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厦门大学

硕 士 学 位 论 文

# 盐度胁迫对马拉瓜丽体鱼生理生化的影响

# Effect of salinity stress on the physiological and biochemical characteristics of Managua Cichlid, *Cichlasoma managuense*

陈曦飞

指导教师姓名：艾 春 香 副 教 授
专 业 名 称：海 洋 生 物 学
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# 摘 要

采用实验生态学方法，探讨盐度[0(对照组，S0 组)、3(S3 组)、6(S6 组)、9(S9 组)、12(S12 组)和 15(S15 组)]胁迫对平均体质量为(79.33±14.61)g，平均体长为(12.71±2.84)cm 的马拉瓜丽体鱼(*Cichlasoma managuense*)血清血糖、乳酸和皮质醇激素含量、肌肉 RNA/DNA 比值、鳃 ATPase 活性、肝脏和肌肉热休克蛋白 90(HSP90)含量以及肝脏、肌肉、鳃和血清抗氧化酶与磷酸酶的活性等影响，以期探明马拉瓜丽体鱼在盐度胁迫下的生理响应和适应策略，为建立其低盐养殖模式提供理论依据。本实验的主要结果和结论如下：

1. 盐度胁迫对马拉瓜丽体鱼血清葡萄糖与乳酸含量以及肌肉 RNA/DNA 比值的影响

马拉瓜丽体鱼血清葡萄糖含量，胁迫至 96 h 时，S3 组、S6 组和 S9 组显著低于 S0 组[S0 组稳定在(3.0±0.27)mmol/L 左右] ($P$<0.05)，S9 组最低，胁迫 192 h 时降至(1.77±0.084)mmol/L，为相同时间下 S0 组的 62.32%；S15 组升高，最大值达(4.48±0.193)mmol/L，胁迫至 192h 时比 S0 组升高了 8.1%。鱼血清乳酸含量，胁迫至 192h 时，S3 组、S6 组和 S9 组显著低于 S0 组($P$<0.05)，S6 组最小[(5.97±0.45)mmol/L]，仅为相同时间下 S0 组的 63.4%，S6 组与 S9 组间差异不显著($P$>0.05)；S12 组和 S15 组显著高于相同时间下 S0 组($P$<0.05)，胁迫至 144 h 时达到最大值，分别为(14.36±1.64) mmol/L、(17.16±1.88)mmol/L，比 S0 组升高了 21.3%、44.9%，胁迫至 192 h 时有所降低。

盐度胁迫显著影响鱼肌肉 RNA/DNA 比值。胁迫 144 h 后，S0 组、S3 组与 S6 组间差异不显著($P$>0.05)；胁迫至 96 h 时，S15 组降至 0.53±0.094，显著低于相同时间下 S0 组($P$<0.05)，胁迫至 144 h 时回升，至 192 h 时降至最低；S3 组、S6 组、S9 组与 S0 组差异逐渐缩小，表明这 3 组的鱼生长正在恢复，S12 组、S15 组持续显著降低，表明其生长受阻。

2. 盐度胁迫对马拉瓜丽体鱼 ATPase 活性、HSP90 及皮质醇激素含量的影响

盐度胁迫显著影响鱼鳃 $Na^+$-$K^+$-ATPase(NKA)和 $Ca^{2+}$-$Mg^{2+}$- ATPase(CMA)活性($P$<0.05)。马拉瓜丽体鱼鳃 NKA 活性随盐度的升高表现出先降低后升高，最

小值出现在 S6 组和 S9 组，CMA 变化则相反，表明 NKA 与 CMA 在渗透压调节作用中具有互补性，同时又互相竞争。随胁迫时间的延长，NKA 表现为先升高后降低，CMA 则保持稳定，表明 NKA 是马拉瓜丽体鱼渗透压调节以适应环境的关键酶，CMA 则起补充和辅助作用，NKA 与 CMA 的共同作用是马拉瓜丽体鱼适应新盐度环境的重要途径。

鱼肝脏和肌肉 HSP90 含量，胁迫至 144 h 时，S3 组、S6 组和 S9 组显著低于 S0 组($P$<0.05)，至 192 h 时，S0 组、S3 组、S6 组和 S9 组间差异不显著($P$>0.05)；S12 组和 S15 组的则持续升高，S15 组在胁迫 48 h 时分别达到最大值(3741.35±372.64) ng/gprot (肝脏)和(4488.11±493.74) ng/gprot (肌肉)，显著高于相同时间下 S0 组($P$<0.05)，分别升高了 16.0%和 37.5%。

鱼血清皮质醇激素含量，S3 组、S6 组和 S9 组在整个实验过程中逐渐降低，这 3 组间差异不显著($P$>0.05)，均显著低于 S0 组($P$<0.05)；S12 组和 S15 组在整个实验过程中持续显著升高，胁迫至 192 h 时分别达到最大值(192.76±18.57) ng/mL 和(203.63±15.11) ng/mL，比 S0 组升高了 104.7%和 116.2%($P$<0.05)。

3. 盐度胁迫对马拉瓜丽体鱼抗氧化酶及磷酸酶活性的影响

S3 组、S6 组和 S9 组的鱼肝脏、肌肉和血清 SOD、CAT 活性均被激活，S12 组和 S15 组则被抑制，鳃 SOD、CAT 活性变化却相反，这表明低盐胁迫能激活该鱼的抗氧化能力，盐度超过一定的范围，抗氧化能力受到抑制，产生氧化损伤。鱼各组织 GPX 活性变化均与 CAT 相反，二者在鱼体内既互相补偿又互相竞争。胁迫至 192 h 时，S3 组、S6 组和 S9 组的均能恢复至接近 S0 组，S12 组和 S15 组显著低于 S0 组($P$<0.05)。这表明超过该鱼耐受能力的盐度变化将导致其体内抗氧化酶系统发生异常。

鱼肝脏 ACP 与 AKP 均随盐度的升高表现为先降低后升高，胁迫至 192h 时 S3 组、S6 组和 S9 组与 S0 组差异不显著($P$>0.05)，S12 组和 S15 组则在全程中均显著高于 S0 组($P$<0.05)；鱼肌肉 ACP 与 AKP 活性在胁迫至 48h 时，各组随盐度升高表现为先降低后升高，胁迫至 192h 后各组之间差异不显著($P$>0.05)。这表明低盐度组的鱼体内磷酸酶活性受到抑制，但随着鱼对环境的适应而逐渐恢复，S12 组和 S15 组的鱼体内磷酸酶活性显著升高，可能是环境盐度超过了鱼耐受能力使其组织结构受到一定程度的损伤所致。

**关键词**：马拉瓜丽体鱼；盐度胁迫；生理生化指标；RNA/DNA 比值；HSP90

# Abstract

An experimental ecology was conducted to determine the effect of different salinities (0,Control group (fresh water), 3(S3 group), 6(S6 group), 9(S9 group), 12(S12 group) and 15(S15 group)) stress on the physiology response of Managua Cichlid (*Cichlasoma managuense*),with an average initial body weight of(79.33±14.61) g and body length of (12.71±2.84) cm including the glucose, lactic acid and cortisol content in serum, RNA/DNA ratio in muscle, two kinds of ATPase in gills, HSP90 content in muscle and liver, antioxidant enzyme and phosphotase enzyme in liver, muscle, gills and serum. To investigate the physiological response and adaptive strategies of *C. managuense* to the salinity stress, which provide a theoretical basis for the establishment of the low salinity aquaculture patterns for *C. managuense*. The majority results and conclusion of this study were as followed:

1. Effect of salinity stress on the content of glucose and lactic acid in serum and RNA/DNA ratio in muscle of *C. managuense*.

The glucose content in serum of *C. managuense* in S3 group, S6 group and S9 group were significantly lower than that in S0 group ($P$<0.05), S0 group decreased down to about (3.0±0.27) mmol/L and remained stable at 96 h stressed. S9 group decreased down to (1.77±0.084) mmol/L at 192 h stressed, it was 62.32% of S0 group under the same stress time. There was an increase in S15 group, and it was up to the maximum at (4.48±0.193) mmol/L at 48 h stressed, and it was 8.1% higher than that of S0 group. lactic acid content in serum of *C. managuense* in S3 group, S6 group and S9 group were significantly lower than S0 group ($P$<0.05), but the minimum was (5.97±0.45) mmol/L in S6 group, and it was 63.4% of S0 group, there was no significant difference between S6 group and S9 group ($P$>0.05) at 192 h stressed. lactic acid content in serum of *C. managuens* in S12 group and S15 group were significantly higher than that of S0 group under the same stress time ($P$<0.05), elevated to the maximum at (14.36±1.64) mmol/L and (17.16±1.88) mmol/L

respectively at 144 h stressed, and they were 21.3%, 44.9% higher than that of S0 group, and then they decreased to some extent.

Salinity stress significantly reduced the RNA/DNA ratio in muscle of *C. managuense* ($P$<0.05). The differences of RNA/DNA ratio were not significant among S0 group, S3 group and S6 group, also that was between S9 group and S12 group ($P$>0.05), but S9 group and S12 group were significantly lower than S0 group ($P$<0.05); The RNA/DNA ratio in S15 group decreased down to the lowest at (0.53±0.094) at 96 h stressed and remained stable; the differences were reducing with the stress time in S3 group, S6 group and S9 group compared to S0 group, it indicated a recover of fish in these groups, but the RNA/DNA ratio in S12 group and S15 group continuously decreased significantly ($P$<0.05), it showed a inhibition of growth in the two groups.

2. Effect of salinity stress on the osmoregulation, HSP90 content in liver and muscle, and cortisol content in serum

Salinity stress significantly affected the activities of NKA and CMA in gills of *C. managuense* ($P$<0.05), NKA activity first decreased and then increased with the salinity level increasing, but the CMA activity showed conversely. It indicated that NKA and CMA had a mutual compensation mechanism, meanwhile, they competed to each other. NKA activity showed to be first increasing then decreasing but CMA activity basically remained stable in all groups. So it indicated NKA play a key role in osmoregulation of *C. managuense*, while CMA was very critical too, it had supplementary aids for NKA.

HSP90 content in liver and muscle of *C. managuense* in S3 group, S6 group and S9 group were lower significantly than that of S0 group when salinity stress time up to 144 h ($P$<0.05), but there were no significant differences among S0, S3, S6 and S9 groups at 192 h stressed ($P$>0.05). On the contrary, S12 group and S15 group increased continuously, S15 group was up to the maximum at (3741.35±372.64) pg/g (liver) and (4488.11±493.74) pg/g (muscle), they were 16.0% and 37.5% higher than that of S0 group.

Cortisol content in serum of *C. managuense* in S3 group, S6 group and S9 group

decreased gradually, and the differences were not significant among the three groups ($P$>0.05), but significantly lower than that of S0 group ($P$<0.05). On the contrary, S12 group and S15 group continuously increased significantly ($P$<0.05), up to the maximum at (192.76±18.57) and (203.63±15.11) ng/mL respectively at 192 h stressed, it was 104.7% and 116.2% higher than that of S0 group ($P$<0.05).

3. Effect of acute salinity stress on the antioxidant enzymes and phosphotase in liver, muscle, gills and serum

The activities of SOD and CAT were stimulated in liver, serum and muscle of *C. managuense* in S3 group, S6 group and S9 group, but inhibited in S12 group and S15 group, and the activities of SOD and CAT showed an opposite variation in gills. It indicated that promoted the antioxidant capacity of the fish under salinity 3-9, but it may inhibit the antioxidant enzyme and cause oxidant damage when water salinity exceeded the tolerance ability of fish. GPX activity showed an opposite way against CAT activity in different tissues and organs, it indicated that they had a mutual compensation mechanism, meanwhile, they competed to each other. S3 group, S6 group and S9 group could recover to the level of S0 group, but S12 group and S15 group were lower significantly than that of S0 group ($P$<0.05). It indicated that salinity viaration exceed the tolence ability of the fish could lead to abnormal occur.

The activities of ACP and AKP in liver of *C. managuense* decreased at first and then increased. The activities of ACP and AKP in liver of S0 group, S3 group, S6 group and S9 group had no significant differences ($P$>0.05), but those of S12 group and S15 group were higher significantly than that of S0 group ($P$<0.05) at 192 h stressed. However, the activities of ACP and AKP in muscle decreased at first and then increased with the salinity level elevating, but it showed no significant difference among treatments ($P$>0.05) at 192 h stressed. It indicated that there was some metabolic inhibiting when it was transferred into salinity 3-9 treatments but it could recover soon; S12 and S15 group increasing abnormally, maybe they injuried cell structure to some extent when the environmental salinity exceed the tolence ability of *C. managuense*.

**Key words**: *C. managuense*, salinity stress, physiological and biochemical index, RNA/DNA ratio, HSP90

# 缩略词表

| 缩略词 | 英文全称 | 中文全称 |
|---|---|---|
| GH | growth hormone | 生长激素 |
| IGF-1 | insulin-like growth factor1 | 胰岛素类生长因子 1 |
| RNA | ribonucleic acid | 核糖核酸 |
| DNA | deoxyribonucleic acid | 脱氧核糖核酸 |
| MRCs | mitochondrion-rich cells | 富含线粒体细胞 |
| CCs | Secrete chlorine cells | 泌氯细胞 |
| ATPase | triphosphatase triphosphatase | 腺嘌呤核苷三磷酸酶 |
| NKA | Na+-K+-ATPase | 钠钾-腺嘌呤核苷三磷酸酶 |
| CMA | Ca2+-Mg2+-ATPase | 钙镁-腺嘌呤核苷三磷酸酶 |
| ROS | reactive oxygen species | 活性氧物质 |
| SOD | Superoxide dismutase | 超氧化物歧化酶 |
| CAT | Catalase | 过氧化氢酶 |
| GPX | Glutathione peroxidase | 谷胱甘肽过氧化物酶 |
| GSTs | Glutathione S-tranferases | 谷胱甘肽 S-转移酶 |
| GRs | Glutathione reductase | 谷胱甘肽还原酶 |
| AA | Ascorbate peroxidase | 抗坏血酸过氧化物酶 |
| ACP | Acid phosphatase | 酸性磷酸酶 |
| AKP | Alkaline phosphatase | 碱性磷酸酶 |
| HSP | heat shock protein | 热休克蛋白 |
| CFTR | cystic fibrosis transmembrane conductance regulator | 囊性纤维化跨膜转运调节器 |
| GR | glucocorticoid receptor | 糖皮质激素受体 |
| MR | mineralocorticoid receptor | 盐皮质激素受体 |
| CRH | corticotropin releasing hormone | 促肾上腺皮质激素释放激素 |
| ACTH | adrenocorticotropin | 促肾上腺皮质激素 |

# 第一章 绪论

水中溶解盐类的总量称盐度或者矿化度，它取决于溶解在水体中的盐类，其中包括92种基本化学元素中的62种(Riley，1965)。一般认为，海水盐度为35左右，主要元素为$Na^+$、$Mg^{2+}$和$Cl^-$，纯淡水盐度为0，淡水并非去离子水，其主要离子为$CO_3^{2-}$、$HCO_3^-$和$SO_4^{2-}$等，具有一定的渗透压。实验发现，鱼类无法在去离子水中生长，甚至导致其死亡(Boeuf and Payan，2001)。盐度与鱼类的生长、发育、繁殖和渗透压调节关系密切，鱼类通过一系列生理过程来应对盐度变化，以维持自身体内外渗透压的动态平衡(张龙岗，2011)。研究表明，淡水鱼类可通过调节组织细胞膜的通透性(Palacios et al，2004)、营养代谢途径(Rosas，2001)和机体离子浓度(Lignot et al，2000)等多种方式来应对水体盐度变化。机体主动调节渗透压的过程中所需的额外能量和一些中间代谢物，均直接或间接地由摄入的饲料养分中分解和转化而得。盐度变化除了影响机体消化酶活性(Li et al，2008)和免疫功能(Alvarez et al.，2004)等机体生理反应外，也影响到机体的正常发育、生长、呼吸代谢、能量收支、健康和繁殖。

鱼类由淡水进入海水后，机体通过吞饮海水、减少尿量和排出离子等方式来调节渗透压，其中排出离子是一种重要的方式，在这个过程中，鱼类鳃上的泌氯细胞发挥着主要作用。泌氯细胞主要依靠增强 $Na^+$-$K^+$-ATPase(NKA，EC 3.6.1.3)为排出氯化钠提供能量，随着盐度升高，机体 NKA 活性逐渐增强，且 NKA 活性受激素调控(Hachim et al.，1996；马惠等，2012)，其中，生长激素(Growth hormone，GH)能够使鱼类鳃泌氯细胞数量增加、体积增大，同时使鳃 NKA 活性增强(McCormick，2001)。胰岛素类生长因子-I(Insulin like growth factor-1，IGF-I)能够使虹鳟(*Oncorhynchus mykiss*)的耐盐能力大大提高(Mancera，1998)，而 GH/IGF-I 调节中枢是鱼类渗透压调节的重要组成部分(Yousefian，2011)。

盐度胁迫是人为地改变水生动物生活水体的盐度，导致其机体生理状态改变的实验手段。盐度胁迫分为急性胁迫和慢性胁迫：急性胁迫即把鱼体直接从自然生活环境移入设定盐度的水体中，鱼体生理状态的变化能够比较迅速地表现出来，主要用于研究鱼类对盐度变化的生理响应及其机制；慢性胁迫即在一定时间段内逐级地改变鱼体生活环境的盐度，使其逐渐适应环境盐度并保持正常生理状