EFFECTS OF EGG INCUBATION TEMPERATURE ON CARDIAC FUNCTION AND MORPHOLOGY IN ATLANTIC SALMON

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HIGHLIGHTS

Marked differences were observed in **cardiac function** and **morphology** in Atlantic salmon larvae from **eggs incubated at 4** and **8°C**.

These differences **persisted** until sea transfer and seems to be exacerbated by higher water temperatures in the hatchery.

INTRODUCTION

Deviating heart morphology is frequently observed in farmed salmon and is believed to contribute to the high mortality rates observed during stressful events, such as delousing (1). **Heart deformities** have previously been linked to elevated rearing temperatures during the freshwater stage post hatching (2). However, lower water temperatures lead to longer production cycles thereby increasing production costs. Recently, **egg incubation temperature** emerged as a promising target for reducing the prevalence of cardiac maladaptation in a less costly manner. Thus, we examined the effects of low and high egg incubation temperatures on **cardiac development** by assessing heart function and morphology from six weeks post hatching to smoltification.

METHODS

Experimental animals: Eggs were incubated at **4-5** and **7-8°C.** Both groups were kept at 6°C until start feeding. Following start feeding, the cold group was separated into two cohorts with average temperatures of **8°C** and **12.5°C**. The warm eggs were reared at **13.4°C**.

Cardiac function: Six weeks post-hatching, unanesthetized larvae were placed in 2% methylcellulose in a petri dish under a microscope and video-recorded (30 sec, 25 frames/sec). Ventricular function was then analyzed by the prolate spheroid model as described by Perrichon et al. (3). At smoltification, anaesthetised fish (MS-222) underwent echocardiographic examination as described by Becker et al. (4) at 10°C using a linear ultrasound probe.

Heart morphology: Following *in vivo* experiments, fish were euthanized and hearts fixated for more detailed morphological analyses as described by Engdal et al. (5).

Statistics: Statistical analyses were performed in GraphPad Prism v.9. After checking for normality, variables were tested by unpaired or Welch's t-test. One-way ANOVA or Brown-Forsythe and Welch ANOVA with multiple comparisons were used for three-group analyses.

RESULTS

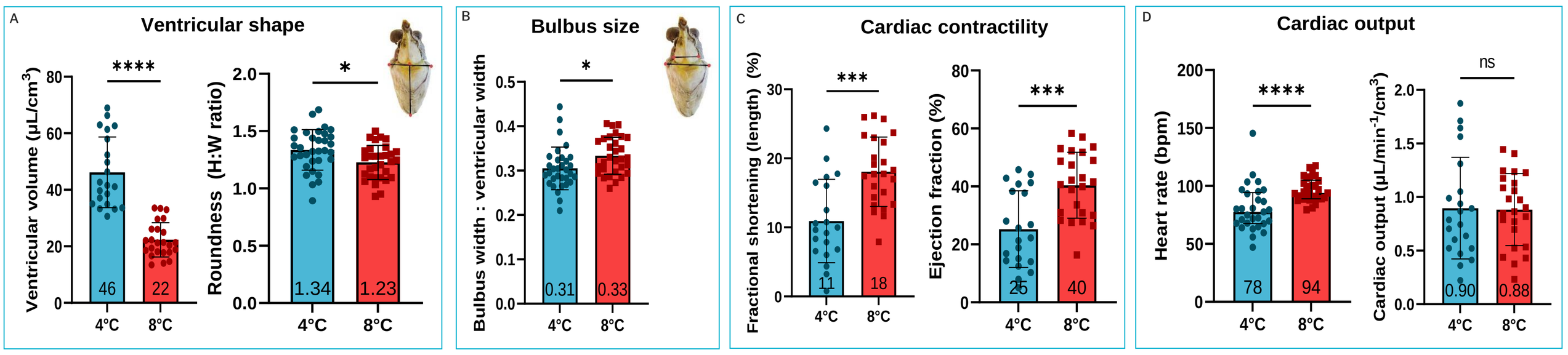

Figure 1: **Egg incubation temperature influences heart morphology and cardiac function.** Fish from eggs incubated at 8°C (warm) had smaller and rounder ventricles **(A)** and larger bulbi **(B)** compared to those incubated at 4°C (cold). As a seemingly compensatory response to the smaller ventricles, the warm group exhibited larger cardiac contractility **(C)** and increased heart rates to maintain cardiac output **(D)**. All figures are presented as mean ± SD. Illustrations of heart measurements are from Vindas et al. (in press) (2). n_{cold}=30, n_{warm}=31. * = p < 0.05, *** = p < 0.001, **** = p < 0.0001. Of note, more anatomical anomalies (anemia, scoliosis) were observed in the warm group.

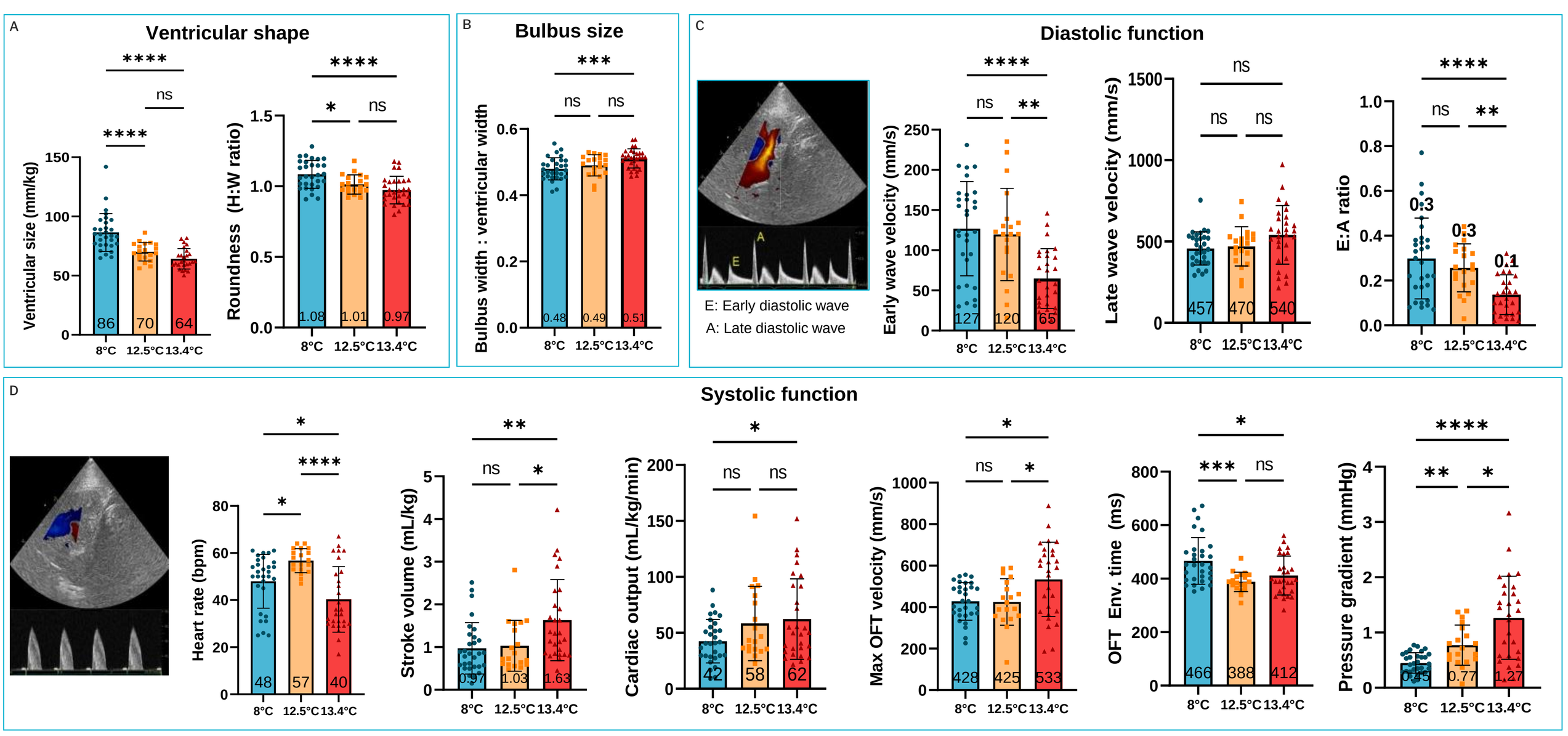

Figure 2: The combination of low egg incubation and freshwater temperatures yields more elongated ventricles with improved diastolic function at smoltification. Echocardiography was used to examine cardiac function immediately before sea transfer. Following start feeding, eggs incubated at 4°C were split into two groups in the remaining period in the hatchery: one reared at 8°C and the other at 12.5°C. Eggs incubated at 8°C were reared at 13.4°C. **(A)** Both groups reared at warmer temperatures had smaller and rounder ventricles, with a tendency towards more severe rounding in the 13.4°C group. **(B)** Only the 13.4°C group exhibited larger bulbi. **(C)** Diastolic function was assessed by measurement of early (E) and late (A) diastolic blood flow velocities. E-velocity was reduced in 13.4°C and the A-wave was unchanged. Consequently, E:A ratio was lower in the 13.4°C group, indicative of diastolic dysfunction**. (D)** Fish at 13.4°C had lower heart rates that were compensated for by larger stroke volume. This resulted in significantly higher cardiac output. These individuals also produced faster outflow tract velocities. Likely, the combination of faster ventricular contraction (OFT envelope time) and the steeper pressure gradient were responsible for the systolic differences observed. In summary, our results indicate that low egg incubation temperatures protect against early onset diastolic dysfunction as measured by E:A ratio. In addition, colder temperatures in the hatchery seems to reduce the hearts' workload. All figures are presented as mean ± SD. Echocardiographic images are taken from Becker et al. (4). $n_{8°C}$=30, $n_{12.5°C}$=20, $n_{13.4°C}$=28. * = p < 0.05, ** = p < 0.01, *** = p < 0.001, **** = p < 0.0001, ns = not significant.

CONCLUSIONS

Our results indicate that both reduced egg incubation and hatchery temperatures are beneficial for cardiac morphology and function. More experiments are needed to fully elucidate the effects of reduced temperatures in early life stages of Atlantic salmon.

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