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Brief Report

Elevated Embryonic Temperature Has Persistent Adverse Effects on Zebrafish Swimming Capacity

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Abstract: In recent years, global warming of anthropogenic origin and its impacts on biodiversity have increasingly gained public awareness. Here, we demonstrate that embryonic temperature can have persistent and crucial effects on zebrafish swimming capacity and cardiac shape. Three different embryonic temperature treatments (T_E = 24, 28 or 32 °C) were applied to zebrafish embryos until hatching. Fish were then raised in common conditions (28 °C) until adulthood. Ventricle roundness was found to increase significantly with a rise in T_E in juvenile (10% increase) and male (8% increase), but not female fish. T_E and sex significantly affected zebrafish swimming performance. Juveniles, males and females raised in cold (24 °C) presented significantly greater swimming capacity than those raised at 28 and 32 °C T_E. Our results represent a direct link between the physical capacity of adult fish and embryonic temperature fluctuations that add to the emerging rationale of the potential climate change scenarios on wild fish populations.

Keywords: fish early life stages; aerobic performance; ventricle; micro-ct; thermal plasticity

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1. Introduction

Anthropogenically driven climate change is causing ocean global warming, and regionally, Marine Heat Waves (MHWs) with harmful impacts on marine ecosystems [1]. In wild-stock populations, fish early life stages (ELS) could be even more vulnerable to temperature fluctuations given their limited dispersal activity in the water column. The susceptibility of fish to temperature experienced during the early life stages garners increasing attention due to phenotypic plasticity, a physiological process that alters fish development in response to their environment [2]. Still, despite the ongoing significance of phenotypic responses of fish to recent climate change [3], there is relatively little evidence concerning the plastic responses of fish against short temperature alterations experienced during fish ELS.

In fish, developmental temperature has been identified as a critical regulating factor in acclimation capacity, swimming muscle phenotype and sex [4–6]. To our knowledge, the plastic responses of fish swimming capacity to embryonic temperature remain unexplored. Thus, more evidence is needed in order to unravel fish plastic responses to temperature associated with climate change scenarios and impacts on wild populations. Here, we examined whether embryonic temperature (3–5 days post fertilization, dpf) has persistent effects on the swimming performance of later life stages (juveniles and adults, 4 and 10 weeks post hatching, respectively). Cardiac ventricle shape (VL/VD) was examined given the existing linkage with fish aerobic capacity [7].

2. Materials and Methods

2.1. Experimental Treatments

Zebrafish (*Danio rerio*) eggs were collected from the same subset of wild type breeders (AB strain) and subjected to three different embryonic temperatures of 24, 28, or 32 °C (±0.2 °C) until hatching (3–5 dpf). After hatching, all groups were raised in common conditions (28 °C) (Figure 1A).

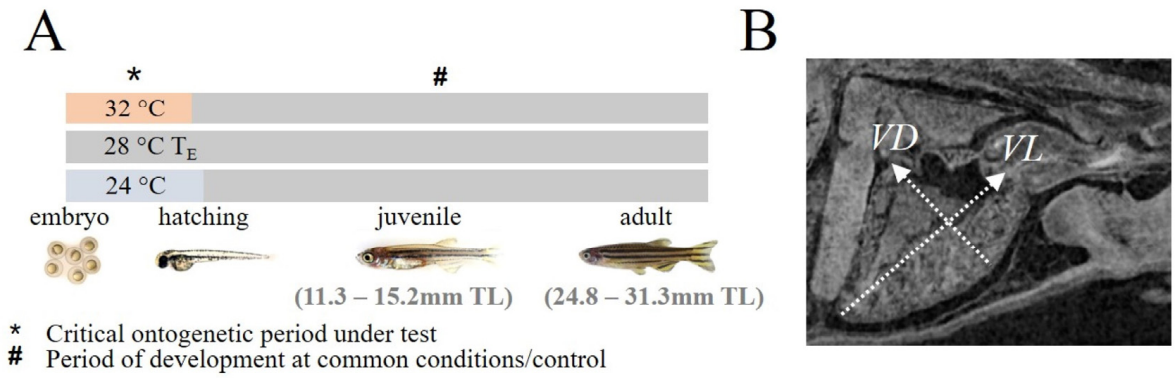

Figure 1. (**A**) Experimental design of the study, (**B**) Representative cross-section of the heart. T_E = embryonic temperature; TL = total length; VD = ventricle depth; VL = ventricle length.

Egg incubation and acclimation to the three thermal regimes (24, 28, or 32 ± 0.2 °C) was performed in 100 mL glass beakers (60 eggs per beaker, 6 beakers per thermal condition). Acclimation to the 24 °C and 32 °C treatments was performed at a rate of 1 °C.

After hatching, larvae were maintained at a common temperature of 28 ± 1 °C (Eheim, Thermopreset 150 aquarium heaters), 14 h light:10 h dark, 470–510 µS/cm and 80–97% oxygen saturation in closed recirculation systems (100% water exchange rate 100% of the aquarium volume per hour). At 12–14 mm TL (Total Length), fish were transferred to 9 L aquaria (ZebTEC, Tecniplast, Buguggiate, Italy) at a stocking density of 8 individuals L^{-1} and maintained at 28 ± 1 °C until adulthood. Two independent sets of identical experimental replicates were conducted for each of the thermal regimes.

2.2. Swimming Performance

Swimming performance was assessed by measuring the sustained critical swimming speed (U_{crit}) [8] via a custom-designed swimming apparatus with a swimming channel. A screen of plastic straws helped to maintain laminar flow and to prevent fish from escaping. Following a food deprivation period of 18–20 h, fish were individually placed in the swimming channel (static water) and left to acclimate for 5 min. Then, water velocity was raised at 2 (TL s^{-1} for 10 min. Subsequently, water velocity would be raised by 3 TL s^{-1} for a full interval of 10 min. When fish left the channel, this was determined as fatigue [9]. U_{crit} was calculated according to the equation:

$$U_{crit} = U_i + (U_{ii} \cdot t_i / t_{ii})$$

where U_i is defined as the highest swimming velocity (mm s^{-1}) achieved for 10 min, U_{ii} is the velocity raise (1 TL s^{-1}), t_i is the time that each individual swam at the fatigue velocity, and t_{ii} is the time between velocity changes (i.e., 10 min) [8]. Temperature and oxygen saturation in the swimming channel was 28.0 °C and 100%, respectively.

Exhausted fish were measured for TL after being anaesthetized (MS222, 100 mg/L^{-1}). Fish with morphological abnormalities were excluded from the analysis. For the juveniles, 21–27 individuals per experimental condition (10–15 per replicate, 12.9 ± 0.9 mm TL) were selected. For the adults, 15–20 females per treatment (5–12 per replicate, 28.2 ± 1.3 mm TL) and 21–27 males per treatment (7–16 per replicate, 27.7 ± 1.4 mm TL) were subjected to swimming tests (Table S1). Relative critical swimming speed (RU_{crit}) was calculated as the ratio of U_{crit} to the TL of each specimen.

2.3. Heart Morphology

Fourteen to sixteen individuals per experimental condition for the juveniles (5–11 per replicate, 12.7 ± 0.9 mm TL), 5–7 females and 6 males per experimental condition for the adults (3–4 per replicate, 28 ± 1.6 mm TL and 2–4 per replicate, 27.6 ± 1.6 mm TL) (Table S2) were euthanatized, fixed in formalin (5% phosphate buffered), stained for

6 days with 2.5% PMA (phosphomolybdic acid [10]), and underwent dehydration in 70% ethanol. Specimens were scanned individually (199 μA, 50 kV, 4–5 μm, 0.4° rotational step, 180° and 650 ms) by use of a micro-CT scanner (Skyscan 1172, Bruker micro-CT, Kontich, Belgium). Projection images of the scanning process were further reconstructed (NRecon software, SkyScan) into cross sections and saved as image stacks (TIFF). TIFF images were then imported into the software Amira v.5.2 (Visage Imaging, Berlin, Germany, Burlington, VT, USA) to achieve heart morphometrics processing. To estimate ventricle roundness, we measured the length-to-width ratio as an adequate indication for ventricle shape [11]. Ventricle length (VL) was measured as the maximum distance between the ventriculo-bulbar valve and the apex. Ventricle depth (VD) was measured as the widest distance of the ventricle, perpendicularly to ventricle length (VL) (Figure 1B).

2.4. Data Analysis

Statistical differences between different thermal treatments were tested via the non-parametric Kruskal–Wallis test. Differences between males and females of the same embryonic treatment were tested via the non-parametric Mann–Whitney U test (IBM SPSS Statistics, IBM Corp. in Armonk, NY, USA).

3. Results and Discussion

In juveniles, RU_{crit} values were significantly reduced with the rise in T_E (Embryonic Temperature) ($p < 0.05$, Mann–Whitney U test, Figure 2A). In adults, T_E and sex significantly affected zebrafish swimming performance. Males and females of the 28 and 32 °C groups had decreased swimming velocities compared to the ones incubated at 24 °C ($p < 0.05$, Mann–Whitney U test, Figure 2A). At 24 °C T_E, sex significantly affected swimming performance, with males achieving higher swimming velocities than females ($p < 0.05$, Mann–Whitney U test, Figure 2A).

Heart shape of juveniles and males was significantly modified in response to T_E, while there was no change in female fish. (Figure 2B). In juveniles, VL/VD ratio significantly reduced with T_E elevation, from 1.53 ± 0.05 at 24 °C to 1.39 ± 0.03 at 32 °C ($p < 0.05$, Mann–Whitney U test). A similar effect was detected in male fish, with the groups of 24 and 28 °C presenting a significantly higher ratio (1.26 ± 0.03 and 1.25 ± 0.03, respectively) in comparison to the 32 °C group (1.16 ± 0.03, $p < 0.05$, Mann–Whitney U test) (Figure 2B). In females, no differences were observed in ventricular shape. No sex differences were obtained between males and females of the different temperature treatments.

Despite the existing association between embryonic exposure to pollutants and heart form and function [11], this is the first study demonstrating the inseparable linkage between temperature experienced during the embryonic stage, and the heart shape and aerobic swimming capacity of juvenile and adult fish. In zebrafish, cardiac morphogenesis (5–48 hpf) is characterized by an inseparable linkage between heart form and function [12]. In the present study, the programming of ventricular shape (rounder ventricle) by T_E might be attributed to the early temperature effect on the heart rate which in turn modulates cardiac remodeling [13].

In the only relevant study [5], Scott and Johnston demonstrated the significant effect of T_E on the thermal acclimation capacity of the adults, without however taking into account the sex-related plasticity of swimming performance of the species [14]. In the present study, we also depicted the effect of embryonic temperature on the swimming capacity of the adults, considering the strong sexual dimorphism that zebrafish are characterized by. We concluded that 3–5 days of fish embryo exposure to elevated temperature has persistent adverse effects on zebrafish swimming speed.

In nature, this short exposure is relevant to marine heat waves that are known to last at least five days and reach a temperature threshold well above the normal range [15]. Given the vital ecological significance of sustained swimming (U_{crit}) for wild populations [16], the present study has relevance to global climate change implications and understanding fish remodeling in response to environmental change.

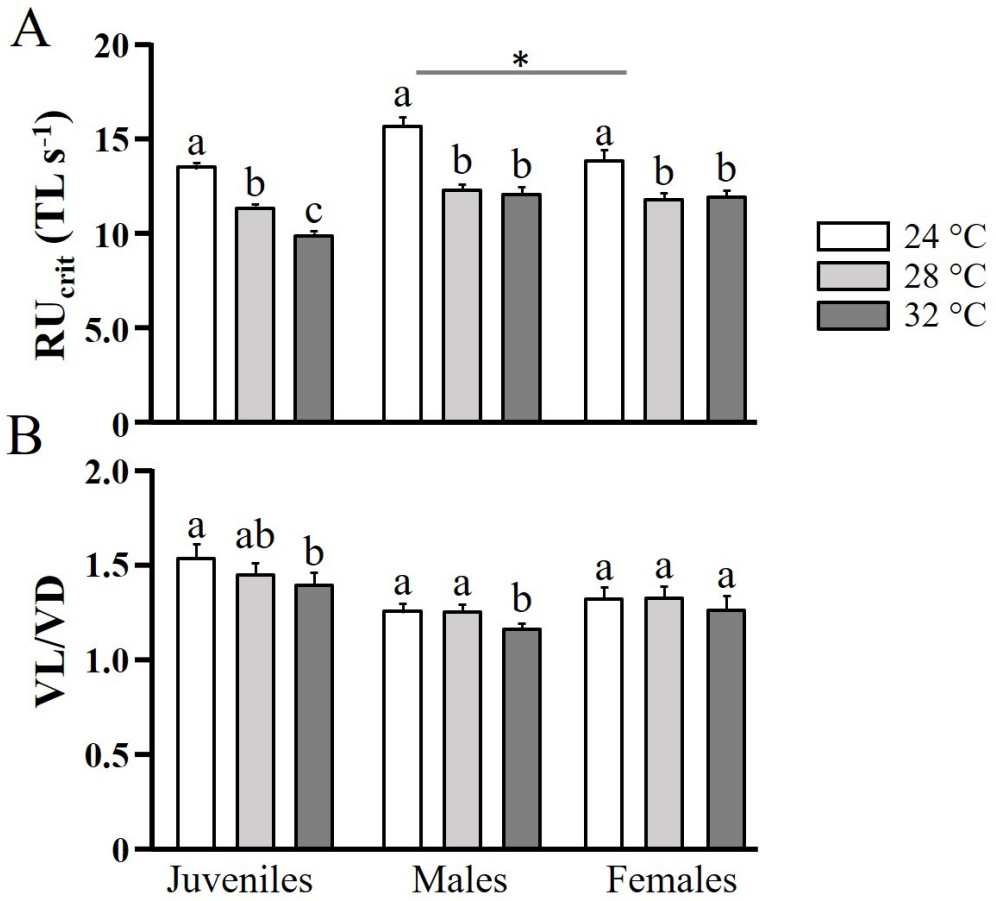

Figure 2. Effect of embryonic temperature on (**A**) the aerobic swimming capacity of zebrafish juveniles (n = 21–29), males (n = 21–27) and females (n = 15–20) and on (**B**) juvenile and adult cardiac anatomy. Ventricle length-to-depth ratio in juveniles (n = 14–16), males (n = 6 in all groups) and females (n = 5–7) for the different thermal treatments. Asterisks represent significant statistical differences between females and males of the same thermal treatment ($p < 0.05$, Mann–Whitney U test). Values without a letter in common are statistically different ($p < 0.05$, Kruskal–Wallis and Mann–Whitney U test. Error bars equal to 1 SEM (Standard Error of Measurement)).

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/fishes7060373/s1, Table S1: Sampling information (per replicate, R) and morphometric measurements (mean total length ± standard deviation) of zebrafish juveniles, males and females studied for swimming capacity; Table S2: Sampling information (per replicate, R) and morphometric measurements (mean total length ± standard deviation) of zebrafish adults studied for heart morphometry.

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Institutional Review Board Statement: All the experimental procedures involving animals were performed in accordance with Greek (PD 56/2013) and EU (Directive 63/2010) legislation for animal experimentation and welfare. All protocols were approved by the Animal Care Committee of the Biology Department of the University of Crete (Permit Number: 3628/17).

Data Availability Statement: Not applicable.

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Conflicts of Interest: The authors declare no conflict of interest.

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