Acidified Freshwater Inhibits Skeletal Ossification and Bone Remodeling in Zebrafish *(Danio rerio)* Larvae

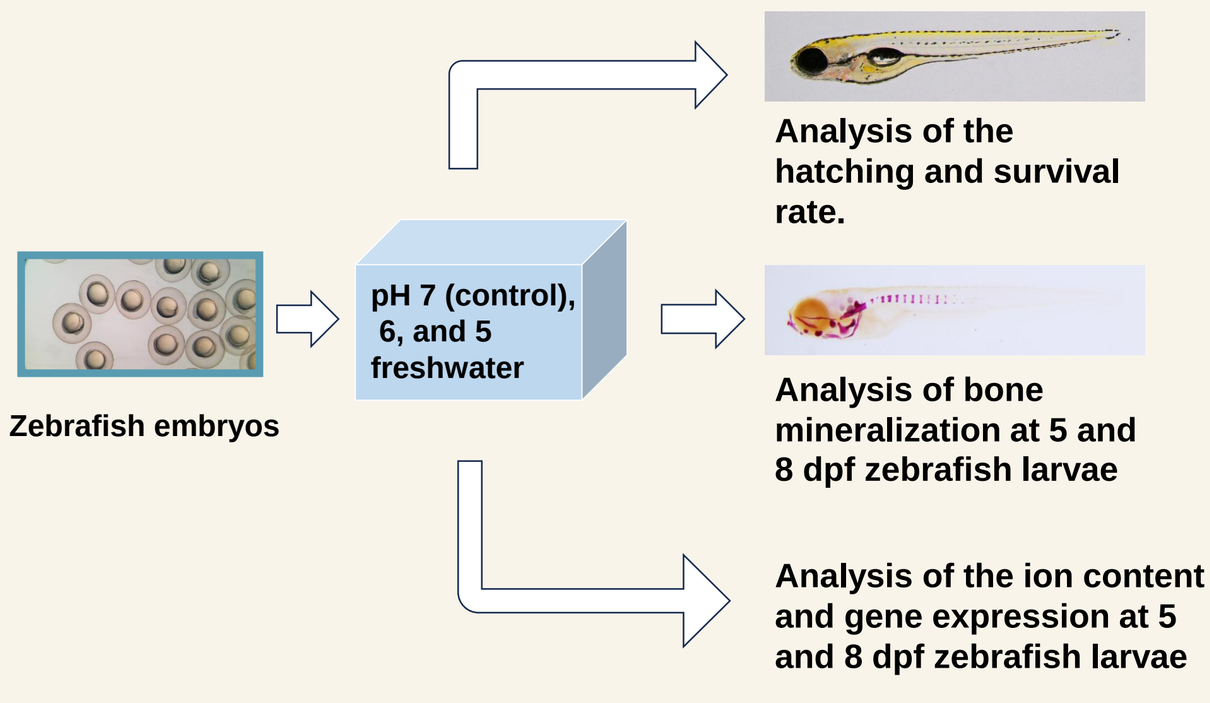
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INTRODUCTION

Acidification in freshwater (FW) aquaculture threatens skeletal development in fish larvae, but the molecular mechanisms remain unclear. We exposed zebrafish (*Danio rerio*) larvae to pH 7 (control), 6, and 5 FW from 0 to 8 days post-fertilization (dpf). Exposure to pH 5 FW significantly reduced body length and impaired vertebral and skull mineralization. Mechanistically, pH 5 FW exposure downregulated osteoblast-related genes and strongly upregulated osteoclast-related genes, thereby accelerating bone resorption and triggering whole-body calcium and phosphorus efflux. While this systemic response likely maintains ion balance, it compromises skeletal integrity to compensate for the loss of calcium and phosphorus in bodily fluid. These findings emphasize the critical need for precise pH regulation in intensive freshwater hatcheries to secure healthy larval skeletal development.

EXPERIMENT DESIGN



RESULTS

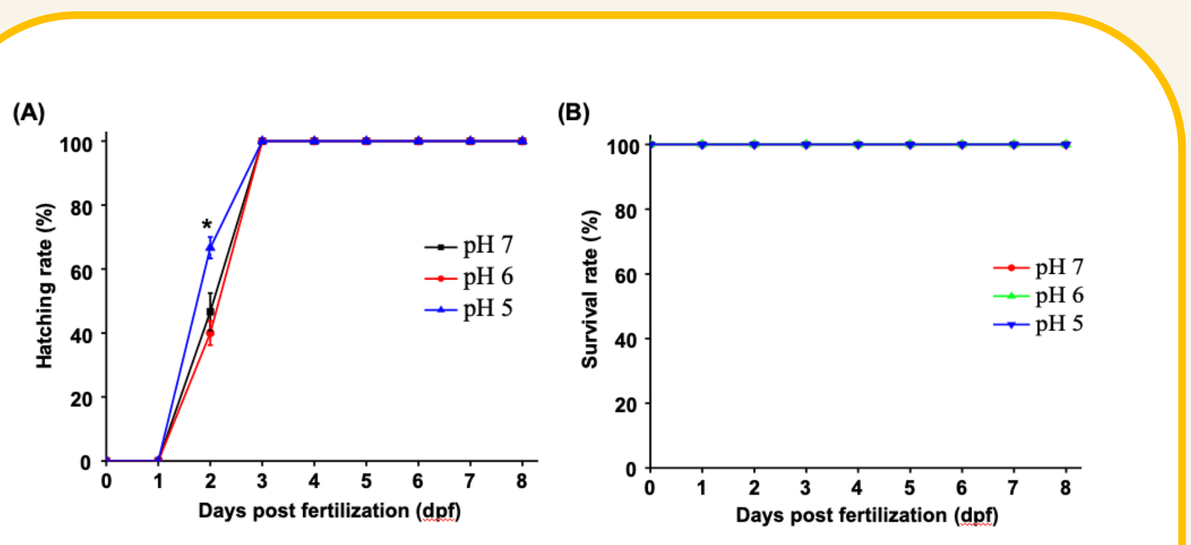


Fig. 1. Effects of acidified freshwater exposure on the (A) hatching and (B) survival rates of zebrafish larvae. Fertilized zebrafish eggs were reared in pH 7, 6, and 5 freshwater (FW) for 8 days post fertilization (dpf). Mean ± SEM (N= 20). *p < 0.01 vs control (ANOVA).

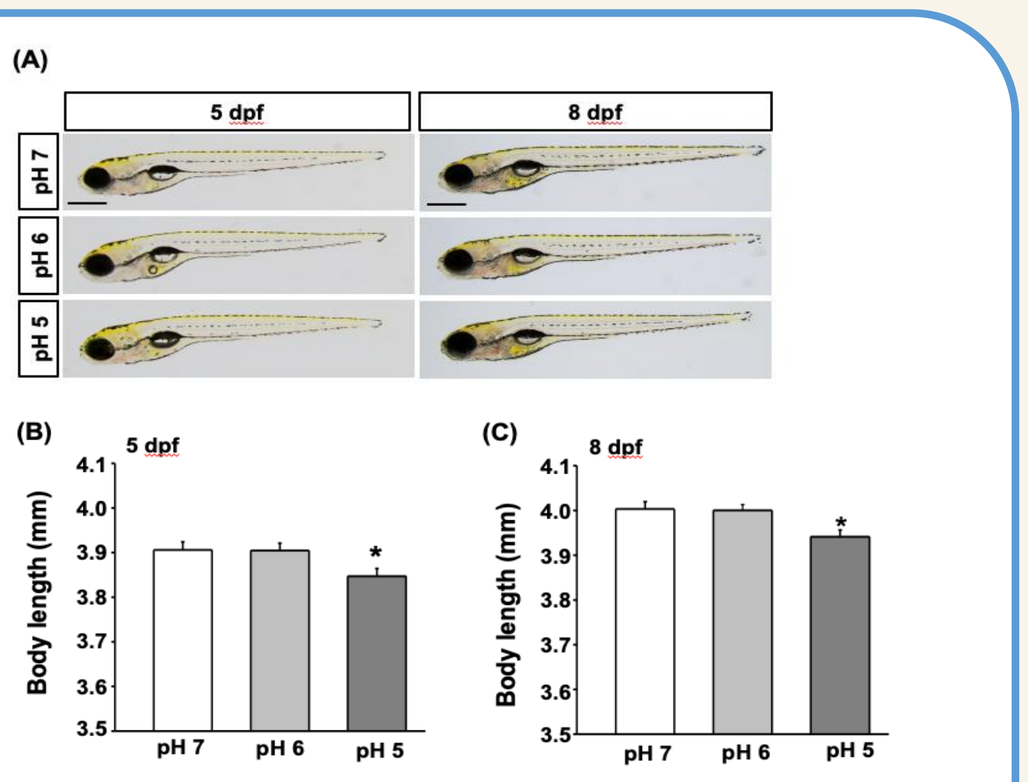


Fig. 2. Effects of acidified freshwater exposure on the body length of zebrafish larvae at 5- and 8 dpf. (A) The representative image of body length. (B) The body length of 5 dpf zebrafish larvae. (C) The body length of 8 dpf zebrafish larvae exposed. N = 20 for each condition. Mean ± SEM (N = 20). *p < 0.05 vs control (ANOVA).

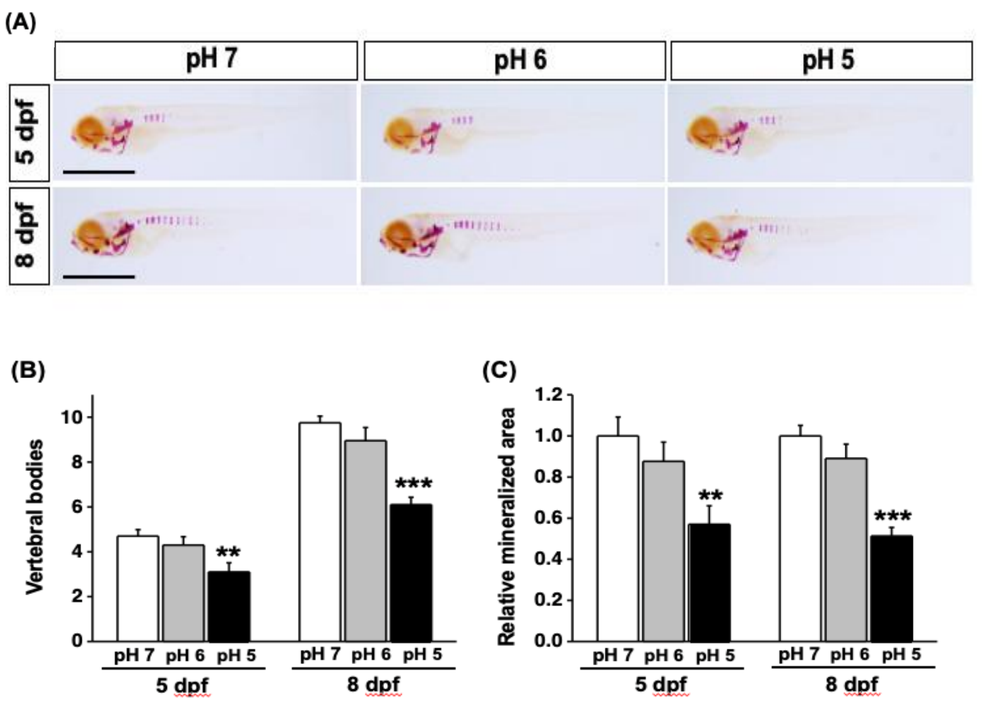


Fig. 3. Effects of acidified freshwater exposure on vertebral bone mineralization of zebrafish larvae at 5- and 8 dpf. (A) The representative image of vertebral bone mineralization. (B) The number of vertebral bodies. (C) Relative mineralized area. Mean ± SEM (N = 20). **p < 0.01, ***p < 0.001 vs control (ANOVA).

Fig. 4. Effects of acidified freshwater exposure on the relative transcript of osteoblast and osteoclast-related genes of zebrafish larvae at 5- and 8 dpf. (A) *runx2b*. (B) *acp5b*. (C) *ctsk*. Mean ± SEM (N = 6). **p < 0.01, ***p < 0.001 vs control (ANOVA).

Fig. 5. Effects of acidified freshwater exposure on the ion content and the transcript of target genes of zebrafish larvae at 5- and 8 dpf. (A) calcium content. (B) phosphorus content. (C) The relative *ecac* transcript. (D) The relative *npt2a* transcript. Mean ± SEM (N = 6). *p < 0.05, **p < 0.01, ***p < 0.001 vs control (ANOVA).

DISCUSSION AND CONCLUSION

1. **Under pH 5 stress, zebrafish larvae prioritize systemic ion homeostasis over skeletal integrity, sacrificing bone minerals to maintain internal ion balance.**
2. **Acidic stress inhibits bone formation (via *runx2b* downregulation) and actively accelerates bone resorption (via *ctsk* and *acp5b* upregulation), leading to reduced skeletal mineralization.**
3. **Increased expression of *ecac* and *npt2a* enhances environmental ion uptake, which supports mineral balance and mitigates excessive bone resorption by reducing the need to mobilize internal minerals.**