Does early-life exposure to a stressor affect ion balance in developing zebrafish, *Danio rerio*?

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Introduction

- When fish are exposed to a stressor, a series of endocrine and nervous responses is initiated that mediates both behavioural and physiological changes. A key mediator of the physiological stress response is the glucocorticoid hormone cortisol, which in fish is also implicated in the regulation of ionic and osmotic balance[1].
- In developing zebrafish *Danio rerio*, exposure to exogenous cortisol increases Na^+ and Ca^{2+} uptake from the aquatic environment[2,3]; freshwater fish actively take up ions from water to balance diffusive ion loss to their dilute environment[4]. However, how stress exposure affects ionoregulatory pathways over developmental time has not been investigated.
- Thus, the present study aimed to determine the effects of acute stress during early development, and the associated elevation of cortisol, on ion balance at later stages of development.
- If acute stress during early development affects ion regulation in later life stages, then zebrafish exposed to early life stress (and the associated acute increase in cortisol) would be predicted to have higher whole-body ion levels than control fish. In turn, higher whole-body ion levels should reflect higher abundance of ion-transporting cells or ionocytes, specifically Na^+-K^+ ATPase-rich (NaR) and H^+-ATPase-rich (HR) cells, which are responsible for Ca^{2+} and Na^+ ion uptake respectively[5].

Materials and methods

- Zebrafish larvae were exposed to air for 2 x 3 min, twice per day for two days, at 4, 7 or 15 days post fertilization (dpf)[6].
- At 7, 15, and 35 dpf, stressed and control fish were sampled for measurement of:
  - Whole-body cortisol concentrations by commercial EIA
  - Whole-body concentrations of Na^+ and Ca^{2+} by flame emission spectroscopy
- Immunohistochemistry was used to quantify ionocyte numbers in 6 dpf fish.

Results

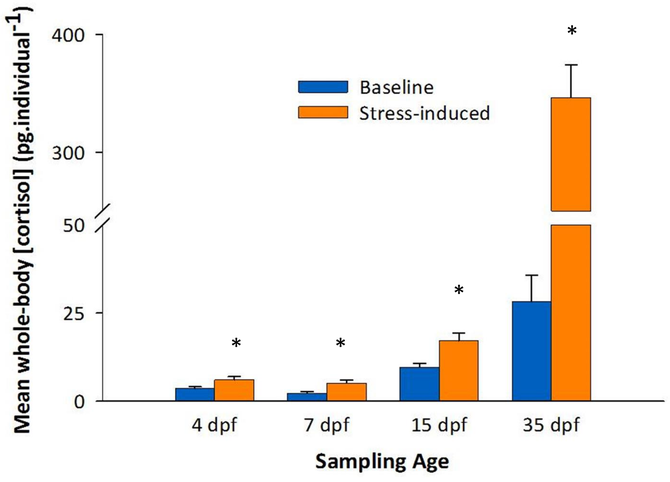

Figure 1. To confirm that exposure to the air-emersion stressor caused a cortisol response, baseline and stress-induced cortisol levels were measured at each treatment time. Exposure to air resulted in a significant elevation of whole-body cortisol concentrations at all treatment times, with older fish exhibiting higher cortisol levels. Additional tests revealed comparable elevation of cortisol across the individual exposures to air (data not shown). Values are means +SEM (n= 5-12, where n = 1 represents a pool of 1 to 20 individuals depending on age). An asterisk indicates a significant difference in whole-body cortisol between baseline and stress-induced values (two-way ANOVA, $F_{1,57}$= 7.0728, P= 0.01014).

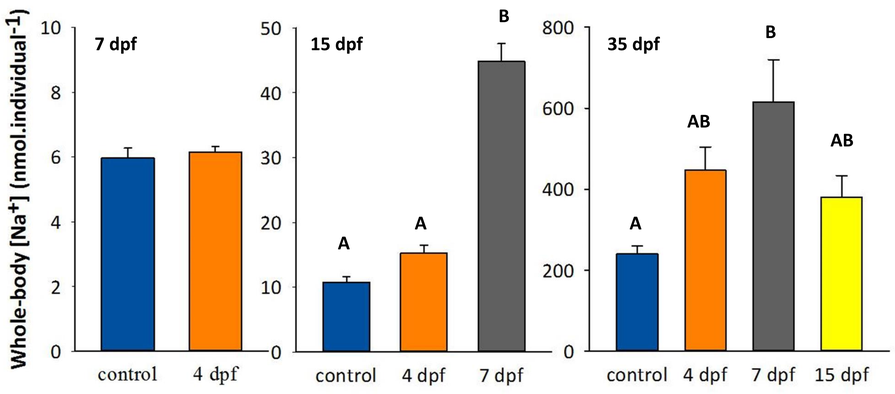

Figure 2. Exposure to stress early in development resulted in significantly higher whole-body Na^+ concentrations at later stages of development. Values are means +SEM (n= 6-16, where n=1 represents a group of 1 to 20 larvae depending on age). Groups that share a letter are not significantly different from one another (7 dpf, one-tailed Student's *t*-test, t= 0.551, P= 0.592; 15 dpf, one-way ANOVA, $F_{2,18}$= 109.78, P< 0.001; 35 dpf, one-way ANOVA, $F_{3,44}$= 5.4623, P= 0.003).

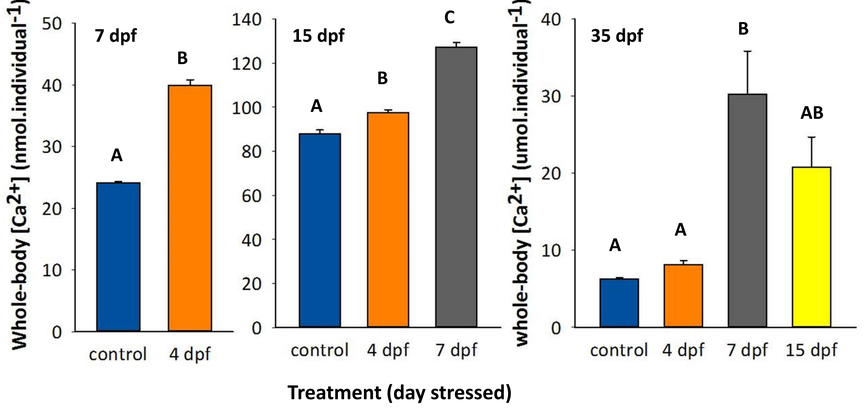

Figure 3. Exposure to stress early in development resulted in significantly higher whole-body Ca^{2+} concentrations at later stages of development. Values are means +SEM (n= 6-16, where n=1 represents a group of 1 to 20 larvae depending on age). Groups that share a letter are not significantly different from one another (7 dpf, one-tailed Student's *t*-test, t= 14.47, P< 0.001, 15 dpf, one-way ANOVA, $F_{2,17}$= 138.91, P< 0.001, 35 dpf, one-way ANOVA, $F_{3,50}$= 8.1052, P< 0.001).

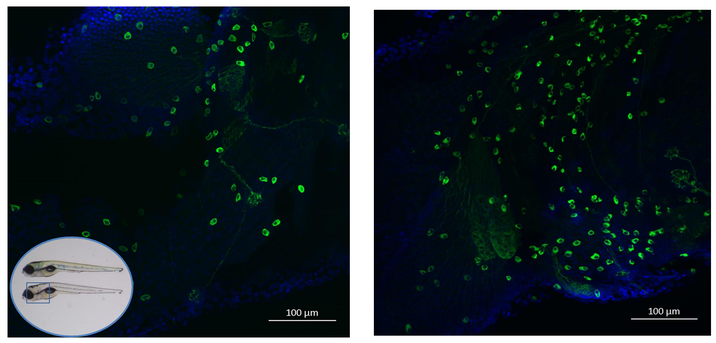

Figure 4. Representative micrographs of the yolk-sac epithelium of control (left) and 4 dpf stress treatment (right) zebrafish larvae imaged at 6 dpf. The Ca^{2+}-transporting ionocytes, the NaR cells (green), were identified by immunofluorescence for Na^+/K^+ ATPase; cell nuclei (blue) were stained using 4',6-diamidino-2-phenylindole 9 (DAPI).

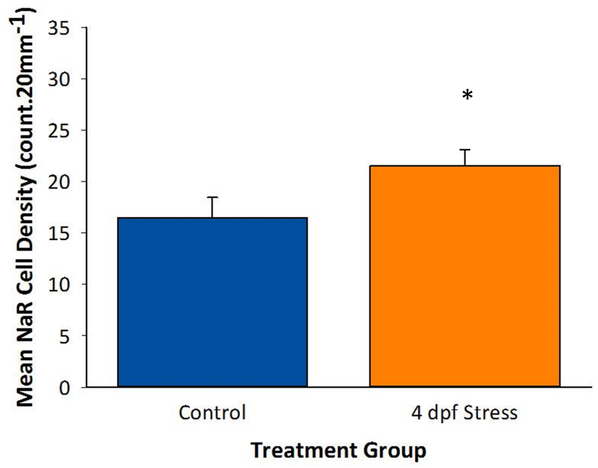

Figure 5. Exposure to stress early in development resulted in a significantly greater abundance of Ca^{2+}-transporting ionocytes or NaR cells in 6 dpf zebrafish larvae. Values are means +SEM (n= 14-16 individual larvae). There was a significant difference in NaR cell density between control and 4 dpf stress-treatment larvae (one-tailed Student's *t*-test, t= 2.062, P= 0.0241). This result suggests a mechanism that explains the observed elevation of whole-body Ca^{2+} concentrations observed in the 4 dpf stress treatment (Fig. 3).

References

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Discussion

- Early-life exposure to an acute stressor and the accompanying rise in endogenous cortisol levels resulted in significantly higher whole-body ion content at later stages of development, with effects on Ca^{2+} content being more marked than effects on Na^+ content.
- Higher whole-body Ca^{2+} levels, in turn, reflect greater abundance of Ca^{2+}-transporting ionocytes (NaR cell) in stressed fish. The mechanism through which stress/elevation of cortisol elicits increases in ionocyte abundance requires further elucidation.
- Although previous work documented effects of exogenous cortisol on ion content, the present study is the first to report an increase in ion content following exposure to a stressor resulting in an endogenous cortisol rise. These results suggest the existence of cross-talk between the stress and ionoregulatory roles of cortisol during early development in zebrafish.
- Future work will examine the effects of acute early-life stress on ionocyte densities at later stages of development as well as the mechanisms underlying changes in ionocyte density.