Impact of Ammonia Exposure on Bone and Cartilage Formation in Zebrafish (*Danio rerio*) Larvae

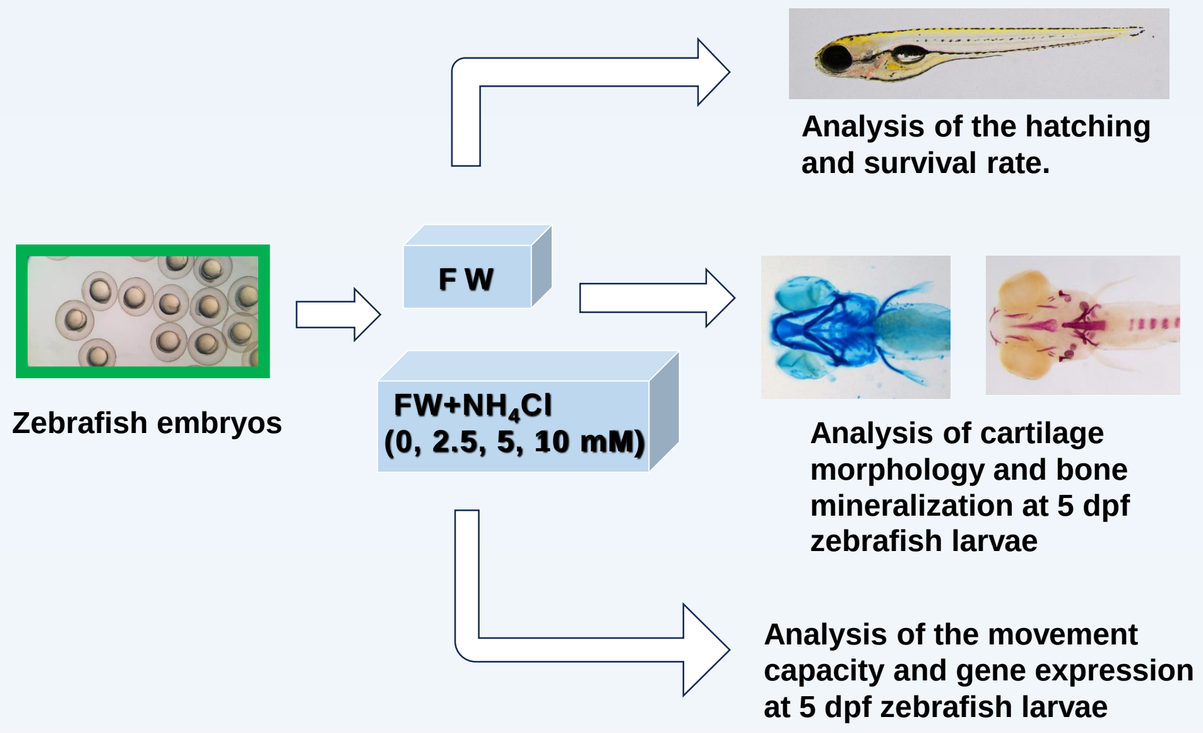
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INTRODUCTION

Ammonia is a pervasive freshwater pollutant from aquaculture and wastewater, reaching tens of mM in certain intensive farming areas and Asian rivers. Although its toxicity in adult fish is well-documented, its effects on early cartilage and skeletal development in larvae remain poorly understood. To address this gap, this study investigated the impacts of chronic NH_4Cl exposure in zebrafish (*Danio rerio*) from 0 dpf under standardized conditions (28 ˚C, pH 7.0, 12-h light/dark cycle). At 5 dpf, we examined craniofacial cartilage formation, skull bone mineralization, swimming behavior, and chondrogenic/osteogenic gene expression, providing new evidence on ammonia toxicity during early fish skeletal development.

EXPERIMENT DESIGN



RESULTS

Survival and Hatching Rate

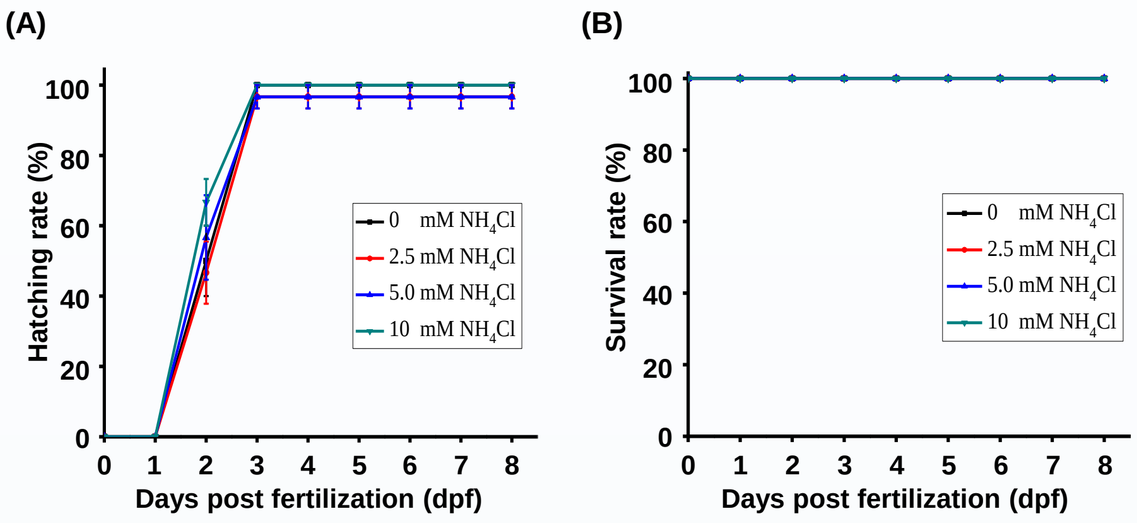


Fig. 1. Effects of NH_4Cl exposure on the (A) hatching and (B) survival rates of zebrafish embryos. Fertilized zebrafish eggs were exposed to freshwater containing 0, 2.5, 5.0, and 10 mM NH_4Cl, respectively, and recorded daily until 8 days post fertilization (dpf). Mean ± SEM (n = 20). No significant differences were observed between groups ($p > 0.05$, ANOVA).

Craniofacial Cartilage Morphology

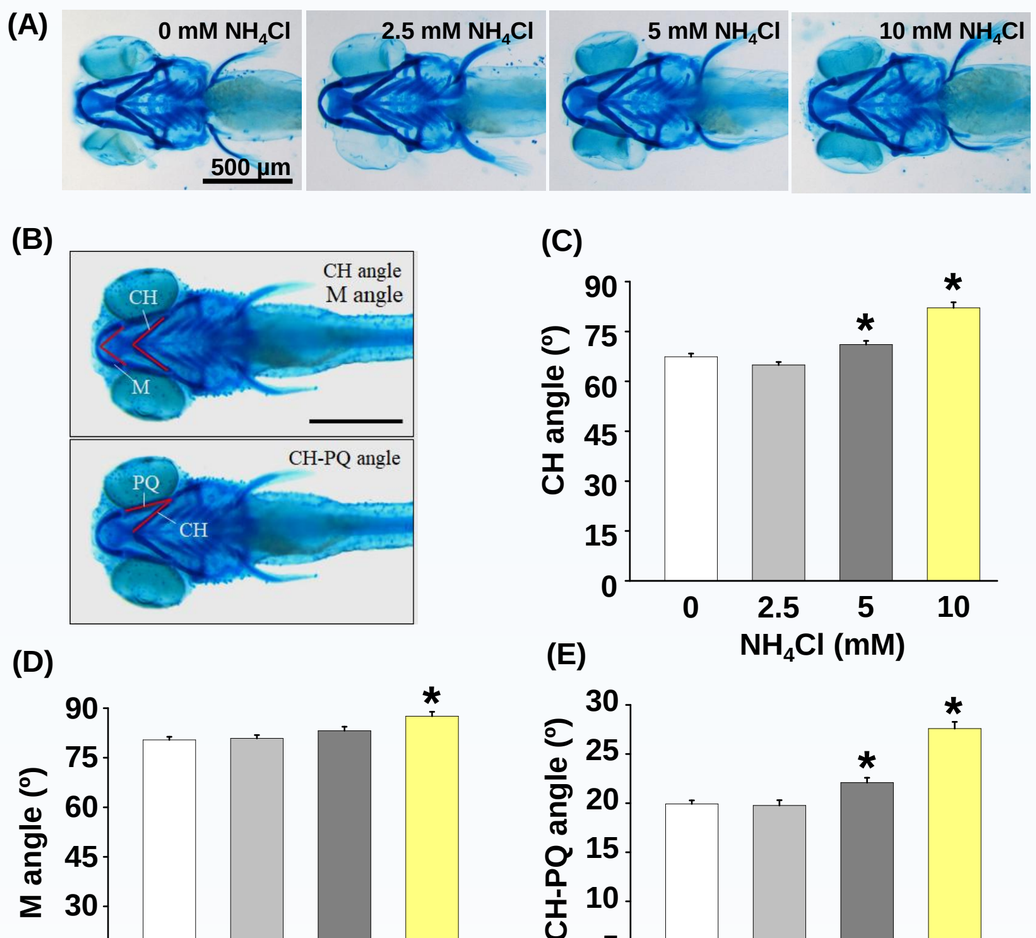


Fig. 2. Effects of NH_4Cl on craniofacial cartilage at 5 dpf. (A) Alcian Blue staining of cartilage. (B) Measurement of cartilage angles (CH, M, CH-PQ). (C) CH angle. (D) M angle. (E) CH and PQ angle. Mean ± SEM (n = 20). *$p < 0.05$ vs control (ANOVA).

Skull Bone Mineralization

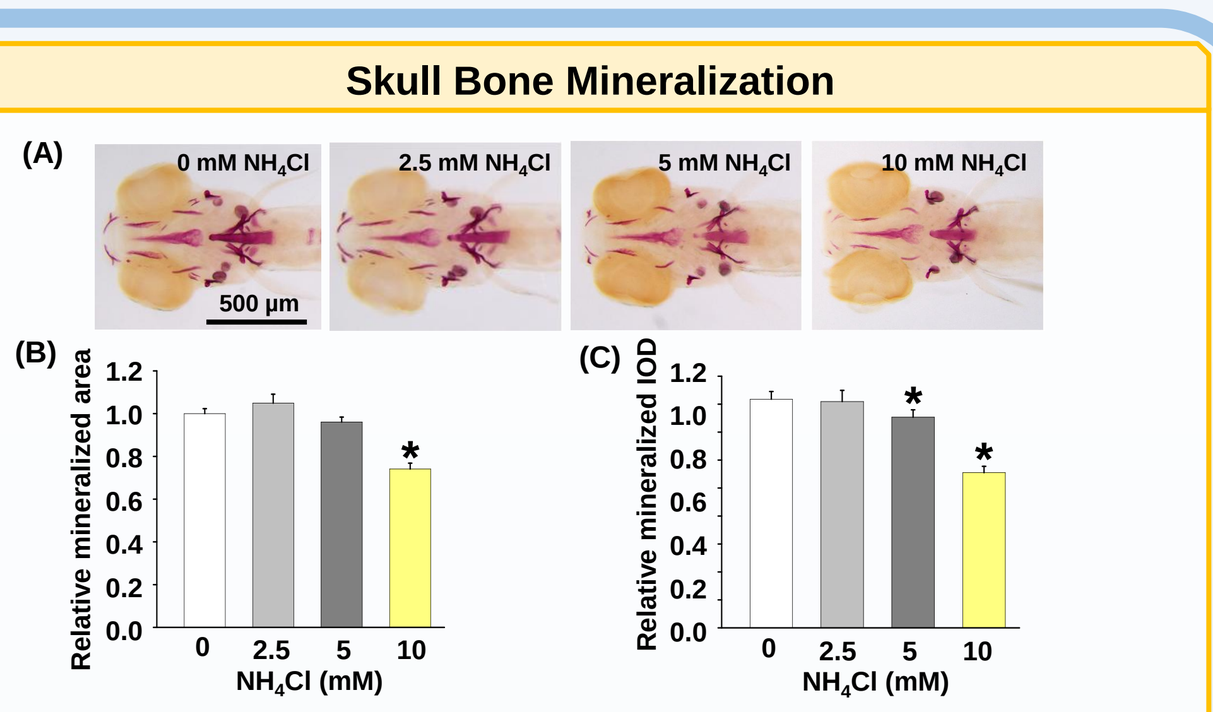


Fig. 3. Effects of NH_4Cl on skull bone mineralization at 5 dpf. (A) Ventral views of Alizarin red staining of skull bone. (B) Relative mineralized area. (C) Relative integrated optical density (IOD). Mean ± SEM (n = 20). *$p < 0.05$ vs control (ANOVA).

Swimming Capacity

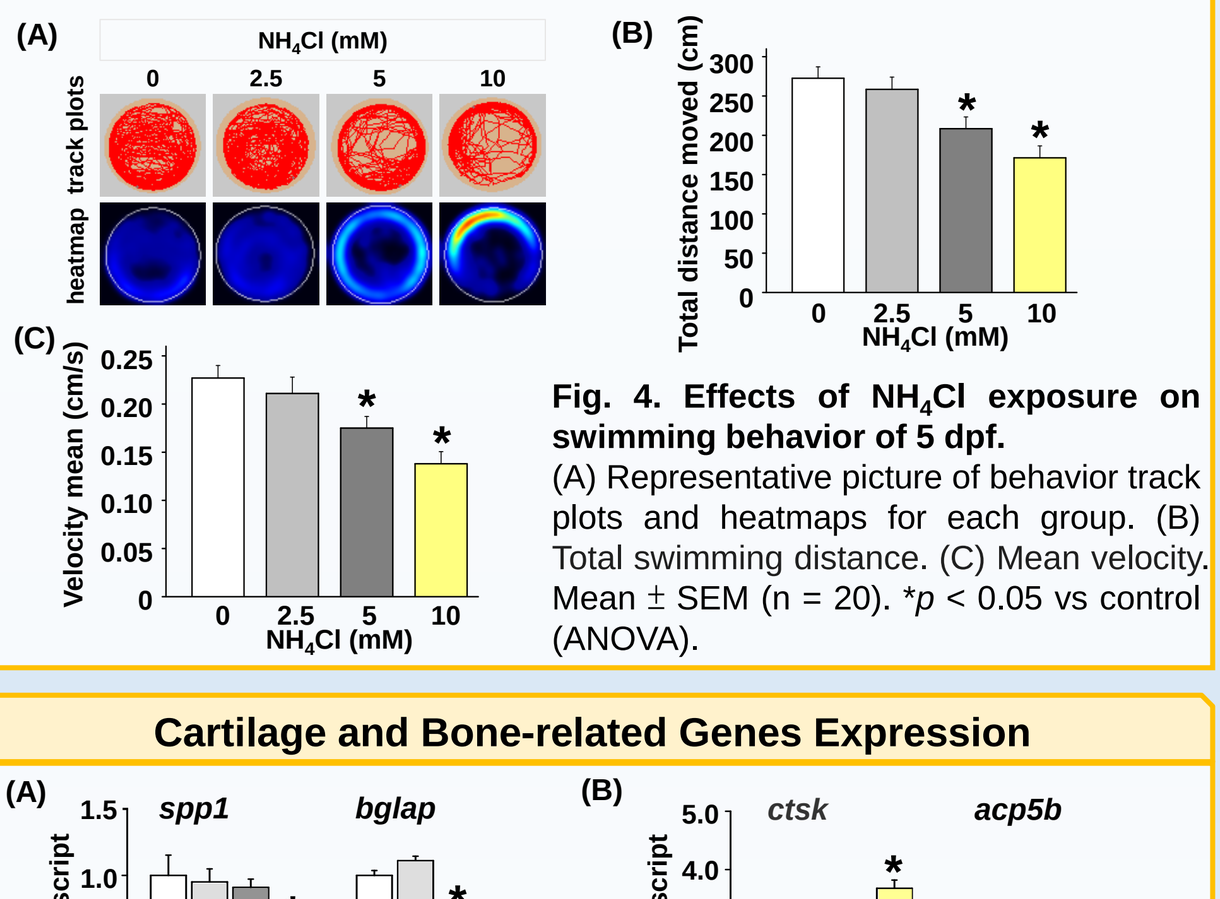


Fig. 4. Effects of NH_4Cl exposure on swimming behavior of 5 dpf. (A) Representative picture of behavior track plots and heatmaps for each group. (B) Total swimming distance. (C) Mean velocity. Mean ± SEM (n = 20). *$p < 0.05$ vs control (ANOVA).

Cartilage and Bone-related Genes Expression

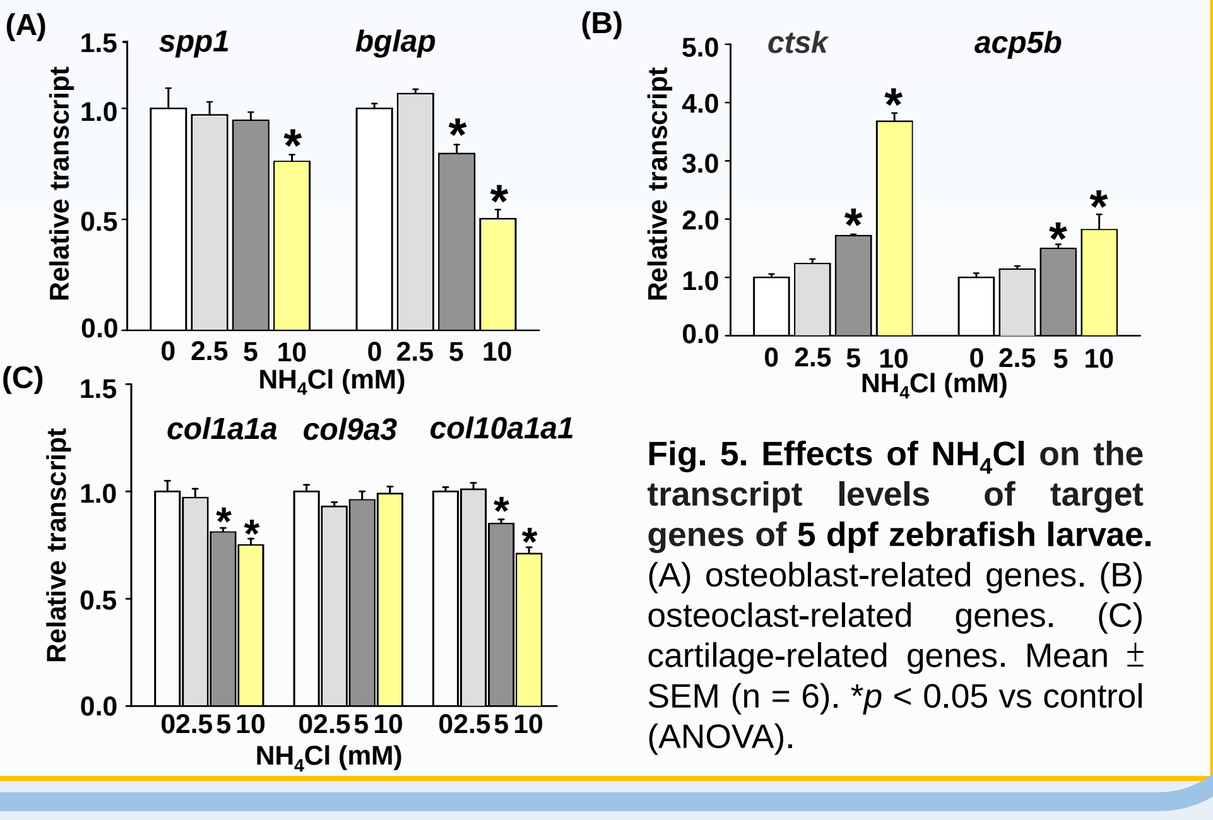


Fig. 5. Effects of NH_4Cl on the transcript levels of target genes of 5 dpf zebrafish larvae. (A) osteoblast-related genes. (B) osteoclast-related genes. (C) cartilage-related genes. Mean ± SEM (n = 6). *$p < 0.05$ vs control (ANOVA).

DISCUSSION AND CONCLUSION

1. **Although zebrafish larvae survived normally in NH_4Cl (up to 10 mM), the treatment impaired cartilage morphology and skull mineralization.**
2. **Mechanistically, 5–10 mM NH_4Cl exposure upregulated the expression of cartilage-related (*col1a1a*, *col10a1a1*) and bone resorption (*ctsk*, *acp5b*) genes, while 10 mM NH_4Cl exposure downregulated the expression of bone formation genes (*spp1*, *bglap*) in zebrafish larvae. These influences may result in defects in cartilage and bone morphology, further impairing swimming capacity in zebrafish larvae.**