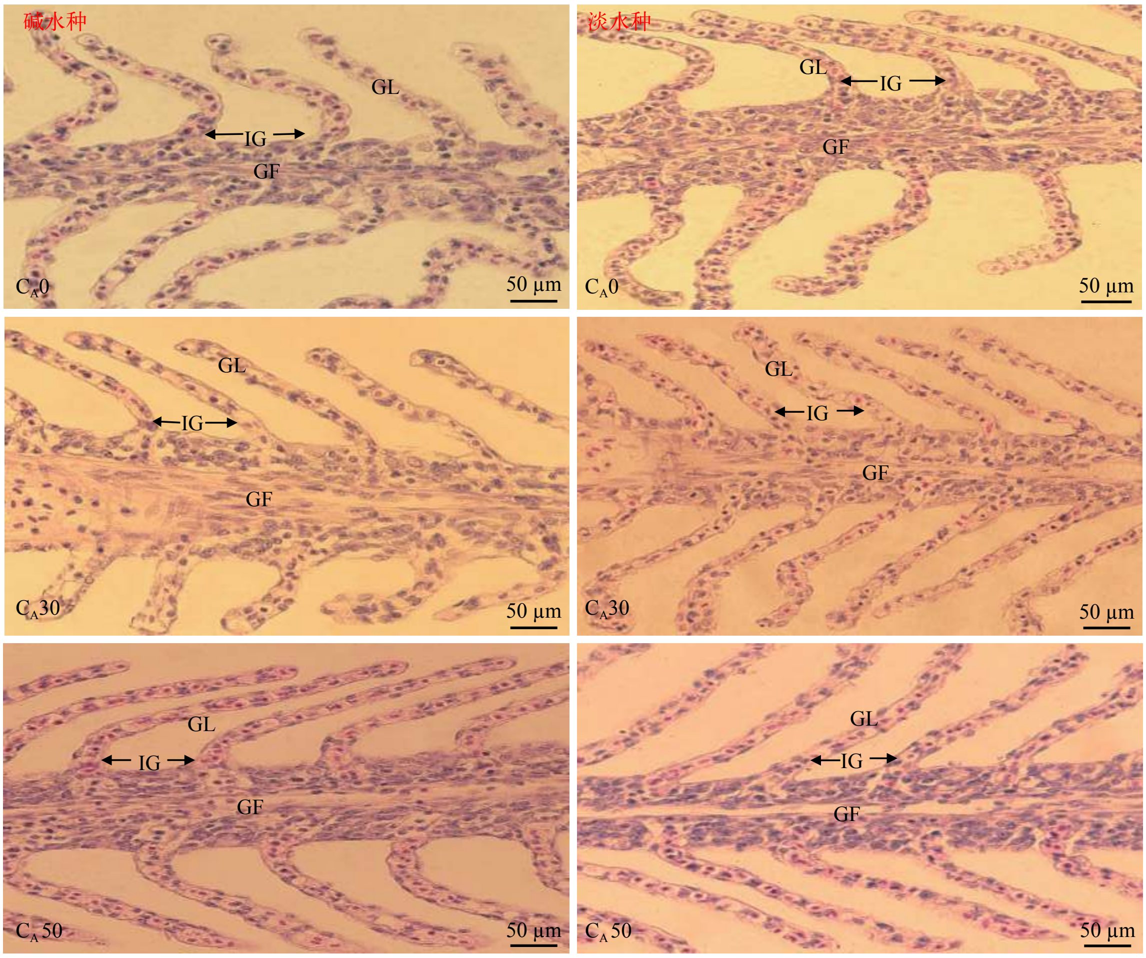

图 1 不同碱度对瓦氏雅罗鱼碱水种和淡水鳃组织结构的影响

Fig. 1 Effects of bicarbonate alkalinity on the gill structure in alkali-adapted species and freshwater species of Amur ide

GF. 鳃丝; GL. 鳃小片; IG. 鳃小片间距

GF. gill filament; GL. secondary gill lamella; IG. the intervals of secondary gill lamella

表 2 $NaHCO_3$碱度对瓦氏雅罗鱼鳃结构的影响

Tab. 2 Effect of $NaHCO_3$ alkalinity on the gill structure of Amur ide (n=9)

指标Index	碱度Alkalinity	碱水种 Alkaliadapted species	淡水种Freshwater species	碱水种*vs.* 淡水种P值Alkali-adapted species *vs*. Freshwater species P value
鳃丝宽度The width of gill filament (μm)	0	41.33±1.35[a]	35.62±1.92[a]	0.0000
	30	50.83±0.95[b]	42.57±2.14[b]	0.0000
	50	55.41±1.23[c]	50.53±0.98[c]	0.0014
鳃小片长度The length of secondary gill lamella (μm)	0	97.83±2.01[a]	100.10±2.65[a]	0.5272
	30	109.16±2.52[b]	109.52±3.01[b]	0.9437
	50	121.97±2.85[c]	101.67±2.56[a]	0.0000
鳃小片间距The intervals of secondary gill lamella (μm)	0	28.82±1.63[a]	26.40±1.25[a]	0.0595
	30	32.63±0.54[b]	29.58±0.63[b]	0.0176
	50	34.99±0.73[c]	32.81±0.49[c]	0.0759

注: 表中数据为9个重复的平均值, 同列数据不同上标字母表示组间差异显著(P<0.05)

Note: Data are means of nine repetitions, different superscript letters in the same column indicate significant differences between groups (P<0.05)

(P<0.05, 表 2)。

2.2　不同$NaHCO_3$碱度对瓦氏雅罗鱼鳃丝上皮细胞结构的影响

鳃丝上皮由多层上皮细胞组成, 包括扁平上皮细胞(Pavement cell, PVC)、氯细胞(Mitochondria-rich cell, MRC)、黏液细胞(Mucous cell, MC)、柱细胞(Pillar cell, PC)等。由图 2可以看出, 氯细胞(Mitochondria-rich cell, MRC)主要位于鳃小片基部, 多为长柱形或卵圆形, 体积膨大, 苏木精-伊红染色(HE)着色较深; 扁平上皮细胞呈薄鳞状覆盖于鳃小片上, 与柱细胞、血细胞交替排列形成血管通道。

与对照组相比, 瓦氏雅罗鱼碱水种在C_A30和C_A50的氯细胞数量明显增加, HE着色较深, C_A50的细胞排列更加紧密, 细胞核变大并且有叠加现象; 扁平上皮细胞变大, 细胞表面增厚。瓦氏雅罗鱼淡水种, C_A30的氯细胞明显多于C_A50; 随着碱度增加, 鳃小片出现破损, 扁平上皮细胞、柱细胞和血细胞融合、脱落现象严重 (图 2)。

2.3　不同$NaHCO_3$碱度对瓦氏雅罗鱼鳃耙上皮黏液细胞的影响

在鳃耙(Gill raker, GR)上皮中发现了黏液细胞, 呈透明状 (Mucous cell, MC, 图 3)。在正常条件下, 黏液细胞呈近似圆的椭圆形, 排列和大小不均匀; 随着碱度的增加, 黏液细胞由大而稀疏变为小而密集, 并且分布均匀。C_A30, 黏液细胞呈长椭圆形, 大小形状相似, 且大都排列在靠外的部位, 排列紧密整齐; C_A50, 黏液细胞大小和形态与C_A30相似, 但排列更加整齐紧密。在相同碱度条件下, 瓦氏雅罗鱼碱水种的黏液细胞数量较淡水种多, 而且排列更为整齐、密集(图 3)。

3　讨论

3.1　$NaHCO_3$碱度对瓦氏雅罗鱼鳃组织结构的影响

鱼类鳃组织直接与外界水体接触, 对水体的物理和化学变化非常敏感。Haaparanta等[17]发现季节性温度及水质是导致鳃组织结构发生变化的主要

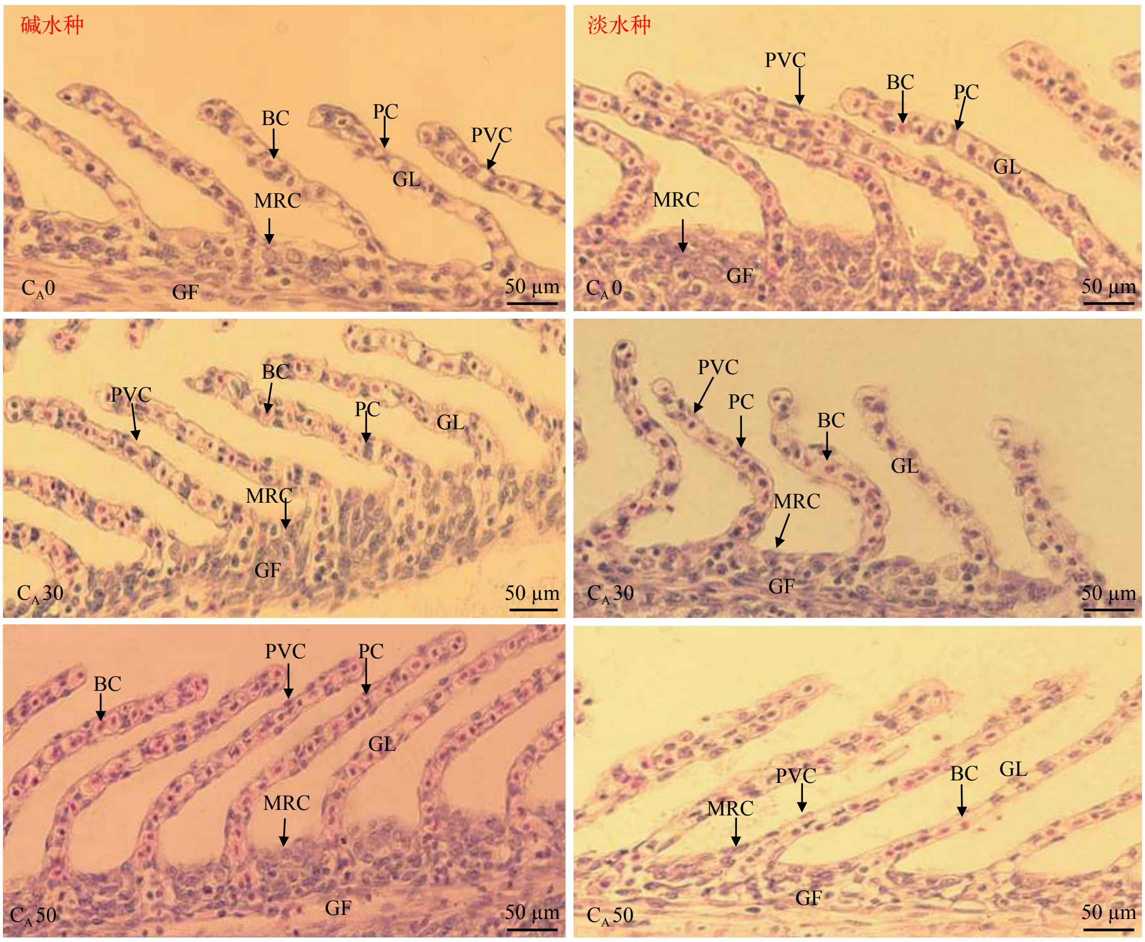

图 2　不同碱度对瓦氏雅罗鱼碱水种和淡水种鳃表皮MRC和PVC细胞的影响

Fig. 2　Effects of bicarbonate alkalinity on the gill MRC and PVC in alkali-adapted species and freshwater species of Amur ide

PVC. 扁平上皮细胞; MRC. 氯细胞; PC. 柱细胞; BC. 血细胞; GF.鳃丝, GL. 鳃小片

PVC. pavement cell; MRC. mitochondria-rich cell; PC. pillar cell; BC. blood cell; GF. gill filament; GL. secondary gill lamella

因素，其中影响程度最为严重的是水体pH。在本研究中，添加$NaHCO_3$导致水体pH处于9.44—9.55，基本与达里诺尔湖的pH 9.6相近。通过胁迫实验发现，瓦氏雅罗鱼碱水种鳃丝主干变宽，鳃小片变长，鳃小片间距变大($P<0.05$)，这与花鲈(*Lateolabrax japonicus*)[18]、鲻(*Mugil cephalus*)[6]、星斑川鲽鱼(*Platichthys stellatus*)[19]在盐度胁迫下(pH 8.0)的鳃丝细胞收缩、鳃小片间距变大的研究结果稍有不同。由此可以看出，碱度与盐度对鱼类鳃组织结构的影响明显不同，即耐盐碱鱼类和海水鱼类鳃组织可能具备不同的生理调节机制。

高碱度高pH是耐盐碱鱼类生存环境的主要特点，因而赋予了这些鱼类一些特殊的生理适应性机制。青海湖裸鲤(*Gymnocypris przewalskii*)为了适应高盐碱的生活习性，其鳃组织形态结构包括鳃耙和鳃丝都发生了适应性改变[20—22]。本研究通过比较瓦氏雅罗鱼碱水种和淡水种在相同碱度处理条件下的鳃组织结构发现，雅罗鱼碱水种鳃丝主干显著变宽($P<0.01$)，鳃小片长度显著变长($P<0.01$)，鳃小片间距变大趋势明显(表 2)。推测这种结构上的适应性变化，有利于增强瓦氏雅罗鱼在碱度胁迫下的气体扩散效率，机体摄氧量，以此促进自身的新陈代谢[5]。另外，从组织结构的完整性来看，随着碱度的升高，瓦氏雅罗鱼碱水种的鳃组织结构较为完整，无脱落、破损的现象；而瓦氏雅罗鱼淡水种随着碱度升高，鳃丝和鳃小片均有一定程度的脱落，这与花鲈[18]、鲻[6]等在盐度胁迫下鳃小片出现断裂、脱落的现象相符。这些研究结果表明，瓦氏雅罗鱼碱水种由于长期生活在高盐碱高pH的环境中，其鳃组织结构已经发生了适应性改变。

3.2 $NaHCO_3$碱度对瓦氏雅罗鱼鳃上皮细胞的影响

鱼类鳃组织直接与外界水体接触，覆盖于鳃丝的表皮细胞对鳃生理功能的正常发挥至关重要。氯细胞在维持机体内环境稳定、离子调节和渗透压平衡方面发挥重要作用[23, 24]。广盐性硬骨鱼类可通过调节鳃丝上皮中氯细胞的数量、分布、内部结构以及Na^+-K^+-ATPase活性等来适应外界的盐度变化[25, 26]。目前已经在莫桑比克罗非鱼(*Oreochromis mossambicus*)、大西洋鲑(*Salmo salar*)等鱼类中鉴定了多种氯细胞类型[27]。研究发现，虹鳟

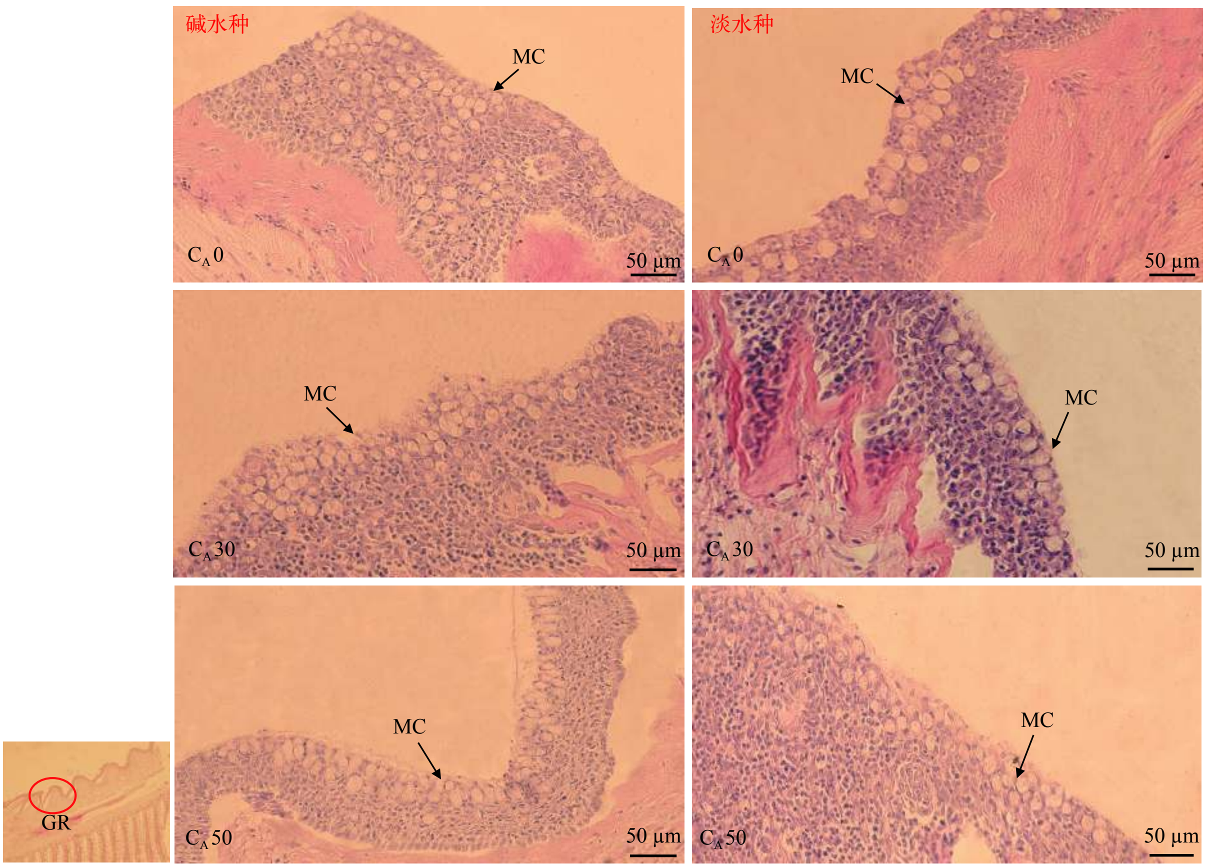

图 3 不同碱度对瓦氏雅罗鱼碱水种和淡水种鳃耙上皮MC细胞的影响

Fig. 3 Effects of bicarbonate alkalinity on the gill MC in alkali-adapted species and freshwater species of Amur ide

GR. 鳃耙; MC. 黏液细胞

GR. gill raker; MC. mucous cell

(*Oncorhynchus mykiss*)鳃氯细胞数量与盐度呈线性正相关[28]。Maina[29]在肯尼亚马加迪湖(Lake Magadi, pH10)罗非鱼(*Oreochromis alacalicus* grahami)鳃上皮发现了2种氯细胞, 其在不同环境刺激下的拓扑结构、超微结构、线粒体数量和大小存在显著差异; 张仁意等[22]观察了青海湖裸鲤鳃氯细胞特征, 发现其数量多于淡水型。本研究发现, 随碱度升高, 瓦氏雅罗鱼碱水种鳃氯细胞数量增多、体积变大并且有叠加现象。这些研究表明耐盐碱鱼类的氯细胞数量和碱度也存在正相关。氯细胞主要位于鳃小片基部, 盐度或碱度胁迫, 鱼类氯细胞数量增多与鳃小片间距变大的研究结果也是相符的。

鳃小片较薄的表皮细胞有利于环境和血液的气体交换和扩散, 但在环境胁迫时因防御反应会造成细胞隆起(Lifting up of the epithelium)和鳃小片融合(Lamellar fusion)现象, 以避免损伤。细胞隆起会增加毒物进入血液的距离, 鳃小片融合则会减少脆弱鳃表面积的数量[30]。本研究在碱度胁迫下未发现鳃小片融合现象, 但发现瓦氏雅罗鱼碱水种鳃小片上的扁平上皮细胞变大增厚, 而淡水种则出现细胞融合脱落现象(图 2), 这与食蚊鱼(*Gambusia affinis*)[30]、大菱鲆幼鱼(*Scophthalmus maximus*)[31]、莫桑比克罗非鱼[32]、黄颡鱼(*Pelteobagrus fulvidraco*)[33]在pH、溴氰菊酯、Cd^{2+}胁迫时的研究结果相似, 进一步表明瓦氏雅罗鱼碱水种可通过细胞隆起阻止HCO_3^-和pH进入血液, 保持细胞结构和生理功能的完整性以适应高碱环境, 而淡水种由于细胞融合, 结构不完整造成生理功能丧失, 因而不能适应高碱环境。

黏液细胞的增加和分泌与鱼类盐度[34]、pH、氨浓度[35]的增加及病原微生物和寄生虫感染[36]显著相关。本研究通过比较瓦氏雅罗鱼碱水种和淡水种在不同碱度胁迫下鳃耙上皮黏液细胞数量、分布、形态等特征发现, 瓦氏雅罗鱼碱水种随着碱度增加, 黏液细胞数量显著增多、大小均匀、排列紧密, 这与王瑞芳等[37]和Oğuz[38]发现盐碱湖泊瓦氏雅罗鱼和卡拉白鱼(*Chalcalburnus tarichi*)黏液细胞数量增多的研究结果相符, 推测其数量增加可能在鱼类渗透压调节中发挥重要作用。也有学者认为黏液细胞的功能是分泌黏液, 形成一个功能表面, 促进水的微循环, 利于气体交换等其他作用[39]。

本研究通过比较瓦氏雅罗鱼碱水种和淡水种在不同碱度胁迫下鳃组织结构的差异, 发现碱水种由于长期适应高碱环境, 其鳃组织发生了适应性变化: 鳃丝宽, 鳃小片长, 鳃小片间距大, 覆盖于鳃丝上皮的氯细胞和黏液细胞数量多, 增强渗透压和离子调节能力, 扁平上皮细胞增厚, 可有效阻止有毒物质进入血液, 并和柱细胞保持鳃小片结构的完整性, 从而实现其在高碱环境下生理功能的正常发挥。

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THE EFFECT OF DIFFERENT BICARBONATE ALKALINITY ON THE GILL STRUCTURE OF AMUR IDE (*LEUCISCUS WALECKII*)

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Abstract: In this study, the differences of branchial epithelium of Amur ide (*Leuciscus waleckii*) originated in Dali Nor Lake (alkal-adapled species) and Songhua River (freshwater species) were observed, which compared under the same bicarbonate stress of 0 (C_A0, pH 7.56), 30 mmol/L (C_A30, pH 9.44) and 50 mmol/L (C_A50, pH 9.55) by histological method. Furthermore, the adaptive relationship between high alkali-tolerance and the micro-adjustment of gill tissue structure was explored. The results showed that the gill structure of the alkaline species changed correspondingly, with the increase of alkalinity, which the gill filament became more full, the gill lamella became longer as well as the interval between lamellae became larger compared to the control (C_A0, $P<0.05$). The freshwater species also changed, which the gill filament became more full and the interval between lamellae became larger ($P<0.05$) than the control, although the gill lamella became longer at C_A30 ($P<0.05$), there was no significant difference between C_A50 and C_A0 ($P>0.05$). Chloride cells were found in the base of lamellae which increased higher in both of species than that of the control, pavement cells were observed in the secondary gill lamella which became larger and thicker in alkal-adapled species than the control, whereas due to the fusion and cell detachment of pavement cells, pillar cell, and blood cells, the secondary gill lamella of fresh water species was damaged severely. In addition, a large number of mucous cells were found in the epithelium of gill raker of alkal-adapled and freshwater species. With the increase of alkalinity, the mucous cells changed from large and sparse to small and dense. Among them, the mucus cells of alkal-adapled species were more than that of freshwater species, and arranged more orderly and tightly. To sum up, the alkal-adapled species could adapt to the high alkali environment for a long time by keeping the integrity of gill structure and physiological function, while the freshwater species vice versa, because of the loss of physiological function caused by the fusion and exfoliation of gill cells. The results of this study can provide basis and guidance for transplantation and acclimation of freshwater fish in saline and alkaline water in the future.

Key words: *Leuciscus waleckii*; Bicarbonate alkalinity; Gill; Histology