# Oxygen limitation is not a major physiological mechanism restricting early life development in zebrafish

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## Abstract

Early life stages are considered particularly vulnerable to warming because tissue oxygen supply is thought to become limiting, given their underdeveloped gill function and reliance on passive oxygen diffusion. Here, we tested whether oxygen availability constrains early development under warming in zebrafish (*Danio rerio*). We exposed embryos and early-stage larvae to a high-resolution factorial design spanning 50 combinations of temperature and oxygen levels, and quantified multiple developmental and physiological responses (including growth- and survival-related performance) as well as carry-over effects on juvenile warming tolerance. Across traits, embryonic and larval performance was less restricted by oxygen availability than expected. Moderate hypoxia did not impair performance across a wide thermal range, while hyperoxia did not rescue performance under warming, indicating that thermal failure was not alleviated by additional oxygen. Developmental failure occurred primarily under the combined effects of severe hypoxia and extreme warming. Severe hypoxia also induced developmental slowing and premature hatching, especially near thermal extremes. Juvenile warming tolerance was reduced by severe hypoxia and extreme developmental temperatures, but small effect sizes indicate limited carry-over effects of developmental plasticity. Together, these findings do not support oxygen limitation as a primary mechanism limiting early-life performance under warming, refining mechanistic expectations for how warming constrains fish performance.

## Introduction

Climate change and eutrophication can alter aquatic environments in many ways, including increasing water temperatures and reducing water oxygen levels. These changes can pose strong physiological challenges for aquatic ectotherms, such as fish, whose thermal tolerance windows and geographic distribution are strongly influenced by water temperature and oxygen availability (Sunday et al., 2012; Comte and Olden, 2017). Ectothermic organisms experience temperature-dependent increases in metabolic rate during warming, which elevate their oxygen demand (Fry and Hart, 1948). The oxygen- and capacity-limited thermal tolerance (OCLTT) hypothesis proposes that aerobic performance is constrained at high temperatures when oxygen transport can no longer meet the rising metabolic demand for standard metabolism (Pörtner, 2010; Pörtner et al., 2017). Evidence for a link between thermal performance and oxygen limitation, however, varies among species and life stages (Ern et al., 2016, 2017; Silva-Garay et al., 2025; Andreassen et al., 2022; McArley et al., 2022; Raby et al., 2025), with most research focusing on juvenile and adult stages. A recent meta-analysis suggested that due to oxygen limitation, fish embryos have narrower thermal windows than larvae and adults (Dahlke et al., 2020), creating a developmental bottleneck under climate change. However, this analysis relies partly on imputed thermal tolerance data due to gaps in the available literature and is influenced by methodological differences among studies (Pottier et al., 2022; Cowan et al., 2023). As a result, further research is needed to clarify the role of oxygen availability during warming in early life stages (Du and Shine , 2022; Pottier et al., 2022; Cowan et al., 2024).

Fish embryos face respiratory constraints distinct from those of juveniles and adults. Elevated temperatures accelerate metabolism, increasing oxygen demand and developmental rates, which can compromise the completion of essential developmental milestones (Kamler, 1994; Barrionuevo and Burggren, 1999). These vulnerabilities may be amplified by underdeveloped respiratory and circulatory systems, and by limited or absent behavioral capacity to escape environmental extremes (Warkentin, 2007). Unlike later life stages that rely on active ventilation, embryos obtain oxygen primarily through passive diffusion across the chorion, perivitelline fluid, and embryonic tissues including the yolk sac membrane, which can act as additional diffusion barriers (Hayes et al., 1951; Rombough, 1989; Warkentin, 2007). After hatching, larvae still rely largely on cutaneous diffusion, as their gills and circulatory systems develop (De Silva, 1974; Wells and Pinder, 1996; Rombough, 1999). Although embryos can initially tolerate low water oxygen levels well, their oxygen requirements generally increase throughout development. It has been suggested that oxygen requirements peak close to hatching when the surrounding egg envelope may become a critical barrier to oxygen diffusion (Rombough, 1989; Czerkies et al., 2001). This diffusion-dependent oxygen uptake could thus create a physiological bottleneck that limits the embryos' ability to meet rising metabolic demands under warming or hypoxic conditions (Hassell et al., 2008). As a result, embryonic development may become oxygen-limited, with severity and duration varying among species and environmental conditions.

Embryos can hatch prematurely in response to external stressors, such as warming and hypoxia, a strategy that can come at the cost of underdeveloped physiological and behavioral functions (Alderdice et al., 1958; Keckeis et al., 1996; Wood et al., 2019). Premature hatching may optimize fitness by balancing the trade-offs of remaining within the protective chorion and emerging as a free-swimming larva, but can also be a maladaptive consequence of a stressor (Cowan et al., 2024; Warkentin, 2011). Despite this flexibility, environmental stressors can still impose substantial developmental constraints (Cowan et al., 2024). Both

elevated temperatures and low water oxygen levels impair yolk conversion efficiency, reducing the energy available for growth and organogenesis (Kamler, 1994; Kamiński et al., 2006). Prolonged or severe hypoxia during embryogenesis can lower metabolic rates, delay organ development, and reduce growth, often resulting in reduced hatching success and the emergence of smaller or malformed larvae (Miller et al., 2008; Garside, 1966, Czerkies et al., 2001). While high temperatures and aquatic hypoxia can restrict development by reducing oxygen supply relative to demand, the effects of hyperoxia are still poorly understood. Because hyperoxia can increase tissue oxygen supply capacity in fish (Skeeles et al., 2022) and may enhance oxygen diffusion across the embryonic and larval respiratory surfaces, it has the potential to alleviate oxygen-limitation at high temperatures, when metabolic demand is greatest.

Many studies have explored the effects of water temperature or oxygen on fish early development in isolation (Kamiński et al., 2006; Hassell et al., 2008; Schnur et al., 2014; Negrete et al., 2024), yet few have examined their interactive impacts, despite the fact that these environmental stressors often co-occur in nature. This study investigates whether and how combinations of temperature and oxygen availability during embryogenic development influence developmental performance in wild-caught zebrafish (*Danio rerio*). Zebrafish inhabit subtropical freshwater habitats in South Asia, where diel oxygen fluctuations and episodic heatwaves are common. Although their embryonic development under normoxic, optimal conditions (~28.5 °C) is well characterized (Kimmel et al., 1995), environmental stressors can have major physiological impacts. Previous work has shown that adult zebrafish upper thermal tolerance limits are unaffected by water oxygen across moderate hypoxia to hyperoxia, regardless of acclimation history (Silva-Garay et al., 2025). However, zebrafish larvae thermal tolerance can be constrained by oxygen availability, due to oxygen-limited brain function (Andreassen et al., 2022), suggesting heightened oxygen sensitivity early in development.

To test whether early fish development is constrained by oxygen availability under warming, we exposed zebrafish embryos to a matrix of 50 temperature-oxygen combinations and quantified developmental rate, heart rate, yolk sac depletion, hatching success, larval growth, and survival to first feeding. We also measured juvenile critical thermal maximum ($CT_{max}$) in fish reared from these embryos to assess developmental carryover effects. Under an oxygen-limitation framework, hypoxia was expected to exacerbate and hyperoxia to alleviate temperature effects if development is oxygen limited. Understanding how oxygen availability constrains early development under warming is essential for improving predictions of fish vulnerability to climate change.

## Materials and methods

### Study Species and Embryo Collection

Zebrafish (*Danio rerio*) originated from a wild population collected in West Bengal, India (2016; Morgan et al., 2019; Sundin et al., 2019) and were maintained in freshwater aquaria at the NTNU Animal Facility (Trondheim, Norway). Two independent breeding groups produced embryos for experiments conducted in April (*Experiment 1*) and September (*Experiment 2*) 2024. Parental fish were kept in well-aerated freshwater at 28 °C under a

14:10 h light:dark cycle and fed dry flakes (TetraPro, Tetra Sales, USA) three times daily. A total of 42 adults were used for breeding in *Experiment 1* and 110 in *Experiment 2*.

Embryos were collected soon after fertilization, photographed, and staged between the one-cell (0 hours post-fertilization, hpf) and cleavage stages (~ ¾ to 2¼ hpf) following Kimmel et al. (1995). From each breeding group, randomly mixed subsets of embryos were distributed among 50 glass beakers (800 mL): 750 embryos (15 per beaker) in *Experiment 1* and 1250 (25 per beaker) in *Experiment 2*. Beakers contained 300 mL water and were sealed with plastic lids fitted with an aeration tube (PE200; 1.4 mm ID) to provide continuous mixing. Embryos were maintained under the same photoperiod and were not fed during embryonic development or early hatching. All procedures were approved by the Norwegian Food Safety Authority (permit 8578).

## Experimental Setup

### *Thermal Gradient Table*

An aluminum thermal gradient table was used to expose embryos to 50 simultaneous temperature-oxygen combinations during development and early larval stages. The system, adapted from Thomas et al. (1963), consisted of a thick aluminium slab (122.2 x 53.8 x 17 cm) with 50 wells (Thomas et al., 1963; Myrvold , 2020; Haugen , 2022) arranged in a 10 × 5 grid, accommodating 800 mL glass beakers. Each row corresponded to a distinct temperature treatment (Fig. 1A; Fig. S15–S18). A stable linear thermal gradient was generated by circulating cooled and heated water at opposite ends using a cooler (Titan 200, Aqua Medic, Germany) and heater circulator (Grant Instruments, GD100), each regulated by built-in thermostats. Water from each bath was pumped to the respective edges of the table with an Eheim Universal 1000 pump (Germany), producing a consistent cold-to-warm gradient across the aluminium slab (Fig. 1B).3

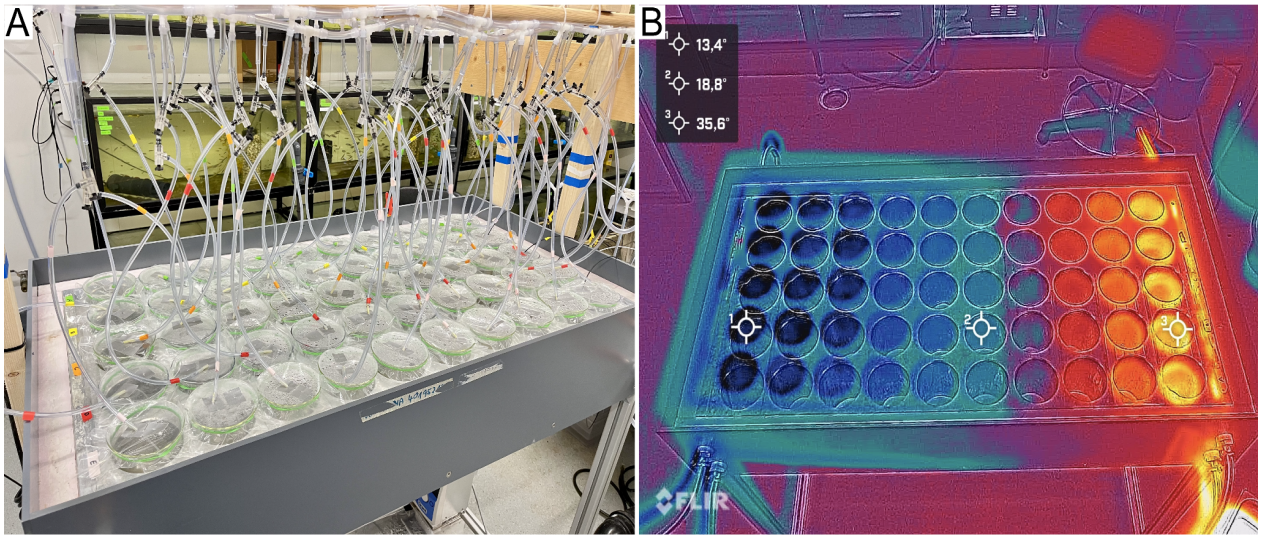


**Fig. 1: Aluminum thermal gradient table used to generate 50 temperature-oxygen combinations. (A)** Ten temperature treatments combined with five oxygen levels (12.5, 25, 50, 100, and 200% air saturation) supplied through gas mixing lines. **(B)** Thermal image of the gradient surface (FLIR One Pro, Teledyne FLIR LLC).

*Oxygen Treatments*

Five water oxygen levels (12.5, 25, 50, 100, and 200% air saturation) were supplied to columns of the thermal gradient table via five independent air lines. Oxygen concentration were regulated using manual gas flow controllers (RS Pro, 500 ml/min) that mixed air with nitrogen (50 L, 99.6% $N_2$; hypoxia) or oxygen (50 L, 99.5% $O_2$, Linde Co.; hyperoxia); while ambient air provided normoxia (Fig. S17). The mixed gas was delivered to each column via a 10 mm diameter outflow tube. Each beaker was aerated through an individual air tube tightly inserted through the lid and fitted with an air valve, providing gentle bubbling. Treatment order was randomized across columns (i.e., *Experiment 1*: 100, 12.5, 25, 200, and 50 % air sat.; *Experiment 2*: 25, 200, 12.5, 50, and 100 % air sat.) Dissolved oxygen ($DO_2$) was continuously monitored at the warmest position using fiber-optic probes (Fig. S2), and $DO_2$ and temperature were verified twice daily in all beakers (FireSting$O_2$, PyroScience GmbH).

**Experimental Protocol**

*Experiment 1 - Wide thermal range*

*Experiment 1 (April 2024)* examined embryo development and hatching across a wide thermal gradient (15.7-39.3 °C: 15.7, 18.1, 20.3, 22.5, 25.2, 27.3, 29.4, 32.0, 35.6, and 39.3 °C) at five oxygen levels (12.5, 25, 50, 100, and 200% air saturation). Embryos (15-16 per beaker) were transferred by pipette into sealed beakers containing 300 mL normoxic water at 28 °C (control). High-resolution images were taken to document initial developmental stage before placement on the gradient table. $DO_2$ levels in the 300 mL water reached target values within ~20 minutes, while temperatures stabilized within 1-2 hours (with extreme ends of the gradient table requiring the longest to equilibrate). Embryos were photographed approximately every 8 hours (see imaging methods). Once hatching began, 30-second videos of larvae were recorded. During each sampling event (2-3 min per beaker), lids were removed only briefly (~30 seconds), minimizing disruption to environmental conditions. The experiment continued for 15 days, until all surviving embryos had hatched.

*Experiment 2 - Upper thermal range*

*Experiment 2 (September 2024)* examined embryo responses over a narrower, warmer thermal range (27.8-37.1 °C: 27.8, 28.4, 29.5, 30.4, 31.4, 32.4, 33.3, 34.5, 35.8, and 37.1 °C). Embryos (24-26 per beaker) were processed as in *Experiment 1*. Target temperatures were reached within ~1 hour (Fig. S1) and target $DO_2$ levels within 20 min after beakers were placed on the gradient table. Embryonic development was documented with video recordings every 12 hours, with additional photographs collected once hatching began. The experiment lasted seven days, concluding when all surviving embryos had hatched.

Dead embryos and larvae were identified by discoloration (e.g., white, cloudy, or opaque appearance) and lack of response to gentle water flow applied via pipette and were removed during sampling. After hatching, larvae remained in their original beakers until just before the onset of exogenous feeding (~2-3 days post-hatching, treatment depending). At that point, a subset of larvae from five temperature treatments across all oxygen levels were transferred to control tanks (28 °C, normoxia) for later assessment of critical thermal maximum ($CT_{max}$) during the juvenile stage.

## Data Collection

### *Imaging Setup*

Two imaging stations were used to capture high-resolution images of embryos and larvae.

*Embryo Imaging*

Embryos were imaged using a custom-built glass platform using a camera (Sony Alpha 7C, Sony Corp.) fitted with a macro lens (Sony FE 2.8/50 Macro, Sony Corp.) mounted below the platform and oriented upward, allowing close-up imaging of embryos resting on the beaker bottom within a 40 mm field of view. Illumination was provided by a side-mounted LED flood light (133×173×59 mm, 30W, 2400 lumens) positioned 12-15 cm from the focal point to enhance image contrast. In *Experiment 1*, still images were collected (6000×4000 pixels; Fig. 2A). In *Experiment 2*, 30-second videos (3840×2160 pixels) were recorded using the same setup (Fig. 2B).

*Larval Imaging*

Larvae were imaged using a larger LED light platform (470×280×9 mm, Lightcraft Ultraslim A4 Lightbox) placed beneath the beaker for uniform illumination. A second camera (Sony Alpha 7C) equipped with a macro lens (Sony FE 2.8/90 Macro G OSS) was positioned above the platform pointing downward, capturing larvae swimming in the water column. Beaker lids were briefly removed (~0.5-1 min) during sampling. In *Experiment 1*, 30-second videos were collected (1280×720 pixels), while *Experiment 2* used still images (6000×4000 pixels).

### ***Data Analysis***

Embryo and larval performance were assessed using fitness-related traits including embryo survival, yolk sac area, and heart rate, hatching success, larval length at hatch, and survival to first feeding stage. Metrics were extracted from time-series images and videos.

*Yolk Sac Area*

Yolk sac surface area (YSA, $mm^2$) was measured at three developmental time points starting at 24 hpf, using *ImageJ* (Abràmoff et al., 2004). For each treatment, five embryos were analyzed and two perpendicular yolk sac diameters (length ($l$) and height ($h$) in mm) were measured. YSA was calculated using the ellipse area formula:

$$\text{YSA}=\frac{\pi \cdot l \cdot h}{4}$$

Image calibration was 390 pixels $mm^{-1}$ (*Experiment 1*) and 325 pixels $mm^{-1}$ (*Experiment 2*).

Thermal performance curves were generated using embryonic yolk consumption, quantified as the percentage reduction in yolk area relative to the mean initial area (at 0 hpf). Yolk area was measured at 24, 48, and 72 hpf (*Experiment 1*), and at 24, 36, and 48 hpf (*Experiment 2*):

$$\%\ \text{yolk consumed} = \frac{(initial\ yolk\ area - yolk\ area\ at\ time\ t)}{initial\ yolk\ area} \times 100$$

*Embryo Heart Rate Measurement*

Embryo heart rate was quantified by amplifying subtle pixel-level motion associated with cardiac contractions using Eulerian video magnification (EVM; Lauridsen et al., 2019). This approach enhances small, periodic intensity changes in a video, such as those caused by heartbeats. Videos (1280x720 pixel) were first stabilized in *iMovie* to reduce camera shake by aligning successive frames. Stabilized videos were then processed in Python using the Laplacian pyramid-based motion magnification approach (Burt and Adelson, 1983). This approach enhances very small movements in the video by analyzing the image at multiple spatial scales and gently amplifying motion while reducing noise, making subtle body movements easier to detect. We used a configuration with eight pyramid levels, an amplification strength of 100. Motion signals with spatial wavelengths above 100 pixels were attenuated, and a mild post-filtering step (attenuation factor 0.4 applied to both the I and Q motion channels) was used to reduce noise while preserving biologically relevant movements. The temporary frequency band was restricted to 1.5-3.5 Hz.

To reduce background noise, videos were temporarily cropped around the embryo's heart, typically to a 10x10 pixel region (Fig. S3A). From this region, a vertical scan line (fixed x-coordinate) positioned over the clearest cardiac signal was used to extract a space-time image representing heartbeat dynamics (Fig. S3B). Heart rate was typically extracted from 5-30 s of recording, limited to periods of high image stability with minimal embryo movement. We then applied Fast Fourier Transform with SciPy 1.15.2 to this space-time image to obtain the frequency spectrum. The dominant peak frequency was taken as heart rate (Hz; Fig. S3C) and converted to beats per minute. Up to six embryos per treatment were analyzed across developmental time points (24-144 hpf) in *Experiment 2*. When background noise prevented reliable frequency detections for six embryos, we analyzed as many individuals as possible.

*Survival and Hatching*

Embryo survival was assessed visually and confirmed using image recordings. Hatching success was calculated as the proportion of hatched embryos relative to the number of fertilized embryos per beaker. Fertilization was verified by observing early cleavage stages, such as blastula or blastocyst formation, which became visible at ~24 hours post-fertilization (hpf) in *Experiment 1* and ~12 hpf in *Experiment 2*, depending on temperature and oxygen conditions. In addition, survival to the first feeding stage was defined as the proportion of fertilized embryos surviving through hatching and yolk sac absorption, at which point larvae were removed and the treatment was terminated. Larvae showing severe pathological stress (e.g., spiral swimming, edema, spinal deformities) were excluded. Exclusions were not applied to larvae exhibiting delayed but otherwise viable development.

Cumulative hatching and survival to first feeding were visualized using 2D plots and 3D surface plots. For the latter, cumulative survival ratios were smoothed using a kernel-based normalization (*image.smoother*, *Fields* package, R Core Team, 2024). This method applies local averaging to the observed data to estimate a continuous surface.

*Larval Length*

Larval total length (mm; head to caudal fin tip) was measured from all post-hatch images and videos using *ImageJ*. Image scale calibration was 7.4 pixels $mm^{-1}$ (*Experiment 1*) and 35.2 pixels $mm^{-1}$ (*Experiment 2*).

*$CT_{max}$ Testing*

To assess developmental effects on upper thermal tolerance, larvae from five developmental temperatures across all oxygen levels were reacclimated to control conditions until reaching the juvenile stage (*Experiment 1*: 28.0 ± 0.5 °C; *Experiment 2*: 27.0 ± 0.5 °C). Surviving individuals were maintained under control conditions for four weeks in *Experiment 1* (20.3, 25.2, 29.4, 32.0, 35.6 °C) and six weeks in *Experiment 2* (27.8, 29.5, 31.4, 33.3, 35.8 °C) prior to critical thermal maximum ($CT_{max}$) testing. During reacclimation, larvae were fed live artemia and commercial larvae feed (Zebrafeed, Sparos I&D, <100 to 600 µm) three times daily and were transitioned to a dry flake diet (TetraPro, Tetra Sales, USA) upon reaching the juvenile stage (~2-3 weeks post-fertilization).

$CT_{max}$ was defined as the temperature (°C) at loss of equilibrium (LOE; inability to remain upright for 3 s; Morgan et al., 2018). Trials followed the protocol of Morgan et al. (2018) and were conducted under control conditions (28 °C, normoxia; 100% air saturation). Groups of 7-9 fish from each rearing condition were transferred from holding tanks to a $CT_{max}$ arena (9 L; 25 × 22 × 18 cm) and allowed to acclimate for 15 min before temperature ramping began. Starting at 28 °C, water temperature was increased at a rate of 0.34 ± 0.02 °C $min^{-1}$. Fish were fasted for 16-20 h prior to testing, and water in the $CT_{max}$ arena was replaced between trials to maintain water quality. Treatment order was randomized to minimize time-of-day and order effects, and trials were completed within a three-day period for *Experiments 1* and *2*. At LOE, fish were immediately removed, and temperature and time were recorded. Individuals were transferred to recovery tanks maintained at 28 °C, where all recovered equilibrium within 2 min. After a 30 min recovery period, fish were euthanized by ice immersion, and body mass (± 0.01 g) and total length (± 0.1 mm) were measured.

*Fulton's Condition Factor*

Using body mass ($W$) and total length ($L$) of the fish tested for $CT_{max}$, we quantified the Fulton Condition Factor ($K$) as follows:

$$K = \frac{W}{L^3}$$

**Statistical Analysis**

*Physiological Parameters Analysis*

Yolk consumption, heart rate, larval length, $CT_{max}$ and Fulton's condition factor were analyzed using linear models in R (*car* package; v4.4.1; R Core Team, 2024). Fixed effects included oxygen, temperature, their interaction, and developmental time when applicable. Temperature was centered to the coldest treatment and included as linear ($Temp_1$) and quadratic ($Temp_2 = Temp_1^2$) within each experiment to facilitate interpretation of model

coefficients and interaction terms: *Response variable ~ Oxygen + $Temp_1$ + $Temp_2$ + Oxygen*$Temp_1$ + Oxygen*$Temp_2$ + hpf*. Type II ANOVA was used for balanced designs and Type III for unbalanced designs or models with interactions. Alternative models were built, removing the quadratic term of temperature, when oxygen or temperature had a linear relationship with the response variable. Models were contrasted using Akaike Information Criterion (AIC) to identify the most parsimonious model structure that best fit the data (*MuMIn* package). We considered a difference of $\Delta AIC > 2$ as evidence of substantial improvement in model fit (Burnham and Anderson, 2004). Estimates, standard errors, and p-values (with significance $p < 0.05$) are reported in the Results section. Model assumptions were evaluated via residual diagnostics and homoscedasticity.

*Hatching Success Analysis*

Hatching and larval survival probability were analyzed using binomial generalized linear models (GLMs, *glm* package; Brooks et al., 2017) with a logit link and bias-reducing estimation (*brglmFit*) to mitigate small-sample and separation issues. Models included oxygen, temperature, their interaction, and log(hpf) as fixed effects, with fertilized embryos specified as the binomial denominator: (*Larval hatched, Initial embryos - Larval hatched*) ~ *Oxygen * Temperature + log(hpf)*. From these models, we estimated the time to 50% hatching ($ET_{50}$) for each temperature–oxygen combination in both experimental runs by interpolating predicted hatching probabilities from the fitted curves using the functions *predict* and *approx*. Treatment effects were assessed by contrasting model predictions against control conditions (normoxia at 27.3 °C in *Experiment 1* and 27.8 °C in *Experiment 2*).

Quadratic temperature models were also fitted to characterize nonlinear responses: (*Larval hatched, Initial embryos - Larval hatched*) ~ *Oxygen + $Temp_1$ + $Temp_2$ + Oxygen*$Temp_1$ + Oxygen*$Temp_2$ + log(hpf)*; where $Temp_1$ and $Temp_2$ represent linear and quadratic temperature terms, respectively. This model was used to assess the effect of oxygen on thermal performance of both hatching success and survival to first feeding across temperatures. Model goodness-of-fit was evaluated using residual deviance (residual deviance/degrees of freedom ≈ 1) and diagnostic plots. Statistical significance was set at $\alpha = 0.05$. Parameter estimates reported in the Supplementary Materials correspond to log-odds coefficients, along with their standard errors, z-values, and associated p-values derived from the fitted GLMs.

## Results

### *Yolk Sac Area*

In *Experiment 1* (15.7 to 39.3 °C), yolk consumption was affected by temperature, oxygen availability, their interaction, and developmental time (all $p < 0.001$; Fig. 2A-C; Fig. S4A-S5A; Table S1). Under normoxia, yolk utilization increased steeply with warming from the coldest treatment ($Temp_1$: $\beta = 5.01 \pm 0.45$, $p < 0.001$) and declined above 32 °C ($Temp_2$: $\beta = -0.195 \pm 0.020$, $p < 0.001$), forming a thermal performance curve with maximal yolk consumption between 22.5 - 32 °C. Oxygen level strongly altered this temperature dependence. At the lowest temperatures, severe hypoxia increased yolk consumption ($\beta = 7.66 \pm 2.88$, $p = 0.008$; $\beta = 6.14 \pm 2.85$, $p = 0.032$), coinciding with delayed development and developmental arrest at 15.7 °C. Above 20.3 °C, however, hypoxia consistently reduced yolk

consumption ($\beta = -10.19 \pm 1.52$, $p < 0.001$ at 12.5%; $\beta = -5.03 \pm 1.51$, $p = 0.001$ at 25%; $\beta = -7.15 \pm 1.54$, $p < 0.001$ at 50%). Severe hypoxia (12.5% and 25%) markedly flattened and narrowed the thermal performance curve by reducing the increase in yolk use with warming ($O_2 \times Temp_1$: $\beta = -4.39 \pm 0.65$, $t = -6.78$, $p < 0.001$ for 12.5%; $\beta = -2.68 \pm 0.63$, $p < 0.001$ for 25%). Moderate hypoxia also reduced yolk use at warm temperatures ($\beta = -1.34 \pm 0.67$, $p = 0.048$), whereas hyperoxia did not differ from normoxia ($\beta = -1.16 \pm 3.29$, $p = 0.47$).

In *Experiment 2* (27.8 - 37.1 °C), yolk sac dynamics mirrored those observed in *Experiment 1* ($p < 0.001$ all; Fig. 2B-D; Fig. S4B-S5B; Table S2). Yolk consumption increased from 27.8 °C ($Temp_1$: $\beta = 2.74 \pm 0.74$, $p < 0.001$) and declined at the highest temperatures ($Temp_2$: $\beta = -0.325 \pm 0.078$, $p < 0.001$). Severe hypoxia (12.5 and 25% air saturation) reduced yolk consumption across temperatures ($\beta = -21.62 \pm 1.91$, $p < 0.001$ at 12.5%; $\beta = -8.60 \pm 1.91$, $p < 0.001$ at 25%), whereas moderate hypoxia and hyperoxia did not differ from normoxia (Table S2, Fig. 3B). Unlike *Experiment 1*, oxygen-temperature interactions were weak in this warmer range, with only moderate hypoxia showing an interaction with temperature at high temperatures ($O_2 \times Temp_2$: $\beta = 0.296 \pm 0.110$, $p = 0.007$).

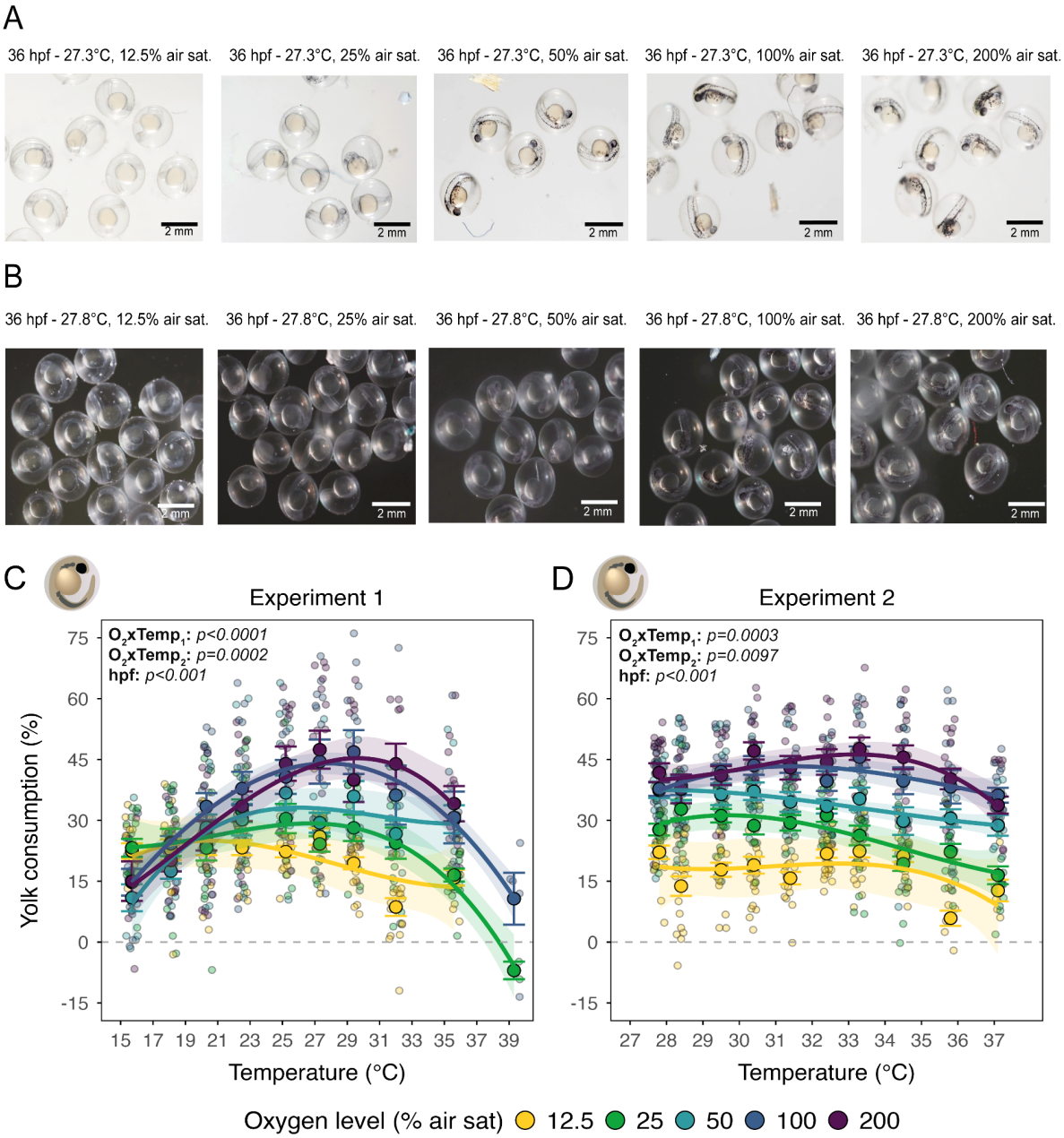


**Fig. 2: Yolk consumption (%) of embryos across temperature and oxygen treatment.** **(A)** Embryonic development progress at 36 hpf across oxygen levels at 27.3 °C in *Experiment 1*. **(B)** Equivalent data at 27.8 °C in *Experiment 2*. **(C-D)** Yolk consumption (% of initial area, $mm^2$) across temperatures (x-axis) and oxygen levels (colors) in *Experiment 1* (**C**; n = 2–9) and *Experiment 2* (**D**; n = 2–11). Points show individual embryos; circles: means ± s.e, lines: quadratic fits by oxygen level. Anova (type III) results in Table S1-S2.

***Heart Rate***

*Experiment 2*

Embryonic heart rate exhibited nonlinear temporal dynamics and was strongly influenced by oxygen level and temperature, but not by their interaction (Fig. 3A-B; Table S3). Centered at 27.8 °C under normoxia, heart rate increased with warming from the coldest treatment

(Temp$_1$: β = 19.60 ± 3.72, $p < 0.001$) and declined above ~33.3 °C (Temp$_2$: β = -2.19 ± 0.41, $p < 0.001$), showing a unimodal thermal response. Heart rate increased with developmental time (β = 1.63 ± 0.08, $p < 0.001$) from its onset at ~24 hpf and approaching a plateau prior to hatching.

Severe hypoxia (12.5% air saturation) delayed the onset of heart activity and markedly reduced heart rate across temperatures (β = -96.20 ± 8.72, $p < 0.001$), while 25% air saturation also reduced heart rates, with stronger effects at warmer temperatures (β = -19.15 ± 8.91, $p = 0.032$). Heart rates under 50% and 200% air saturation did not differ from normoxia. Oxygen-temperature interactions were generally weak, indicating that oxygen mainly shifted overall heart rate rather than altering its temperature dependence; only 50% air saturation showed detectable interaction effects (Temp$_1$: β = -12.96 ± 5.84, $p = 0.027$; Temp$_2$: β = 1.44 ± 0.67, $p = 0.032$). Increased variability in heart rate above 33.3 °C likely reflects measurements from embryos that failed to hatch on time (~48 hpf) and exhibited delayed or abnormal development.

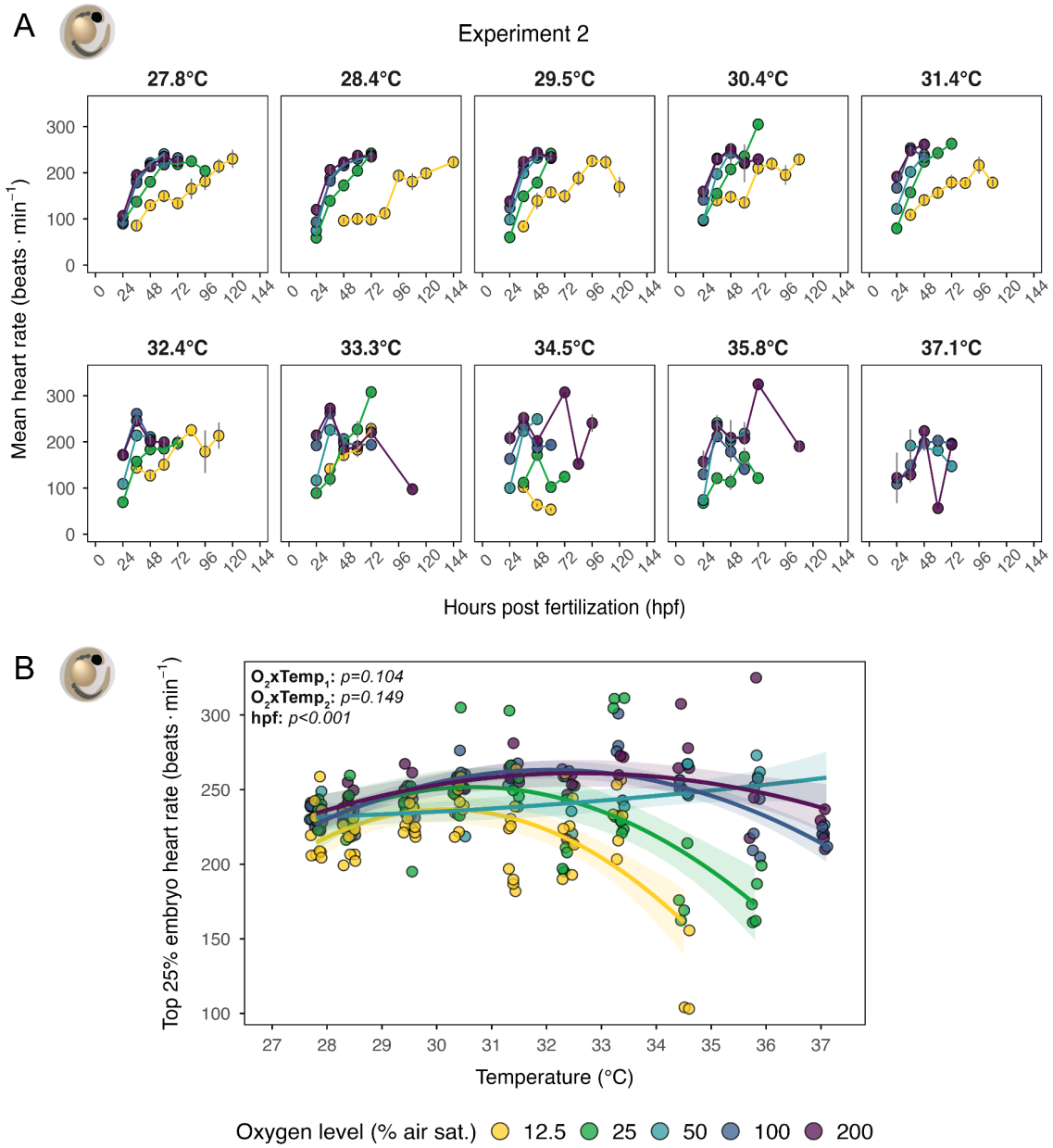

**Fig. 3: Embryonic heart rate across temperature and oxygen treatments.**
**(A)** Mean heart rate (beats $min^{-1}$) of embryos in *Experiment 2* over developmental time, calculated as the average of individual embryo heart rates within each temperature-oxygen treatment (n = 1-8 embryos per time point). Colors indicate oxygen levels. Points show mean ± s.e. **(B)** Top $25^{th}$ percentile of embryo heart rates (beats $min^{-1}$) by temperature-oxygen treatment, calculated from measurements pooled across developmental time. Colored points representing individual measurements, lines show quadratic fits by oxygen level. P-values from type III Anova (Table S3).

***Hatching Success***

*Experiment 1*

Across the wide thermal range (15.7-39.3 °C), hatching rate was influenced by temperature, oxygen level, their interaction, and developmental time (GLM, all $p < 0.0001$; Fig. 4A; Table S4). Under normoxia, embryos showed a clear thermal window for successful development: hatching was delayed and reduced at 18.1 °C, increasing sharply from 20.3 to 35.6 °C (>75% hatching), and was fastest at 27.3-32 °C. No hatching occurred at the extreme temperatures (15.7 °C and 39.3 °C). Oxygen availability strongly modified this thermal response (Fig. 4C; see Fig. S6A-S7A-S8). At 18.1 °C, hatching was delayed and reduced across oxygen treatments, and especially under hypoxia (≤ 50% air saturation; $p < 0.001$) and hyperoxia (200% air saturation; $p < 0.001$). Severe hypoxia (12.5% air saturation) showed hatching rates comparable to normoxia (~80-100%) between 20.3 and 27.3 °C but declined at 29.4 °C (~75%) and ceased at 32.0 °C. Hypoxia (25% air saturation) showed a similar pattern, with reduced hatching at 32.0 °C (~75% ; $p = 0.001$) and complete suppression at higher temperatures. Both moderate hypoxia (50%) and hyperoxia (200%) reduced hatching at the warmest temperature (35.6 °C) only, where hatching declined to ~50% (both $p < 0.001$; Table S5).

A GLM including the temperature-oxygen interaction provided the best fit for estimating time to 50% hatch ($ET_{50}$; Table S5; Fig. S9A). Hatching time decreased steeply with warming, from: ~224-279 hpf at 18.1 °C and 126-200 hpf at 20.3 °C, to 51-79 hpf at 27.3 °C and 41-78 hpf at 32 °C (Table S6). Oxygen availability further modified this temperature dependence. At cooler temperatures (≤ 27.3 °C), severe hypoxia (12.5-25%) accelerated hatching by ~20-70 h relative to normoxia, whereas at warmer temperatures hypoxia progressively delayed hatching by ~25-50 h. Hyperoxia delayed hatching only at the warmest temperature (35.6 °C).

*Experiment 2*

Within the warmer developmental range (27.8-37.1 °C), hatching success was again influenced by temperature, oxygen level, their interaction, and developmental time (GLM, all $p < 0.001$; Fig. 4B; Table S6). Under normoxia, hatching remained high (90-100%) from 27.8 to 35.8 °C and failed entirely at 37.1 °C. Oxygen strongly modified this thermal window (Fig. 4C; see Fig. S6B-S7B-S8). Severe hypoxia (12.5%) consistently reduced and delayed

hatching across most temperatures ($p < 0.001$), with complete failure at 34.5 °C ($p = 0.0002$; Table S7). Hypoxia (25%) produced hatching rates comparable to normoxia at 27.8-28.4 °C, but reduced hatching at 32.4 and 33.3 °C (both $p < 0.001$) and suppressed hatching at 34.5 °C, consistent with *Experiment 1*. Moderate hypoxia (50%) had little effect except at 35.8 °C, where hatching was strongly reduced and delayed ($p = 0.001$). Hyperoxia (200%) did not improve hatching success relative to normoxia across this temperature range (27.8-35.8 °C); but reduced hatching to a ~50% at 35.8 °C ($p < 0.001$), again mirroring *Experiment 1*.

Consistent with these patterns, $ET_{50}$ estimates (GLM; Table S7; Fig. S9B) revealed strong oxygen effects across 27.8-37.1 °C. Severe hypoxia (12.5%) markedly delayed hatching throughout this thermal range, with $ET_{50}$ occurring ~68-136 hpf later than under normoxia (41-62 hpf; Table S6). Hypoxia (25%) produced $ET_{50}$ values comparable to normoxia at cooler temperatures (~56 hpf at 27.8-28.4 °C), but increasingly delayed hatching at warmer temperatures ($ET_{50} \approx$ 59-80 hpf), consistent with the temperature-dependent shift observed in *Experiment 1*. Hyperoxia delayed the hatching only at the warmest temperature (35.8 °C).

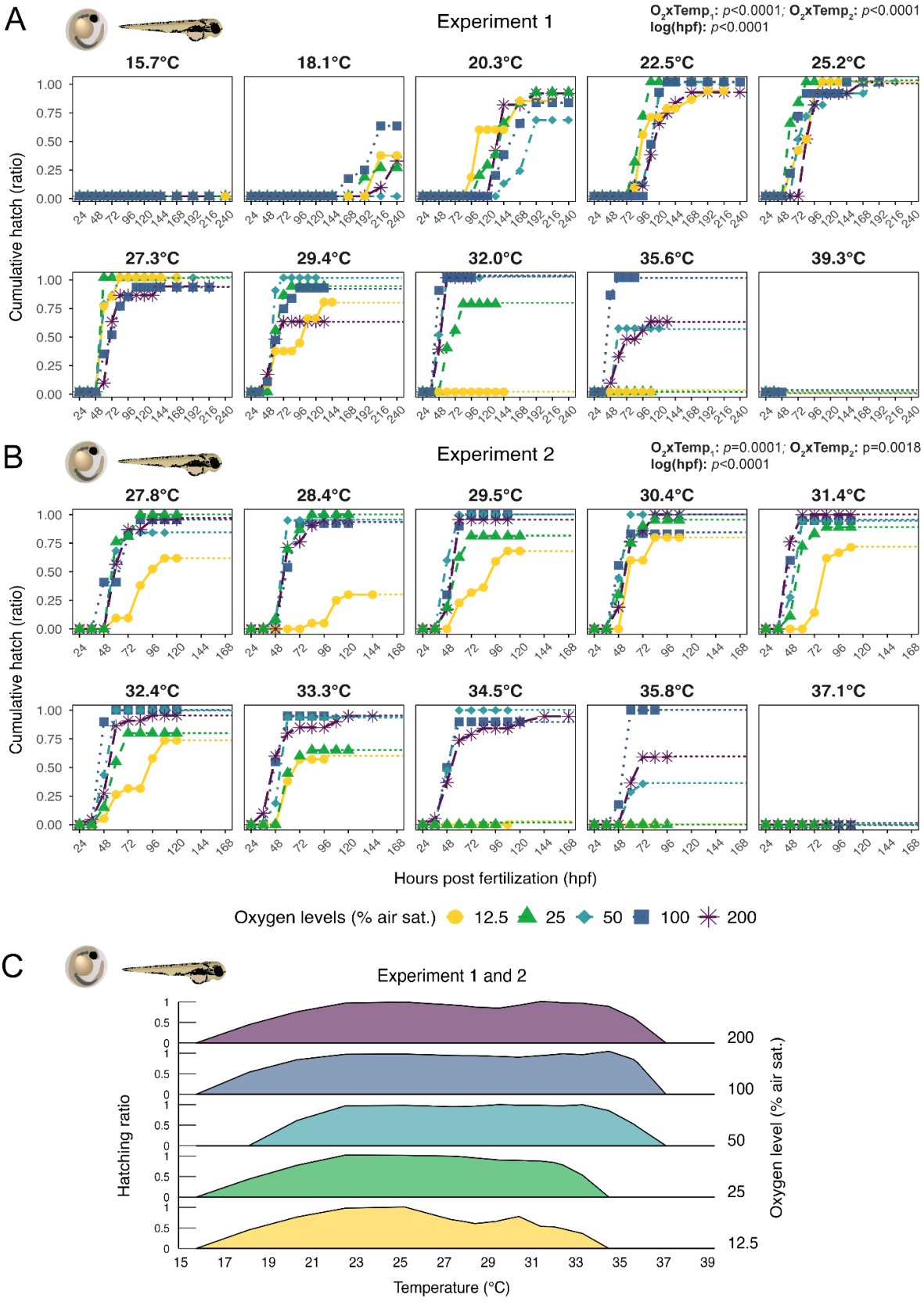


**Fig. 4: Hatching dynamics across temperature and oxygen treatments. (A)** Cumulative hatching ratio over time (hpf) across temperatures (panels) and oxygen levels (colors) in *Experiment 1* (n = 8-15). **(B)** Equivalent data for *Experiment 2* (n = 13-23). Symbols denote observed hatching ratios; dotted lines indicate periods when larvae were removed following

mortality or completion of hatching. P-values from binomial GLM (Table S4-S6). **(C)** Maximum hatching ratio across temperatures combining *Experiment 1* and *2*. The ridgeline shows *LOESS*-smoothed maxima (span = 0.45).

***Larvae Length***

In *Experiment 1*, larval length varied with temperature, oxygen level, and developmental time, but not with the interaction temperature-oxygen ($p < 0.001$; Fig. 5A; Table S9; Fig. S10A-S11). Under normoxia and centered at 18.1 °C, length increased with warming from the coldest treatment ($Temp_1$: $\beta = 0.085 \pm 0.017$, $p < 0.001$) and declined slightly at higher temperatures ($Temp_2$: $\beta = -0.0042 \pm 0.0009$, $p < 0.001$). Severe hypoxia (12.5% air saturation) produced the largest reduction in length across temperatures ($\beta = -0.423 \pm 0.139$, $p = 0.002$), while hyperoxia caused a moderate decrease ($\beta = -0.250 \pm 0.117$, $p = 0.033$). Hypoxia (25% air saturation) reduced length only above 22.5 °C ($\beta = -0.145 \pm 0.042$, $p = 0.001$), whereas 50% air saturation did not differ from normoxia. Oxygen-temperature interactions were weak, suggesting that oxygen primarily shifted mean larval length without substantially altering its temperature response. Larval length increased modestly with developmental time ($\beta = 0.00094 \pm 0.00025$, $p < 0.001$).

In *Experiment 2*, larval length again varied with oxygen level and developmental time but showed no temperature effects ($p < 0.001$; Fig. 5B; Table S10; Fig. S10B-S12). Length was no different across temperatures ($Temp_1$: $p = 0.44$; $Temp_2$: $p = 0.46$). Oxygen effects were more pronounced: larvae reared in hypoxia were consistently smaller across temperatures, with the strongest reduction at 12.5% ($\beta = -0.410 \pm 0.062$, $p < 0.001$), followed by moderate reduction at 25%, 50%, and 200% air saturation (all $p \leq 0.02$). As in *Experiment 1*, oxygen-temperature interactions were weak, and mostly driven by responses under hyperoxia at 27.8 °C. Larval length also increased with developmental time ($\beta = 0.00445 \pm 0.00042$, $p < 0.001$).

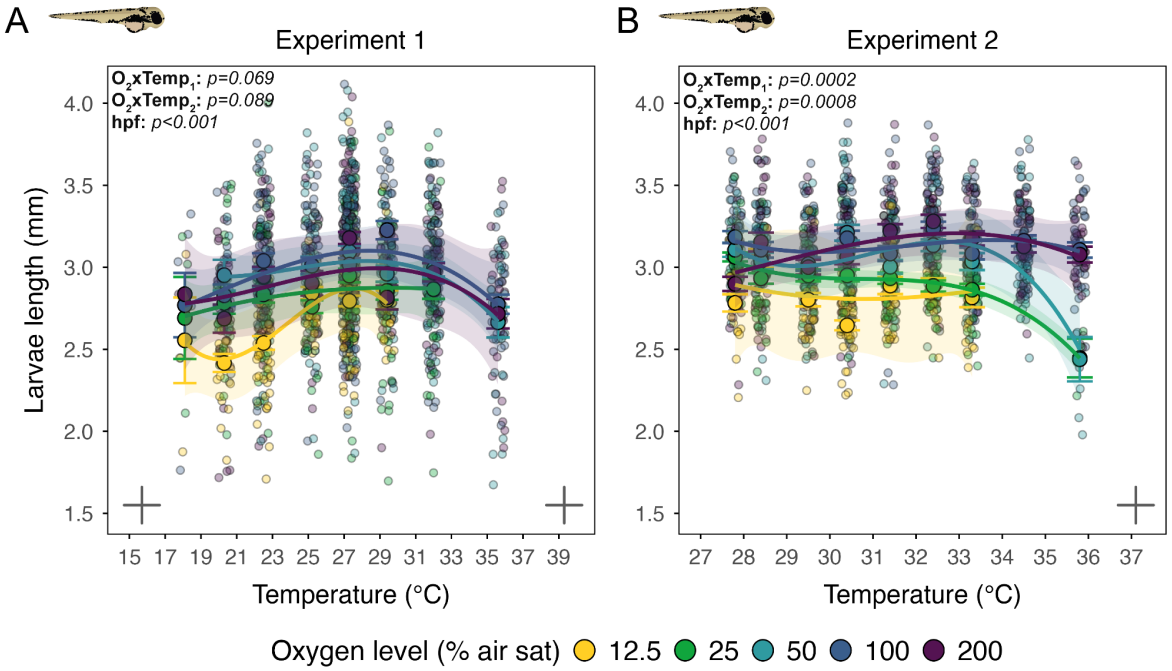


**Fig. 5: Larval length across temperature and oxygen treatments. (A)** Larval length (mm) in *Experiment 1* (n = 2-15). **(B)** Equivalent data for *Experiment 2* (n = 2-24). Points represent

individual larvae; colored circles indicate means ± s.e., lines show third-degree polynomial fits. P-values from type III Anova (Table S9-S10).

***Survival To First Feeding Stage***

Survival to the first feeding stage showed significant main and interactive effects of temperature and oxygen level when results from *Experiments 1* and *2* were combined (Fig. 6; Table S11). At 18.1 °C, hatching was substantially delayed (~225-275 hpf), and surviving larvae exhibited retarded development, retaining large yolk sacs for an additional 48-72 h after hatching. Because the experiment was terminated at this point, these individuals were included in survival-to-first-feeding estimates, as they reflected delayed development rather than mortality. In contrast, under extreme warming (~35.6-35.8 °C) and hyperoxia, larvae that had consumed the yolk sac but exhibited severe deformities (e.g., spinal malformations or tissue swelling) were excluded from survival estimates.

Under normoxia, survival remained high (>80%) across a broad thermal range (~20-35 °C), declining toward the warmest temperatures (Fig. S8). At lower temperatures, larvae successfully completed endogenous feeding, resulting in high survival-to-first-feeding across all oxygen levels. In contrast, elevated temperatures caused substantial mortality that increased progressively with hypoxia severity (all $p < 0.001$; Table S11). Survival declined below 50% above 34 °C under 50% air saturation, above 32 °C under 25%, and above 27 °C under 12.5%. Notably, extreme heat (~35°C) combined with hyperoxia also markedly reduced survival ($p < 0.001$), whereas under normoxia survival closely matched hatching success. The slight valley at intermediate-high temperatures and hyperoxia appears to be an artifact of combining experiments rather than a biologically meaningful pattern, as survival remained relatively high across that range.

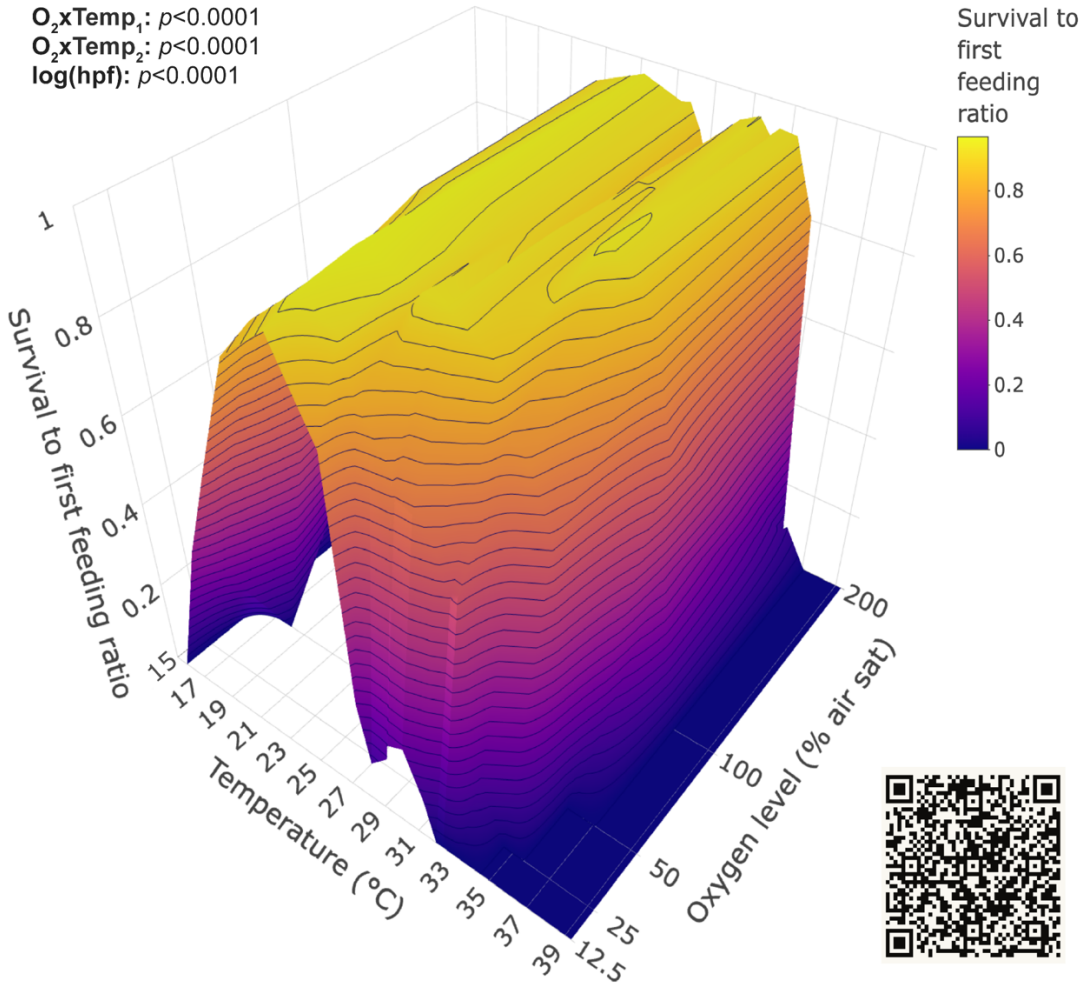


**Fig. 6: Three-dimensional surface plot of larval survival across temperature and oxygen treatments.** 3D surface shows the proportion of fertilized embryos surviving to first feeding across temperatures (15.7-39.3 °C) and oxygen levels, combining *Experiments 1* and *2*. Survival ratios > 0.6 were smoothed using kernel-based normalization (*aRange*, *theta* = 0.9). P-values from binomial GLM-Tmb (Table S11). An interactive version is available at: https://loresilvag.github.io/Interactive-3Dplot/3D_SurvivalToFirstFeeding_Plot.html

### *Fulton's Condition Factor and $CT_{max}$*

In *Experiment 1*, larvae from five temperatures and oxygen levels were re-acclimated to control conditions before $CT_{max}$ testing (Table S12). Larvae hatched at 12.5% air saturation in the warmer treatments died before transfer, and post-transfer mortality regardless of the rearing oxygen level was high at the hottest temperatures, preventing full factorial testing (Fig. S13A). Fulton's condition factor of juvenile fish did not differ across rearing temperatures or oxygen levels ($p = 0.076$ and $p = 0.150$, respectively; Table S15, Fig. S14A). $CT_{max}$ varied significantly with rearing temperature (Anova type III: $p < 0.001$), but not with oxygen or the oxygen-temperature interaction (Fig. 7A; Table S13). Post hoc comparisons centered on normoxia and 20.3 °C showed that early exposure to severe hypoxia (12.5%) reduced $CT_{max}$ ($\beta = -0.304 \pm 0.138$, $p = 0.031$), while 25%, 50%, and 200% air saturation did not differ from normoxia. $CT_{max}$ was also lower in fish reared at 20.3 °C ($\beta = -0.324 \pm 0.122$,

$p = 0.010$), unchanged at 25.2 and 32 °C, and marginally reduced at 35.6 °C (β = -0.436 ± 0.222, $p = 0.053$).

In *Experiment 2*, early mortality under severe hypoxia and warm temperatures similarly limited the number of treatment combinations available for testing. (Fig. S13B). Fulton's condition factor again showed no effect of temperature or oxygen ($p = 0.387$ and $p = 0.426$, respectively; Table S15, Fig. S14B). $CT_{max}$ varied with both oxygen level and rearing temperature (Anova type II: both $p < 0.001$), with a border-line interaction ($p = 0.056$; Fig. 7B, Table S14). Developmental hypoxia (25% air saturation) reduced $CT_{max}$ (β = -0.483 ± 0.09, $p < 0.001$), same in the 50% air saturation fish (β = -0.158 ± 0.073, $p = 0.032$), while hyperoxia (200%) did not differ from normoxia. Relative to 27.8 °C, $CT_{max}$ was lower in fish reared at 33.3 °C (β = -0.164 ± 0.082, $p = 0.04$), while intermediate temperatures produced no difference. Although overall interactions were weak, moderate hypoxia at higher temperatures produced small but detectable reductions in $CT_{max}$ (e.g., at 31.4 °C and 33.3 °C).

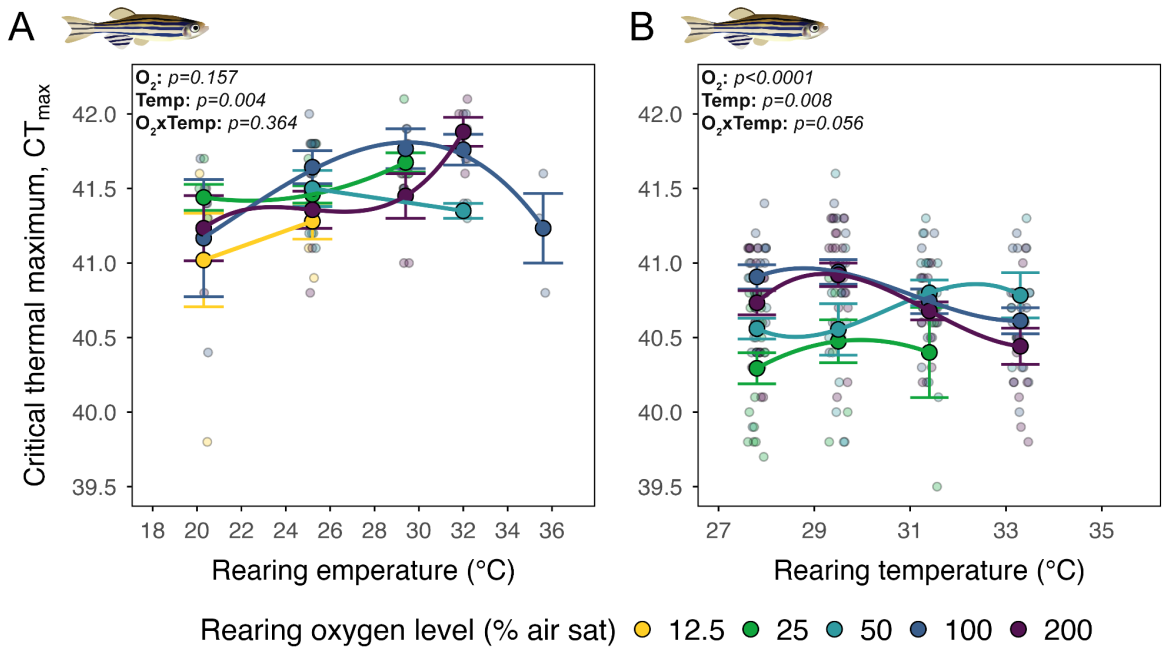


**Fig. 7: Critical thermal maximum ($CT_{max}$) of juvenile zebrafish following early-life exposure to temperature and oxygen treatment. (A)** $CT_{max}$ of juveniles in *Experiment 1*. **(B)** Equivalent data for *Experiment 2*. Points represent individual fish (jittered); circles show means ± s.e., and lines depict polynomial fits. P-values from type III Anova (Table S13-S14).

## Discussion

### *Negligible effects of water oxygen availability on embryo development and hatching*

By leveraging the many treatment combinations of the thermal gradient table, we examined how the thermal-oxygen landscape influences zebrafish early development. Because embryos and early larvae rely on passive diffusion of oxygen across the chorion and perivitelline fluid,

hyperoxia was hypothesized to buffer against oxygen limitation during thermal stress. We therefore predicted that oxygen availability would play a dominant role in shaping thermal performance, especially at supraoptimal temperatures. Contrary to these predictions, the role of oxygen on developmental performance was relatively minor. Increasing oxygen availability through hyperoxic water did not alleviate key symptoms of thermal stress. Even at the highest temperatures, hyperoxic water did not improve yolk consumption, hatching, or larval growth and survival. This lack of benefit from hyperoxia is consistent with observations in rainbow trout (*Oncorhynchus mykiss*) embryos, where growth was similar under hyperoxia and normoxia rearing (Ciuhandu et al., 2005), and in Atlantic salmon (*Salmo salar)* where hyperoxic rearing (150% air saturation) through embryonic and alevin development did not enhance growth or aerobic metabolism (Wood et al., 2019). Together, these findings indicate that developmental constraints under warming are not readily explained by OCLTT predictions (Dahlke et al., 2020).

Hyperoxia delayed hatching in zebrafish (by 26-50 h), but only at the highest temperatures. Similar delays were reported in rainbow trout (*Oncorhynchus mykiss*) and Atlantic killifish (*Fundulus heteroclitus,* Dimichele and Taylor, 1980; Latham and Just, 1989). As hatching has been hypothesized to be triggered by hypoxia in the embryo (Czerkies et al., 2001; Teletchea and Pauly 2024), hyperoxic water that reduces diffusive constraints across the chorion and perivitelline fluid would accordingly delay hatching, although empirical support for this mechanism remains limited. We found delayed hatching from hyperoxia only at the highest temperatures, meaning it does not appear to delay hatching in general. The hypothesis is also not supported by our moderate hypoxia groups, where earlier hatching would have been predicted. As this was not found, and because hyperoxia did not delay hatching at most temperatures, we don't see evidence for embryonic oxygen limitation as a universal trigger for hatching.

Similar to the relatively minor effects of hyperoxia, mild hypoxia (50% air saturation) did not dramatically exacerbate the thermal performance of embryos and larvae in most of our measurements. There appeared to be a slight slowing of yolk consumption at higher temperatures, but heart rate, hatching success, and larvae length were mostly unaffected until the very highest temperatures. Taken together, the limited effects of hyperoxia and mild hypoxia suggest that tissue oxygen availability is not a major physiological mechanism restricting development in zebrafish.

*Severe hypoxia allows early development but imposes physiological costs*

Remarkably, embryos reared under severe hypoxia (12.5 and 25% air saturation) showed high hatching success (60-100%) across a broad thermal range (~20-33 °C), indicating substantial tolerance to low oxygen levels. Yet, severe hypoxia produced clear signs of developmental disturbance, including reduced heart rate, slowed yolk consumption, reduced body length, and delayed and underdeveloped hatching, particularly near thermal limits. These patterns are consistent with hypoxia-induced metabolic depression, a response observed in many teleosts embryos (Shumway et al., 1964; Hassell et al., 2008; Mueller et al., 2011; Marks et al., 2012). Edema and diminished skin pigmentation were also observed in the severe hypoxia-exposed embryos but were not quantified. Notably, embryos exposed to severe hypoxia exhibited lower heart rates than those reared at higher oxygen levels from the

onset of cardiac activity (~24 hpf). Although they eventually reached peak heart rates comparable to those observed in normoxic embryos, this occurred several days later under severe hypoxia. Because this pattern is consistent with observed trajectories of cardiac development in zebrafish (Gierten et al., 2020), it likely reflects delayed cardiac development rather than persistent bradycardia. While heart rate itself is unlikely to directly limit oxygen uptake at this stage, delayed cardiac and organ development can nonetheless impair overall performance. Hatching under severe hypoxia is a common and well-documented response in fish embryos exposed to suboptimal environmental conditions, including hypoxia and elevated temperatures (Czerkies et al., 2001; Cowan et al., 2024). Mechanistically, sustained hypoxia during late embryogenesis can trigger the release of chorionase from hatching gland cells, softening the chorion and facilitating earlier escape (Czerkies et al., 2001, 2002). However, if embryos are weakened or hatching glands remain immature, hatching may fail (Czerkies et al., 2001; Mueller et al., 2011). Thus, while stress-induced hatching may enable embryos to exit unfavorable environments, it can occur before completion of key developmental milestones required for post-hatching function.

Despite the developmental stress observed before hatching, survival to the first feeding stage remained high under severe hypoxia at cooler temperatures (18-27 °C), but declined sharply when hypoxia was combined with supraoptimal temperatures. As in embryos, early larval oxygen uptake relies primarily on cutaneous diffusion because gills are not yet functional (De Silva, 1974; Wells and Pinder, 1996). In zebrafish, neuroepithelial cells that facilitate gill-based oxygen uptake begin developing in the gill filaments at ~5 days post-fertilization at 28 °C (Jonz and Nurse, 2005), coinciding with rising metabolic demands that may render cutaneous respiration insufficient. This likely explains the time-limited survival and elevated mortality observed in larvae exposed to severe hypoxia and warming, particularly after transfer to normoxia for $CT_{max}$ testing (Fig. S13). In contrast, larvae reared under severe hypoxia at cooler temperatures showed high survival after transfer and attained body sizes comparable to normoxia-reared larvae. Together, these results identify the early post-hatching transition as a critical window in which rising metabolic demands can outpace diffusive oxygen uptake under combined hypoxia and warming.

Temperature is a key regulator of fish embryonic development (Kamler, 1994; Kamiński et al., 2006), and in our study, warming predictably accelerated yolk consumption, development and hatching. Furthermore, severe hypoxia had a temperature-dependent and non-linear effect on hatching time. When compared to normoxia, the hatching under severe hypoxia occurred sooner under cooler temperatures (20-50 hours earlier) and was delayed under warming (30-50 hours later; Fig. 4, Fig. S9). This suggests that at warm temperatures and hypoxia, oxygen can become limiting to metabolic processes, leading to slowed development and delayed hatching. At low temperatures, however, where metabolic demands are reduced, the low oxygen saturation in hypoxia may be sufficient to sustain development, but can still induce premature hatching. Together, these results demonstrate a complex interaction between temperature and oxygen availability, whereby severe hypoxia accelerates hatching under cooler temperatures but delays hatching under warming.

*Thermal performance curves reveal a broad thermal window for embryonic development*

The thermal window for successful zebrafish embryonic development and hatching (>80% success) ranged from 20 to 36 °C under normoxia. This is a broader thermal window than what has previously been reported for zebrafish (~22-34 °C) (Schirone and Gross, 1968; Schnurr et al., 2014; Urushibata et al., 2021). A possible explanation for this discrepancy is that the current experiment used the JU strain of wild-caught zebrafish that have previously been found to be more plastic than the domesticated AB strain (Morgan et al., 2022; Sundin et al., 2019). The thermal range is also narrower, especially on the cold side, than the established thermal window found for juveniles and adults under chronic conditions (Åsheim et al., 2020; Morgan et al., 2019, 2022). A sharp decline in hatching success and complete failure below 18 °C and above 36 °C under all water oxygen levels marks clear thermal limits for the zebrafish embryogenesis. Thermal performance curves further revealed that intermediate temperatures (25-34 °C) maximized yolk sac consumption and larval size, suggesting that this range may support the most efficient energy allocation to growth. Taken together, the embryonic development of zebrafish has a broad thermal window and is surprisingly robust to thermal challenges.

*Developmental heat and severe hypoxia marginally shape juvenile thermal limits*

Developmental plasticity, whereby early life conditions confer lasting effects across subsequent life stages, has been suggested to be an important mechanism shaping ectotherm thermal tolerance (Scott and Johnston, 2012; Noble et al., 2018). Acute thermal tolerance tests of juveniles showed that early-life exposure to severe hypoxia and non-optimal temperatures can have lasting, albeit modest, effects. Fish reared under severe hypoxia exhibited slightly lower $CT_{max}$ than those reared under normoxia (0.3-0.4 °C average difference; Fig. 7). Similarly, fish reared at colder or warmer temperature extremes showed reduced thermal tolerance relative to those reared near the thermal optimum (0.3 and 0.4 °C average difference, respectively). These effects should be interpreted cautiously, as severe hypoxia and warming caused substantial mortality during early development, such that $CT_{max}$ estimates reflect only individuals that survived to the juvenile stage and do not capture responses across the full range of developmental conditions. Notably, higher developmental temperatures did not enhance juvenile heat tolerance, indicating limited capacity for beneficial developmental plasticity under warming. Overall, the effect sizes associated with developmental plasticity were small, consistent with meta-analyses showing that ectotherm thermal tolerance is only weakly influenced by developmental temperature (Pottier et al., 2022). Together, these results suggest that while early-life oxygen and thermal stress can influence later thermal limits, their effects are modest and do not confer increased tolerance to acute warming.

## Conclusion

Early life stages have been viewed as a thermal bottleneck in the fish life cycle, with warming expected to constrain performance through oxygen limitation. Our results challenge the view that oxygen limitation sets the upper thermal boundary for early fish development. Hyperoxia failed to improve early-life performance and even reduced hatching success at the upper thermal extremes, suggesting potential physiological costs of elevated oxygen under

heat stress (e.g. ROS production or oxygen toxicity; Birnie-Gauvin et al., 2016; Tunç et al., 2025), although these were not directly assessed. Overall, responses to hyperoxia appear species- and context-dependent.

In contrast, zebrafish embryos tolerated moderate hypoxia remarkably well, and only severe hypoxia combined with warming impaired development and reduced larval survival. While signs of oxygen limitation emerged under these extreme conditions, developmental failure at high temperatures was not alleviated by additional oxygen. This indicates that mechanisms other than oxygen supply, likely involving cellular and molecular limits, constrain the upper thermal tolerance in early life. The broad temperature range supporting successful hatching further highlights the plasticity of embryonic development in zebrafish. Our study also indicates only limited carry over effects of early developmental conditions on later thermal tolerance. Together, these findings underscore the need to understand how multiple interacting stressors affect early developmental stages to more accurately predict species resilience and vulnerability in a warming world.


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## CRediT authorship contribution statement

**Lorena Silva-Garay:** Conceptualization, Methodology, Investigation, Data collection, Writing – original draft, Data curation, Visualization, Formal analysis, Project administration. **Moa Metz:** Conceptualization, Methodology, Data collection, Review & Editing. **Henning H. Kristiansen:** Methodology, Data collection, Data curation, Visualization, Software, Review & Editing. **Leon Pfeufer:** Methodology, Data collection, Review & Editing. **Emily R. Lechner:** Methodology, Data collection, Review & Editing. **Rasmus Ern:** Conceptualization, Methodology, Review & Editing. **Anna H. Andreassen:** Conceptualization, Methodology, Review & Editing. **Fredrik Jutfelt:** Conceptualization, Methodology, Investigation, Writing – Review & Editing, Validation, Supervision, Resources, Funding acquisition.

## Conflict of interest declaration

We declare we have no competing interests.

## Data availability statement

The data supporting the findings of this study is deposited in Zenodo and will be made publicly available upon acceptance of the manuscript. Data are available to editors and reviewers upon request during peer review. [10.5281/zenodo.18500503]

## Code availability statement

Custom code used to process the data, perform statistical analyses, and generate figures is deposited in Zenodo and will be made publicly available upon acceptance of the manuscript. Code will be provided to editors and reviewers upon request during peer review.

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# Supporting Information for

Oxygen limitation is not a major physiological mechanism restricting early life development in zebrafish

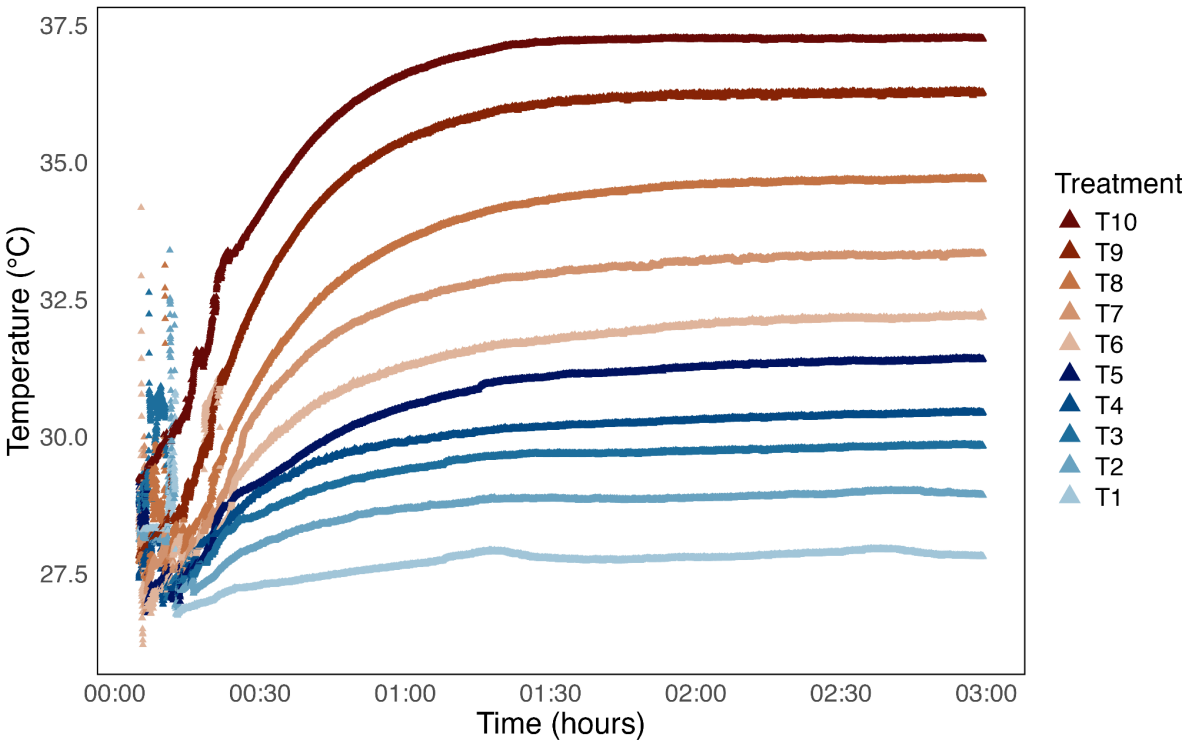


**Fig. S1: Heating rate across treatments in the thermal gradient table.**
Heating rate (°C $h^{-1}$) over time in beakers used in *Experiment 2*. Treatments differ by color and correspond to distinct temperature change profiles established in the thermal gradient table.

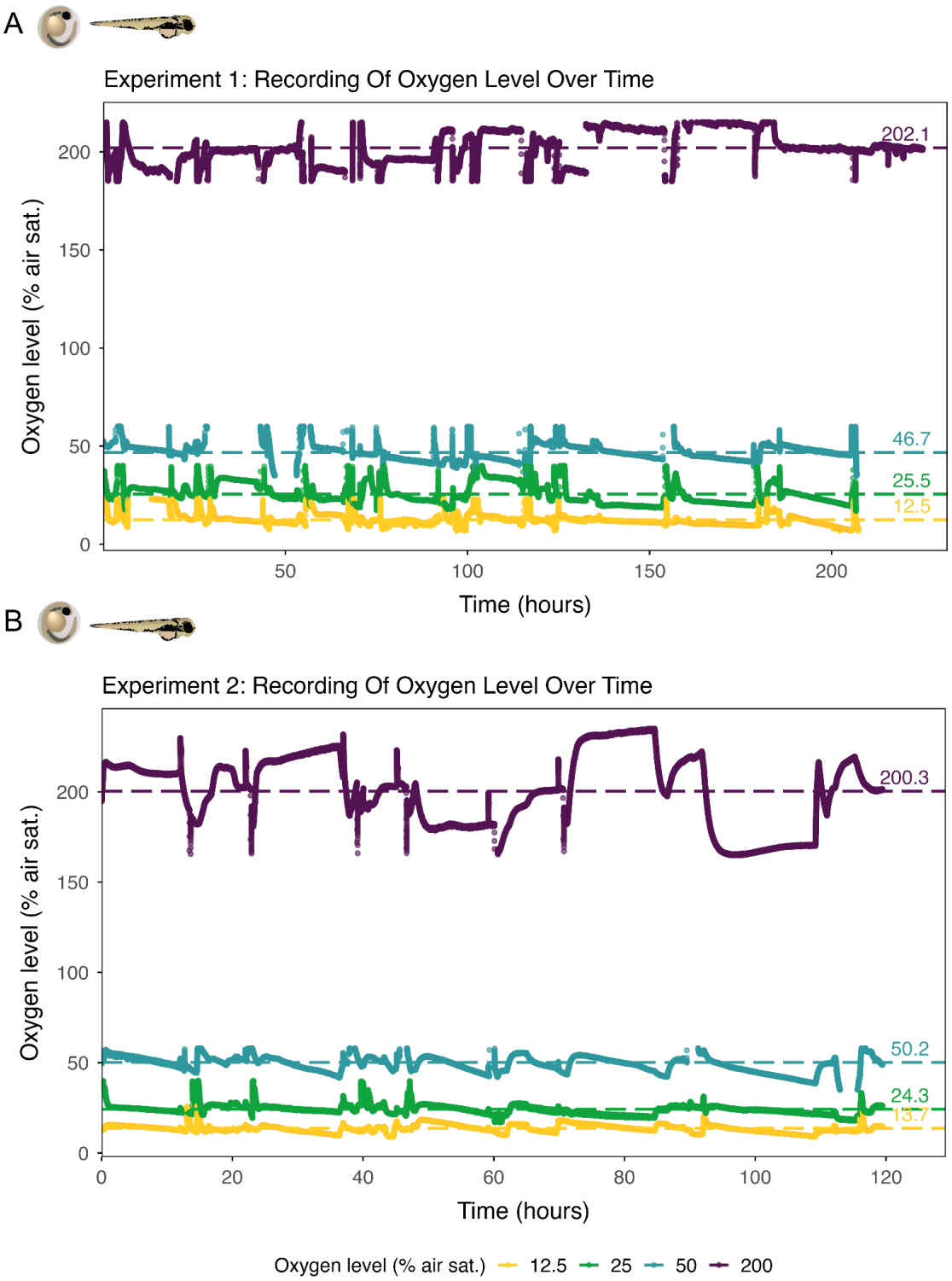


**Fig. S2: Oxygen records over the total experimental time (in hours) of *Experiment 1* and *Experiment 2*.** Raw data and means of the target oxygen treatments during experiments are depicted in colors. *Experiment 1* lasted ~ 10 days and *Experiment 2* lasted ~ 6 days.

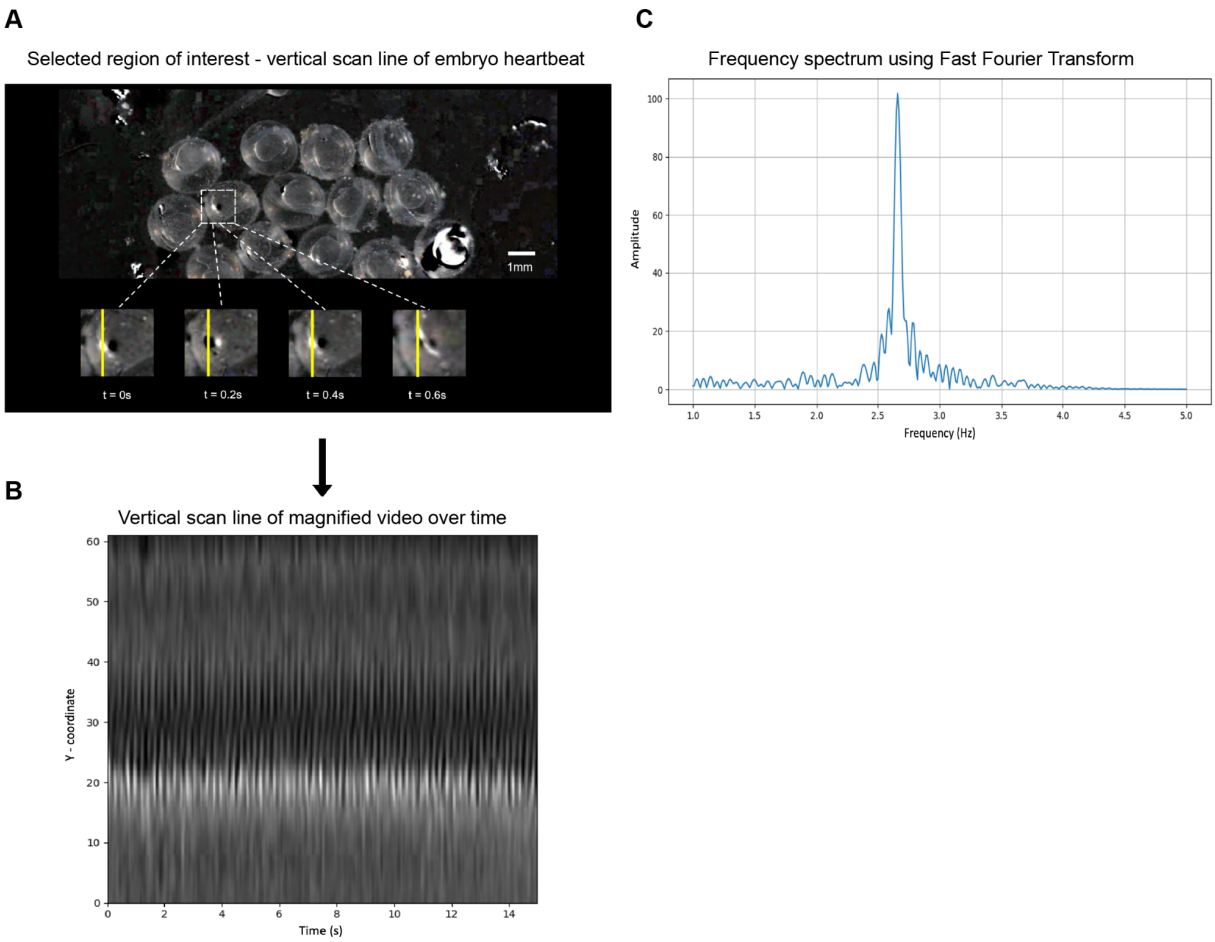


**Fig. S3: Measurement of heart rate in zebrafish embryos using Eulerian video magnification. (A)** Cropped video frame showing the embryonic heart of *Danio rerio*. The yellow vertical line marks the region where the heartbeat is most visible across the recording, with periodic pixel contrast shifts (white to black) reflecting heartbeat frequency. **(B)** Time-series plot of pixel contrast variation along the yellow line over a 15 s recording, capturing rhythmic heartbeat oscillations. **(C)** Frequency spectrum derived from the same region using a Fast Fourier Transform, showing the dominant heartbeat frequency (Hz). A sharp amplitude peak indicates a strong signal with low noise and a stable heart rate.

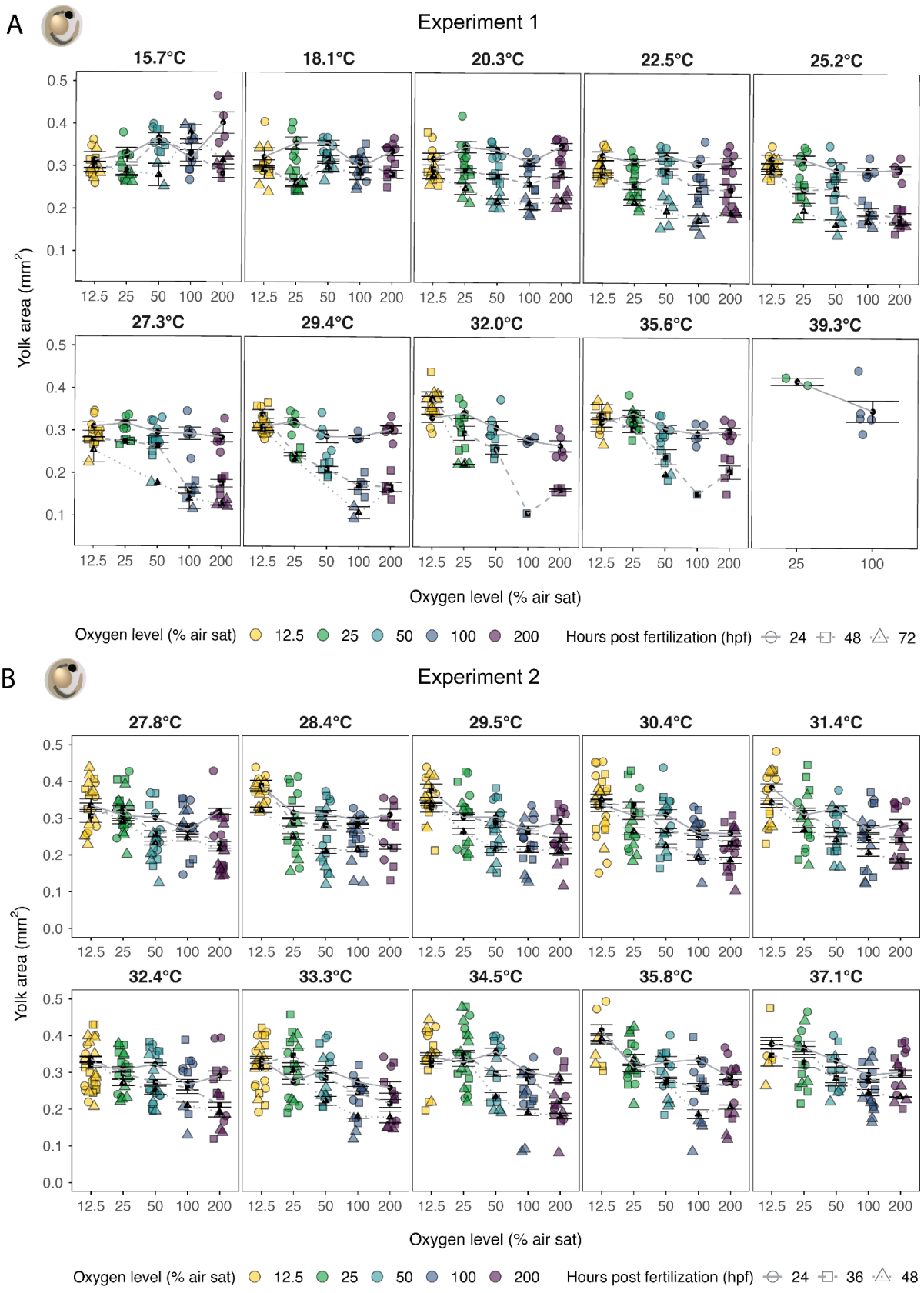


**Fig. S4: Yolk sac area of zebrafish embryos across temperature, oxygen, and developmental time.** Yolk sac area (mm²) of *Danio rerio* embryos across oxygen levels (colors) and temperatures (vertical panels) over time post-fertilization. Measurements were

taken at 24, 48, and 72 hpf in *Experiment 1*, and at 24, 36, and 48 hpf in *Experiment 2*. Data points represent individual embryos; black triangles indicate group means ± s.e., and grey dashed lines connect mean values across sampling times.

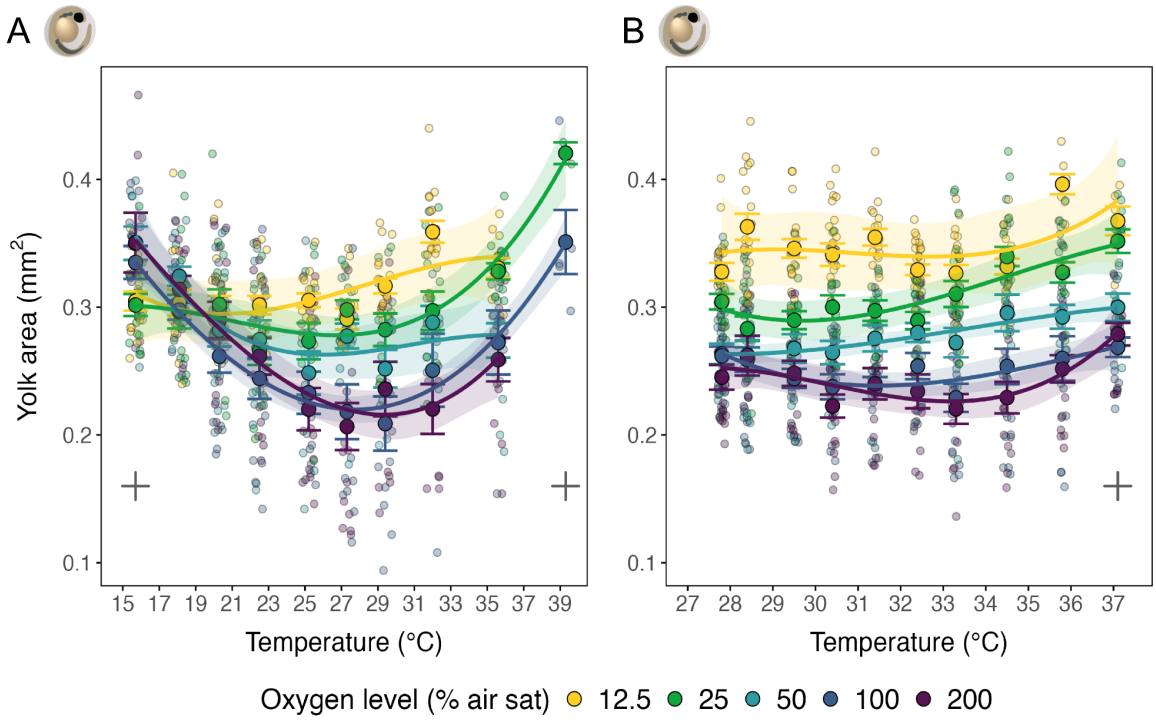


**Fig. S5: Yolk sac area (mm²) of zebrafish embryos across temperatures (x-axis) and oxygen levels (colors) in *Experiment 1*** (A; n = 2–9) **and *Experiment 2*** (B; n = 2–11). Points show individual embryos; colored circles indicate means ± s.e., and colored lines indicating second-degree polynomial fits.

**Table S1: Yolk sac consumption (%) of zebrafish embryos across temperature, oxygen, and developmental time in *Experiment 1***. Anova (type III) and linear model (LM) includes oxygen level, temperature centered to 15.7 °C ($Temp_1$), and its quadratic term ($Temp_2$), their interaction, and developmental time (hpf): Yolk consumption ~ $O_2$ + $Temp_1$ + $Temp_2$ + $O_2$×$Temp_1$ + $O_2$×$Temp_2$ + hpf. Significance was assessed relative to 15.7 °C and 100% air saturation. Estimates (β), standard errors (SE), t-values, and p-values are shown for each predictor.

| *Parameters (Anova, type III)* | *Sum Squares* | *Df* | *F-value* | *P-value* |
|---|---|---|---|---|
| (Intercept) | 581 | 1 | 6.4054 | 0.0116* |
| $O_2$ | 1688 | 4 | 4.6523 | 0.0011** |
| $Temp_1$ | 11172 | 1 | 123.1568 | < 0.0001 *** |
| $Temp_2$ | 8455 | 1 | 93.2098 | < 0.0001 *** |
| $O_2$:$Temp_1$ | 5232 | 4 | 14.4203 | < 0.0001 *** |
| $O_2$:$Temp_2$ | 2006 | 4 | 5.5294 | 0.0002*** |

| | | | | |
|---|---|---|---|---|
| hpf | 37213 | 1 | 410.2245 | < 0.0001 *** |
| Residuals | 53975 | 595 | | |

| *Parameters (LM)* | *Estimate (B)* | *Std. Error* | *t-value* | *P-value* |
|---|---|---|---|---|
| (Intercept) | -5.860 | 2.315 | -2.531 | 0.012 * |
| $O_2$(12.5%) | 7.661 | 2.879 | 2.661 | 0.008 ** |
| $O_2$(25%) | 6.136 | 2.853 | 2.151 | 0.032 * |
| $O_2$(50%) | -2.182 | 3.021 | -0.722 | 0.470 |
| $O_2$(200%) | -1.163 | 3.292 | -0.353 | 0.724 |
| $Temp_1$ | 5.014 | 0.452 | 11.098 | < 0.0001 *** |
| $Temp_2$ | -0.195 | 0.020 | -9.655 | < 0.0001 *** |
| hpf | 0.429 | 0.021 | 20.254 | < 0.0001 *** |
| $O_2$(12.5%):$Temp_1$ | -4.391 | 0.648 | -6.779 | < 0.0001 *** |
| $O_2$(25%):$Temp_1$ | -2.675 | 0.635 | -4.215 | < 0.0001 *** |
| $O_2$(50%):$Temp_1$ | -1.341 | 0.678 | -1.978 | 0.048 * |
| $O_2$(200%):$Temp_1$ | -0.410 | 0.720 | -0.568 | 0.570 |
| $O_2$(12.5%):$Temp_2$ | 0.140 | 0.031 | 4.571 | < 0.0001 *** |
| $O_2$(25%):$Temp_2$ | 0.073 | 0.029 | 2.502 | 0.013 * |
| $O_2$(50%):$Temp_2$ | 0.064 | 0.032 | 2.011 | 0.045 * |
| $O_2$(200%):$Temp_2$ | 0.035 | 0.033 | 1.051 | 0.294 |

| | |
|---|---|
| Residual standard error: | 9.524 on 595 degrees of freedom |
| Multiple R-squared: | 0.6189 |
| F-statistic: | 64.41 on 15 and 595 DF, p-value: < 0.001 *** |

**Table S2: Yolk sac consumption (%) of zebrafish embryos across temperature, oxygen, and developmental time in *Experiment 2*.** Anova (type III) and linear model (LM) includes oxygen level, temperature centered to 27.8 °C ($Temp_1$), and its quadratic term ($Temp_2$), their interaction, and developmental time (hpf): Yolk consumption ~ $O_2$ + $Temp_1$ + $Temp_2$ + $O_2$×$Temp_1$ + $O_2$×$Temp_2$ + hpf. Significance was assessed relative to 27.8 °C and 100% air saturation. Estimates (β), standard errors (SE), t-values, and p-values are shown for each predictor.

| *Parameters (Anova, type III)* | *Sum Squares* | *Df* | *F-value* | *P-value* |
|---|---|---|---|---|
| (Intercept) | 7128 | 1 | 118.067 | < 0.0001 *** |

| | | | | |
|---|---|---|---|---|
| $O_2$ | 13295 | 4 | 55.053 | < 0.0001 *** |
| $Temp_1$ | 828 | 1 | 13.718 | 0.0002*** |
| $Temp_2$ | 1046 | 1 | 17.325 | 0.00003*** |
| $O_2$:$Temp_1$ | 1288 | 4 | 5.334 | 0.0003*** |
| $O_2$:$Temp_2$ | 811 | 4 | 3.359 | 0.0097** |
| hpf | 21395 | 1 | 354.368 | < 0.0001 *** |
| Residuals | 48482 | 803 | | |

| ***Parameters (LM)*** | ***Estimate (B)*** | ***Std. Error*** | ***t value*** | ***P-value*** |
|---|---|---|---|---|
| (Intercept) | 18.766 | 1.727 | 10.866 | < 0.0001 *** |
| $O_2$(12.5%) | -21.622 | 1.907 | -11.336 | < 0.0001 *** |
| $O_2$(25%) | -8.598 | 1.910 | -4.503 | < 0.0001 *** |
| $O_2$(50%) | 0.032 | 1.918 | 0.016 | 0.987 |
| $O_2$(200%) | 1.702 | 1.955 | 0.871 | 0.384 |
| $Temp_1$ | 2.745 | 0.741 | 3.704 | 0.0002 *** |
| $Temp_2$ | -0.325 | 0.078 | -4.162 | < 0.0001 *** |
| hpf | 0.520 | 0.028 | 18.825 | < 0.0001 *** |
| $O_2$(12.5%):$Temp_1$ | -0.245 | 1.031 | -0.238 | 0.812 |
| $O_2$(25%):$Temp_1$ | -1.975 | 1.012 | -1.952 | 0.051 |
| $O_2$(50%):$Temp_1$ | -3.399 | 1.024 | -3.320 | 0.001 ** |
| $O_2$(200%):$Temp_1$ | 0.625 | 1.042 | 0.600 | 0.549 |
| $O_2$(12.5%):$Temp_2$ | 0.008 | 0.116 | 0.073 | 0.942 |
| $O_2$(25%):$Temp_2$ | 0.087 | 0.109 | 0.798 | 0.425 |
| $O_2$(50%):$Temp_2$ | 0.296 | 0.110 | 2.687 | 0.007 ** |
| $O_2$(200%):$Temp_2$ | -0.079 | 0.111 | -0.716 | 0.474 |

| | |
|---|---|
| Residual standard error: | 7.77 on 803 degrees of freedom |
| Multiple R-squared: | 0.659 |
| F-statistic: | 103.2 on 15 and 803 DF, p-value: < 0.001 *** |
| Adjusted R-squared: | 0.652 |

**Table S3: Heart rate of zebrafish embryos across temperature, oxygen, and developmental time in *Experiment 2*.** Anova (type III) and linear model (LM) includes oxygen level, temperature centered to 27.8 °C ($Temp_1$), and its quadratic term ($Temp_2$), their interaction, and developmental time (hpf): Heart rate ~ $O_2$ + $Temp_1$ + $Temp_2$ + $O_2$×$Temp_1$ + $O_2$×$Temp_2$ + hpf. Significance was assessed relative to 27.8 °C and 100% air saturation. Estimates (β), standard errors (SE), t-values, and p-values are shown for each predictor.

| ***Parameters (Anova, type III)*** | ***Sum Squares*** | ***Df*** | ***F-value*** | ***p-value*** |
|---|---|---|---|---|
| (Intercept) | 379095 | 1 | 219.751 | < 0.0001 *** |
| $O_2$ | 338503 | 4 | 49.055 | < 0.0001 *** |
| $Temp_1$ | 47756 | 1 | 27.683 | < 0.0001 *** |
| $Temp_2$ | 48746 | 1 | 28.256 | < 0.0001 *** |
| $O_2$:$Temp_1$ | 13286 | 4 | 1.925 | 0.1042 |
| $O_2$:$Temp_2$ | 11686 | 4 | 1.693 | 0.1494 |
| hpf | 748588 | 1 | 433.935 | < 0.0001 *** |
| Residuals | 1511201 | 876 | | |
| ***Parameters (LM)*** | ***Estimate (B)*** | ***Std. Error*** | ***t-value*** | ***p-value*** |
| (Intercept) | 109.803 | 7.407 | 14.824 | < 0.0001 *** |
| $O_2$(12.5%) | -96.197 | 8.723 | -11.028 | < 0.0001 *** |
| $O_2$(25%) | -19.152 | 8.914 | -2.149 | 0.0319* |
| $O_2$(50%) | 2.098 | 9.916 | 0.212 | 0.8325 |
| $O_2$(200%) | 11.082 | 9.209 | 1.203 | 0.2291 |
| $Temp_1$ | 19.596 | 3.724 | 5.261 | < 0.0001 *** |
| $Temp_2$ | -2.193 | 0.413 | -5.316 | < 0.0001 *** |
| hpf | 1.628 | 0.078 | 20.831 | < 0.0001 *** |
| $O_2$(12.5%):$Temp_1$ | 2.775 | 5.770 | 0.481 | 0.6307 |
| $O_2$(25%):$Temp_1$ | -5.969 | 5.448 | -1.096 | 0.2736 |
| $O_2$(50%):$Temp_1$ | -12.959 | 5.837 | -2.220 | 0.0267* |
| $O_2$(200%):$Temp_1$ | -4.209 | 5.202 | -0.809 | 0.4187 |
| $O_2$(12.5%):$Temp_2$ | -0.301 | 0.842 | -0.357 | 0.7208 |
| $O_2$(25%):$Temp_2$ | -0.211 | 0.658 | -0.321 | 0.7481 |
| $O_2$(50%):$Temp_2$ | 1.435 | 0.669 | 2.144 | 0.0323* |
| $O_2$(200%):$Temp_2$ | 0.182 | 0.573 | 0.318 | 0.7504 |

| | |
|---|---|
| Residual standard error: | 41.53 on 876 degrees of freedom |
| Multiple R-squared: | 0.4287 |
| F-statistic: | 43.82 on 15 and 876 DF, p-value: < 0.0001 *** |
| Adjusted R-squared: | 0.587 |

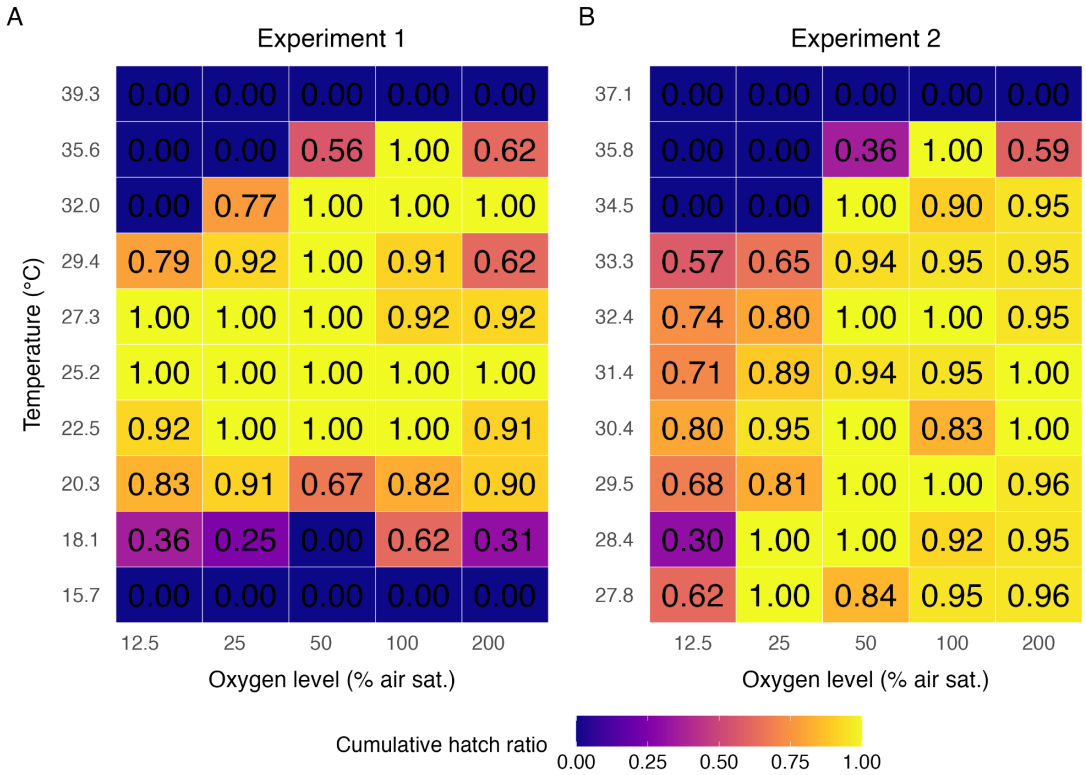


**Fig. S6: Heat map of maximum hatching number across temperature and oxygen treatments.** Heat map showing the accumulated maximum number of hatched *Danio rerio* embryos per temperature and oxygen treatment in (A) *Experiment 1* and (B) *Experiment 2*. Lighter colors (yellow) indicate higher hatching rates.

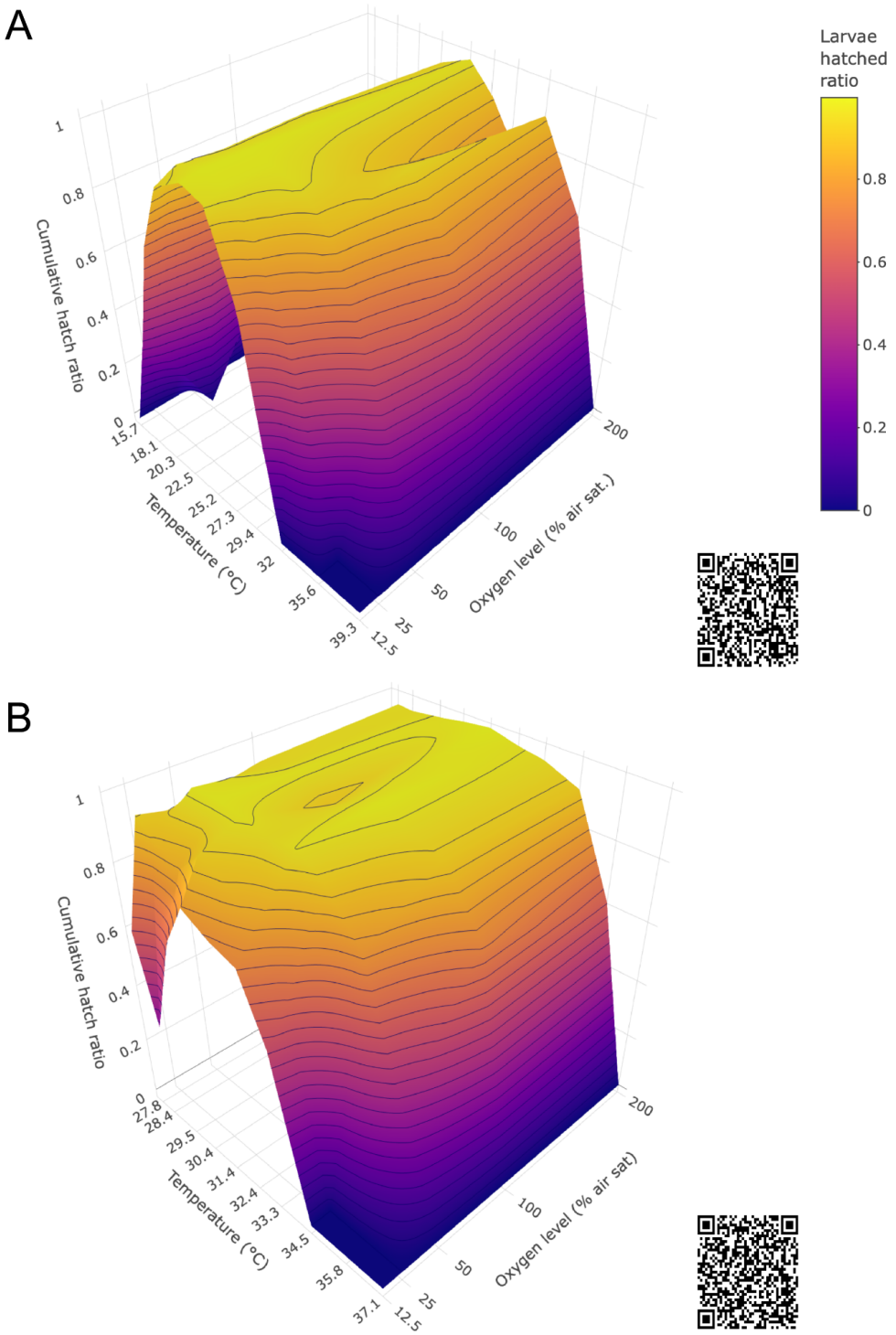


**Fig. S7: Three-dimensional surface plot of cumulative hatching across temperature and oxygen treatments.** 3D surface plots showing cumulative hatching of *Danio rerio* larvae across temperature and oxygen levels in (A) *Experiment 1* and (B) *Experiment 2*. Cumulative hatching values are based on observed data. Survival ratios above 0.6 were smoothed using a kernel-based normalization. (function image.smoother, aRange = 0.75, theta = 0.75, fields package, R), preventing overfitting when hatching nears zero at the extreme temperatures.

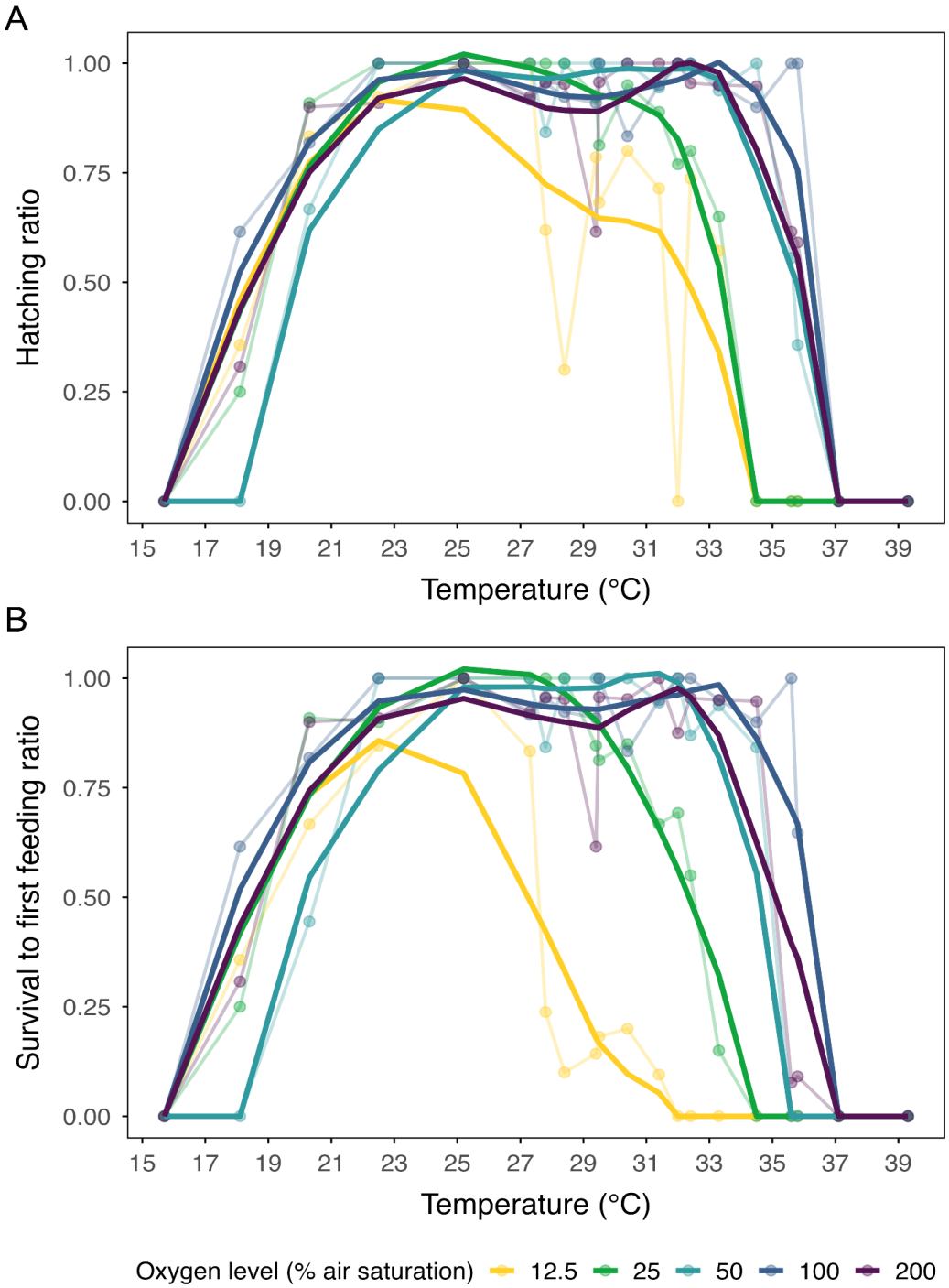


**Fig. S8: Thermal performance curve (TPC) of hatching success (A) and survival to first feeding (B) across temperature and oxygen levels.** Points depict maximum hatching ratio per temperature, and TPC lines were fitted using a smoothed maxima *LOESS* function (span = 0.6).

**Table S4: Effect of oxygen, temperature, and developmental time on hatching success in *Experiment 1*.** GLM includes oxygen level, temperature centered to 18.1 °C ($Temp_1$, lowest temperature at which hatching occurred), and its quadratic term ($Temp_2$), their interaction, and log-transformed developmental time (hpf): Hatching success ~ $O_2$ + $Temp_1$ + $Temp_2$ + $O_2$×$Temp_1$ + $O_2$×$Temp_2$ + log(hpf) + (1 | Experiment). Significance is shown first with

oxygen as numeric relative to 18.1 °C and subsequently relative to 100% air saturation. Estimates (β), standard errors (SE), z-values, and p-values are shown for each predictor.

| ***Parameters (GLM)*** | ***Estimate (B)*** | ***Std. Error*** | ***z-value*** | ***p-value*** |
|---|---|---|---|---|
| (Intercept) | -24.4100 | 0.6950 | -35.128 | < 0.0001 *** |
| $O_2$ | -0.0004 | 0.0015 | -0.288 | 0.773 |
| $Temp_1$ | 1.3150 | 0.0484 | 27.195 | < 0.0001 *** |
| $Temp_2$ | -0.0665 | 0.0027 | -24.564 | < 0.0001 *** |
| $O_2$:$Temp_1$ | -0.0017 | 0.0004 | -4.380 | 0.00001 |
| $O_2$:$Temp_2$ | 0.0002 | 0.0000 | 7.263 | < 0.0001 *** |
| log(hpf) | 4.3400 | 0.1289 | 33.654 | < 0.0001 *** |
| Null deviance: | | 6068.2 on 563 degrees of freedom | | |
| Residual deviance: | | 1498.8 on 557 degrees of freedom | | |
| AIC: | | 1959 | | |
| Num.Fisher Scoring iterations: | | 3 | | |

| ***Parameters (GLM)*** | ***Estimate (B)*** | ***Std. Error*** | ***z-value*** | ***p-value*** |
|---|---|---|---|---|
| (Intercept) | -30.6686 | 0.9320 | -32.905 | < 0.0001 *** |
| $O_2$(12.5%) | -1.1127 | 0.3523 | -3.159 | 0.0016 ** |
| $O_2$(25%) | -1.0823 | 0.3568 | -3.033 | 0.0024 ** |
| $O_2$(50%) | -3.1413 | 0.4516 | -6.956 | < 0.0001 *** |
| $O_2$(200%) | -0.6648 | 0.3208 | -2.072 | 0.0382 * |
| $Temp_1$ | 1.0536 | 0.0597 | 17.661 | < 0.0001 *** |
| $Temp_2$ | -0.0306 | 0.0030 | -10.160 | < 0.0001 *** |
| $O_2$(12.5%):$Temp_1$ | 1.0454 | 0.1155 | 9.048 | < 0.0001 *** |
| $O_2$(25%):$Temp_1$ | 0.9531 | 0.1106 | 8.615 | < 0.0001 *** |
| $O_2$(50%):$Temp_1$ | 0.9264 | 0.1129 | 8.206 | < 0.0001 *** |
| $O_2$(200%):$Temp_1$ | 0.1588 | 0.0833 | 1.907 | 0.0565 · |
| $O_2$(12.5%):$Temp_2$ | -0.1072 | 0.0081 | -13.310 | < 0.0001 *** |
| $O_2$(25%):$Temp_2$ | -0.0774 | 0.0069 | -11.194 | < 0.0001 *** |
| $O_2$(50%):$Temp_2$ | -0.0527 | 0.0059 | -9.004 | < 0.0001 *** |
| $O_2$(200%):$Temp_2$ | -0.0187 | 0.0045 | -4.159 | 0.00003 *** |
| log(hpf) | 5.6246 | 0.1727 | 32.560 | < 0.0001 *** |

| | |
|---|---|
| Null deviance: | 6102.34 on 563 degrees of freedom |
| Residual deviance: | 734.34 on 548 degrees of freedom |
| AIC: | 1212.6 |
| Num.Fisher Scoring iterations: | 4 |

**Table S5: Effect of oxygen, temperature, and developmental time on hatching success in *Experiment 1*.** Effect of oxygen level, temperature, and time post-fertilization (hpf) on hatching success of *Danio rerio* embryos, derived from GLM: Hatching success ~ $O_2$ × Temp + log(hpf). Post hoc comparisons were conducted relative to the control treatment (27.3 °C, normoxia). This model was used to predict time to 50% hatching ($ET_{50}$). Coefficients, standard errors; and p-values are shown for each predictor.

| *Parameters (GLM)* | *Coefficients* | *Std. Error* | *z-value* | *p-value* |
|---|---|---|---|---|
| (Intercept) | -25.7312 | 0.8701 | -29.5740 | < 0.0001 *** |
| $O_2$(12.5%) | 1.8225 | 0.4239 | 4.2990 | 0.00002 *** |
| $O_2$(25%) | 2.3436 | 0.4959 | 4.7260 | < 0.0001 *** |
| $O_2$(50%) | 1.8628 | 0.4155 | 4.4840 | 0.00001 *** |
| $O_2$(200%) | -0.1948 | 0.3792 | -0.5140 | 0.60748 |
| Temp(15.7°C) | -10.1363 | 1.4748 | -6.8730 | < 0.0001 *** |
| Temp(18.1°C) | -6.3693 | 0.4250 | -14.9880 | < 0.0001 *** |
| Temp(20.3°C) | -4.6328 | 0.4008 | -11.5590 | < 0.0001 *** |
| Temp(22.5°C) | -1.9670 | 0.3799 | -5.1780 | < 0.0001 *** |
| Temp(25.2°C) | 0.5331 | 0.4179 | 1.2760 | 0.20201 |
| Temp(29.4°C) | 0.8488 | 0.4150 | 2.0450 | 0.04083 * |
| Temp(32°C) | 3.6409 | 0.5222 | 6.9720 | < 0.0001 *** |
| Temp(35.6°C) | 3.5634 | 0.4647 | 7.6690 | < 0.0001 *** |
| Temp(39.3°C) | 0.2245 | 1.5849 | 0.1420 | 0.88736 |
| $O_2$(12.5%):Temp(15.7°C) | -2.5674 | 2.0789 | -1.2350 | 0.21685 |
| $O_2$(25%):Temp(15.7°C) | -2.8680 | 2.0989 | -1.3660 | 0.17181 |
| $O_2$(50%):Temp(15.7°C) | -2.3014 | 2.0830 | -1.1050 | 0.26922 |
| $O_2$(200%):Temp(15.7°C) | 0.0693 | 2.0836 | 0.0330 | 0.97346 |
| $O_2$(12.5%):Temp(18.1°C) | -2.9697 | 0.6200 | -4.7900 | < 0.0001 *** |
| $O_2$(25%):Temp(18.1°C) | -3.5675 | 0.6918 | -5.1570 | < 0.0001 *** |

| | | | | |
|---|---|---|---|---|
| $O_2$(50%):Temp(18.1°C) | -6.1542 | 1.5286 | -4.0260 | 0.00006 *** |
| $O_2$(200%):Temp(18.1°C) | -1.6456 | 0.6608 | -2.4900 | 0.01276 * |
| $O_2$(12.5%):Temp(20.3°C) | -0.1308 | 0.5530 | -0.2360 | 0.81305 |
| $O_2$(25%):Temp(20.3°C) | -1.3390 | 0.6145 | -2.1790 | 0.02933 * |
| $O_2$(50%):Temp(20.3°C) | -2.9215 | 0.5863 | -4.9830 | < 0.0001 *** |
| $O_2$(200%):Temp(20.3°C) | 0.9848 | 0.5353 | 1.8400 | 0.06582 |
| $O_2$(12.5%):Temp(22.5°C) | -1.9238 | 0.5487 | -3.5060 | 0.00045 *** |
| $O_2$(25%):Temp(22.5°C) | -1.0966 | 0.6247 | -1.7550 | 0.07917 * |
| $O_2$(50%):Temp(22.5°C) | -1.7295 | 0.5650 | -3.0610 | 0.0022 ** |
| $O_2$(200%):Temp(22.5°C) | -0.5116 | 0.5226 | -0.9790 | 0.32755 |
| $O_2$(12.5%):Temp(25.2°C) | -2.2347 | 0.6123 | -3.6500 | 0.00026 *** |
| $O_2$(25%):Temp(25.2°C) | -1.2161 | 0.6700 | -1.8150 | 0.0695 · |
| $O_2$(50%):Temp(25.2°C) | -2.7798 | 0.6009 | -4.6260 | < 0.0001 *** |
| $O_2$(200%):Temp(25.2°C) | -1.1439 | 0.5702 | -2.0060 | 0.04485 * |
| $O_2$(12.5%):Temp(29.4°C) | -3.8866 | 0.5854 | -6.6400 | < 0.0001 *** |
| $O_2$(25%):Temp(29.4°C) | -2.0495 | 0.6520 | -3.1430 | 0.00167 ** |
| $O_2$(50%):Temp(29.4°C) | -0.5201 | 0.6403 | -0.8120 | 0.41659 |
| $O_2$(200%):Temp(29.4°C) | -1.1690 | 0.5560 | -2.1030 | 0.03549 * |
| $O_2$(12.5%):Temp(32°C) | -13.1201 | 1.5821 | -8.2930 | < 0.0001 *** |
| $O_2$(25%):Temp(32°C) | -6.0797 | 0.7280 | -8.3510 | < 0.0001 *** |
| $O_2$(50%):Temp(32°C) | -2.4667 | 0.7134 | -3.4580 | 0.00055 *** |
| $O_2$(200%):Temp(32°C) | -0.6064 | 0.7152 | -0.8480 | 0.39656 |
| $O_2$(12.5%):Temp(35.6°C) | -11.1369 | 1.5641 | -7.1200 | < 0.0001 *** |
| $O_2$(25%):Temp(35.6°C) | -11.7437 | 1.5839 | -7.4140 | < 0.0001 *** |
| $O_2$(50%):Temp(35.6°C) | -5.9753 | 0.6571 | -9.0940 | < 0.0001 *** |
| $O_2$(200%):Temp(35.6°C) | -4.4209 | 0.5949 | -7.4310 | < 0.0001 *** |
| $O_2$(12.5%):Temp(39.3°C) | 0.4634 | 2.2625 | 0.2050 | 0.83770 |
| $O_2$(25%):Temp(39.3°C) | -0.8111 | 2.2380 | -0.3620 | 0.71705 |
| $O_2$(50%):Temp(39.3°C) | -0.2005 | 2.2286 | -0.0900 | 0.92833 |
| $O_2$(200%):Temp(39.3°C) | 2.0225 | 2.2341 | 0.9050 | 0.36531 |
| log(hpf) | 5.9284 | 0.1870 | 31.7010 | < 0.0001 *** |

| | |
|---|---|
| Null deviance: | 6068.18 on 563 degrees of freedom |
| Residual deviance: | 530.22 on 513 degrees of freedom |
| AIC: | 1078.4 |
| Number of Fisher Scoring iterations: | 10 |

**Table S6: Effect of oxygen, temperature, and developmental time on hatching success in *Experiment 2*.** GLM includes oxygen level, temperature centered to 27.8 °C ($Temp_1$, lowest temperature at which hatching occurred), and its quadratic term ($Temp_2$), their interaction, and log-transformed developmental time (hpf): Hatching success ~ $O_2$ + $Temp_1$ + $Temp_2$ + $O_2 \times Temp_1$ + $O_2 \times Temp_2$ + log(hpf) + (1 | Experiment). Significance is shown first with oxygen as numeric relative to 27.8 °C and subsequently relative to 100% air saturation. Estimates (β), standard errors, z-values, and p-values are shown for each predictor.

| ***Parameters (GLM)*** | ***Estimate (B)*** | ***Std. Error*** | ***z-value*** | ***p-value*** |
|---|---|---|---|---|
| (Intercept) | -20.6800 | 0.4985 | -41.4870 | < 0.0001 *** |
| $O_2$ | 0.0062 | 0.0010 | 6.1460 | < 0.0001 *** |
| $Temp_1$ | 0.6726 | 0.0675 | 9.9660 | < 0.0001 *** |
| $Temp_2$ | -0.1184 | 0.0088 | -13.4460 | < 0.0001 *** |
| $O_2$:$Temp_1$ | 0.0024 | 0.0006 | 4.0030 | 0.0001 *** |
| $O_2$:$Temp_2$ | -0.0002 | 0.0001 | -3.1210 | 0.0018 ** |
| log(hpf) | 4.7390 | 0.1118 | 42.3990 | < 0.0001 *** |
| Null deviance: | 7709.4 on 399 degrees of freedom | | | |
| Residual deviance: | 2362.9 on 393 degrees of freedom | | | |
| AIC: | 2885.1 | | | |
| Num.Fisher Scoring iterations: | 3 | | | |

| ***Parameters (GLM)*** | ***Estimate (B)*** | ***Std. Error*** | ***z-value*** | ***p-value*** |
|---|---|---|---|---|
| (Intercept) | -24.5700 | 0.6418 | -38.278 | < 0.0001 *** |
| $O_2$(12.5%) | -3.5550 | 0.2641 | -13.462 | < 0.0001 *** |
| $O_2$(25%) | 0.4599 | 0.2571 | 1.789 | 0.0737 · |
| $O_2$(50%) | 0.3232 | 0.2562 | 1.262 | 0.2071 |
| $O_2$(200%) | -0.0464 | 0.2430 | -0.191 | 0.8486 |
| $Temp_1$ | 1.2810 | 0.1000 | 12.819 | < 0.0001 *** |
| $Temp_2$ | -0.1703 | 0.0110 | -15.494 | < 0.0001 *** |

| | | | | |
|---|---|---|---|---|
| $O_2$(12.5%):$Temp_1$ | 0.1941 | 0.1728 | 1.123 | 0.2615 |
| $O_2$(25%):$Temp_1$ | -0.5149 | 0.1744 | -2.953 | 0.0032 ** |
| $O_2$(50%):$Temp_1$ | -0.1122 | 0.1434 | -0.783 | 0.4338 |
| $O_2$(200%):$Temp_1$ | -0.1242 | 0.1340 | -0.927 | 0.3541 |
| $O_2$(12.5%):$Temp_2$ | -0.0623 | 0.0247 | -2.517 | 0.0118 * |
| $O_2$(25%):$Temp_2$ | -0.0389 | 0.0251 | -1.552 | 0.1208 |
| $O_2$(50%):$Temp_2$ | 0.0016 | 0.0163 | 0.100 | 0.9201 |
| $O_2$(200%):$Temp_2$ | 0.0003 | 0.0148 | 0.021 | 0.9836 |
| log(hpf) | 5.8840 | 0.1459 | 40.318 | < 0.0001 *** |
| Null deviance: | 7709.4 on 399 degrees of freedom | | | |
| Residual deviance: | 1179.7 on 384 degrees of freedom | | | |
| AIC: | 1720 | | | |
| Num.Fisher Scoring iterations: | 3 | | | |

**Table S7: Effect of oxygen, temperature, and developmental time on hatching success in *Experiment 2*.** Effect of oxygen level, temperature, and time post-fertilization (hpf) on hatching success of *Danio rerio* embryos, derived from a GLM: Hatching success ~ $O_2$ × Temp + log(hpf). Post hoc comparisons were conducted relative to the control treatment (27.8 °C, normoxia). This model was used to predict time to 50% hatching ($ET_{50}$). Coefficients, standard errors; and p-values are shown for each predictor.

| ***Parameters (GLM)*** | ***Coefficients*** | ***Std. Error*** | ***z-value*** | ***p-value*** |
|---|---|---|---|---|
| (Intercept) | -25.4008 | 0.6992 | -36.3270 | < 0.0001 *** |
| $O_2$(12.5%) | -3.2721 | 0.3293 | -9.9370 | < 0.0001 *** |
| $O_2$(25%) | 0.1484 | 0.3442 | 0.4310 | 0.66629 |
| $O_2$(50%) | -0.5093 | 0.3468 | -1.4680 | 0.14201 |
| $O_2$(200%) | -0.3443 | 0.3308 | -1.0410 | 0.29791 |
| Temp(28.4°C) | -0.3710 | 0.3862 | -0.9610 | 0.33670 |
| Temp(29.5°C) | 1.0031 | 0.3624 | 2.7680 | 0.00564 ** |
| Temp(30.4°C) | 0.3344 | 0.3626 | 0.9220 | 0.35641 |
| Temp(31.4°C) | 1.2272 | 0.3662 | 3.3510 | 0.00081 *** |
| Temp(32.4°C) | 2.1239 | 0.3832 | 5.5420 | < 0.0001 *** |

| | | | | |
|---|---|---|---|---|
| Temp(33.3°C) | 1.2181 | 0.3671 | 3.3180 | 0.00091 |
| Temp(34.5°C) | 0.5842 | 0.3549 | 1.6460 | 0.09977 |
| Temp(35.8°C) | 0.9323 | 0.3830 | 2.4340 | 0.01493 * |
| Temp(37.1°C) | -7.9833 | 1.4600 | -5.4680 | < 0.0001 *** |
| $O_2$(12.5%):Temp(28.4°C) | -1.6848 | 0.5401 | -3.1190 | 0.00181 ** |
| $O_2$(25%):Temp(28.4°C) | 0.4760 | 0.5169 | 0.9210 | 0.35713 |
| $O_2$(50%):Temp(28.4°C) | 1.5334 | 0.5315 | 2.8850 | 0.00392 ** |
| $O_2$(200%):Temp(28.4°C) | 0.4480 | 0.5090 | 0.8800 | 0.37872 |
| $O_2$(12.5%):Temp(29.5°C) | -0.4888 | 0.4711 | -1.0380 | 0.29939 |
| $O_2$(25%):Temp(29.5°C) | -1.8470 | 0.5152 | -3.5850 | 0.00034 *** |
| $O_2$(50%):Temp(29.5°C) | 1.1248 | 0.5244 | 2.1450 | 0.03197 * |
| $O_2$(200%):Temp(29.5°C) | -0.0036 | 0.4935 | -0.0070 | 0.99413 |
| $O_2$(12.5%):Temp(30.4°C) | 1.9022 | 0.5050 | 3.7670 | 0.00017 *** |
| $O_2$(25%):Temp(30.4°C) | -0.0434 | 0.5102 | -0.0850 | 0.93224 |
| $O_2$(50%):Temp(30.4°C) | 1.5466 | 0.5309 | 2.9130 | 0.00358 ** |
| $O_2$(200%):Temp(30.4°C) | 0.4625 | 0.4982 | 0.9280 | 0.35330 |
| $O_2$(12.5%):Temp(31.4°C) | -0.5664 | 0.4818 | -1.1760 | 0.23969 |
| $O_2$(25%):Temp(31.4°C) | -1.5425 | 0.5149 | -2.9960 | 0.00274 ** |
| $O_2$(50%):Temp(31.4°C) | 0.0625 | 0.5257 | 0.1190 | 0.90530 |
| $O_2$(200%):Temp(31.4°C) | 1.0027 | 0.5157 | 1.9440 | 0.05188 · |
| $O_2$(12.5%):Temp(32.4°C) | -1.0782 | 0.5292 | -2.0370 | 0.04161 * |
| $O_2$(25%):Temp(32.4°C) | -3.1643 | 0.5166 | -6.1250 | < 0.0001 *** |
| $O_2$(50%):Temp(32.4°C) | -0.2523 | 0.5239 | -0.4820 | 0.63008 |
| $O_2$(200%):Temp(32.4°C) | -1.2446 | 0.5091 | -2.4450 | 0.01449 * |
| $O_2$(12.5%):Temp(33.3°C) | 0.9698 | 0.5179 | 1.8730 | 0.0611 · |
| $O_2$(25%):Temp(33.3°C) | -3.3581 | 0.5010 | -6.7030 | < 0.0001 *** |
| $O_2$(50%):Temp(33.3°C) | -0.1684 | 0.5317 | -0.3170 | 0.75145 |
| $O_2$(200%):Temp(33.3°C) | -0.3551 | 0.5038 | -0.7050 | 0.48083 |
| $O_2$(12.5%):Temp(34.5°C) | -5.4563 | 1.4939 | -3.6520 | 0.00026 *** |
| $O_2$(25%):Temp(34.5°C) | -8.2046 | 1.4996 | -5.4710 | < 0.0001 *** |
| $O_2$(50%):Temp(34.5°C) | 1.3427 | 0.5216 | 2.5740 | 0.01005 * |

| | | | | |
|---|---|---|---|---|
| $O_2$(200%):Temp(34.5°C) | -0.4701 | 0.4916 | -0.9560 | 0.33898 |
| $O_2$(12.5%):Temp(35.8°C) | -3.9199 | 1.5096 | -2.5970 | 0.00941 ** |
| $O_2$(25%):Temp(35.8°C) | -8.5087 | 1.5078 | -5.6430 | < 0.0001 *** |
| $O_2$(50%):Temp(35.8°C) | -2.1024 | 0.6062 | -3.4680 | 0.00052 *** |
| $O_2$(200%):Temp(35.8°C) | -2.2404 | 0.5046 | -4.4400 | < 0.0001 *** |
| $O_2$(12.5%):Temp(37.1°C) | 5.3383 | 2.0750 | 2.5730 | 0.01009 * |
| $O_2$(25%):Temp(37.1°C) | 1.5752 | 2.0675 | 0.7620 | 0.44611 |
| $O_2$(50%):Temp(37.1°C) | 1.8417 | 2.0785 | 0.8860 | 0.37559 |
| $O_2$(200%):Temp(37.1°C) | 0.0890 | 2.0571 | 0.0430 | 0.96548 |
| log(hpf) | 6.2491 | 0.1590 | 39.3090 | < 0.0001 *** |
| Null deviance: | 7620.39 on 399 degrees of freedom | | | |
| Residual deviance: | 711.17 on 349 degrees of freedom | | | |
| AIC: | 1321.4 | | | |
| Num.Fisher Scoring iterations: | 5 | | | |

1207

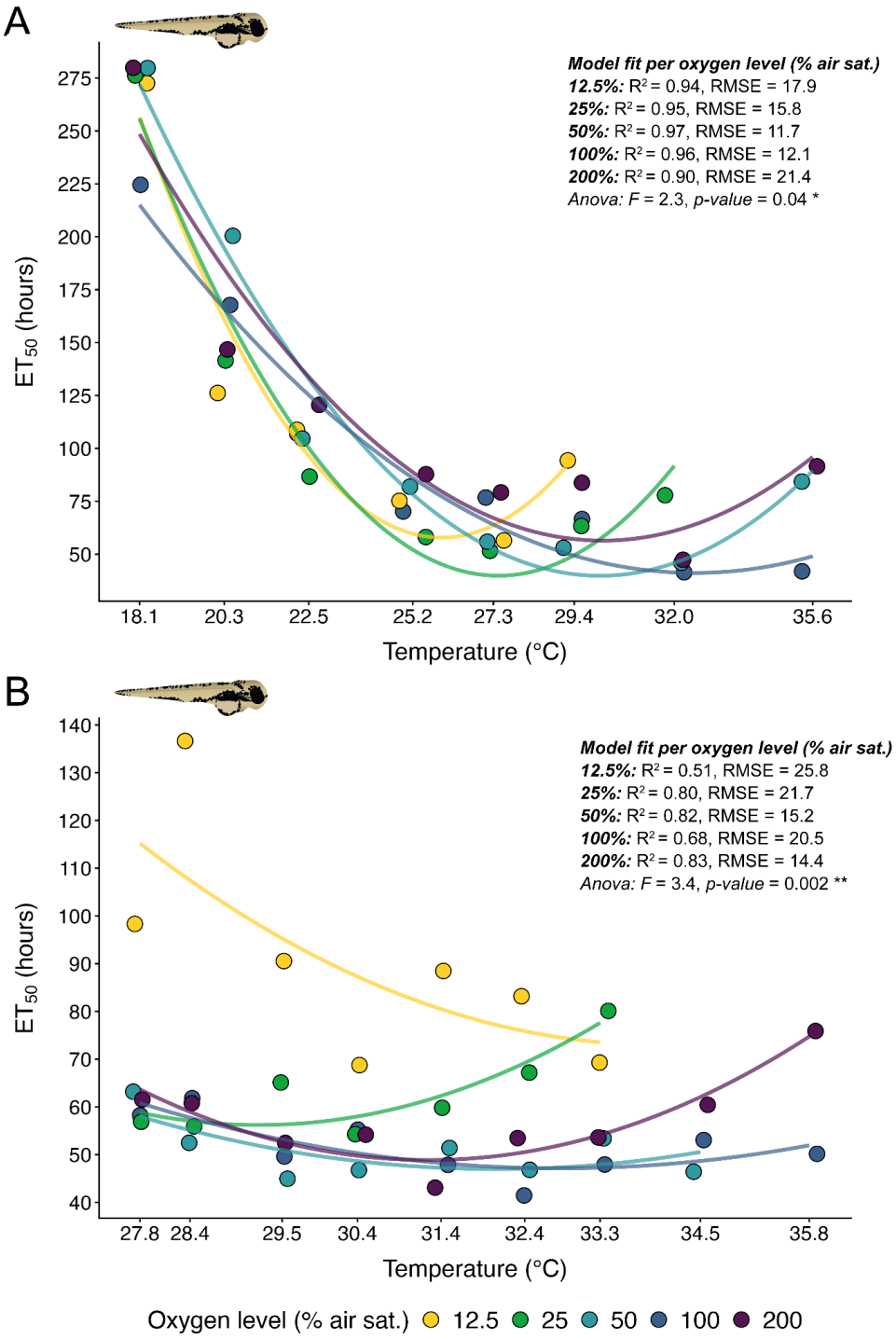


**Fig. S9: Model-predicted time to 50% hatching ($ET_{50}$) in zebrafish (*Danio rerio*).** Predicted $ET_{50}$ (hours) for (A) *Experiment 1* and (B) *Experiment 2* based on GLM: Hatching success ~ Oxygen × Temperature + log(hpf) (Table S5-S7). Oxygen and temperature were included as factors, and hours post-fertilization (hpf) were log-transformed. The models achieved best-fit AIC values. Colored lines represent predicted 50% hatching times for each oxygen level fitted with a second-order polynomial regression (Predicted $ET_{50}$ ~ poly(Temperature, 2)). Coefficients of determination ($R^2$) and root-mean-square deviation (RMSE) indicate regression fit quality per oxygen level. P-values denote significant effects of oxygen treatment on the temperature–hatching time relationship.

**Table S8: Predicted time to 50% hatching ($ET_{50}$ ; hours) for each combination of oxygen level and temperature treatment in *Danio rerio* from *Experiments 1* and *2*.** Predictions were derived from a GLM: Hatching success ~ Oxygen × Temperature + log(hpf) (Table S5-S7), used to generate Fig. S8.

| Experiment 1 | | | Experiment 2 | | |
|---|---|---|---|---|---|
| Temperature (°C) | Oxygen (% air sat) | Predicted $ET_{50}$ | Temperature (°C) | Oxygen (% air sat) | Predicted $ET_{50}$ |
| 15.7 | 12.5 | – | 27.8 | 12.5 | 98.33 |
| 18.1 | 12.5 | 272.66 | 28.4 | 12.5 | 136.65 |
| 20.3 | 12.5 | 126.03 | 29.5 | 12.5 | 90.54 |
| 22.5 | 12.5 | 108.76 | 30.4 | 12.5 | 68.75 |
| 25.2 | 12.5 | 75.18 | 31.4 | 12.5 | 88.47 |
| 27.3 | 12.5 | 56.43 | 32.4 | 12.5 | 83.17 |
| 29.4 | 12.5 | 94.18 | 33.3 | 12.5 | 69.27 |
| 32.0 | 12.5 | – | 34.5 | 12.5 | – |
| 35.6 | 12.5 | – | 35.8 | 12.5 | – |
| 39.3 | 12.5 | – | 37.1 | 12.5 | – |
| 15.7 | 25 | – | 27.8 | 25 | 56.88 |
| 18.1 | 25 | 276.24 | 28.4 | 25 | 55.93 |
| 20.3 | 25 | 141.51 | 29.5 | 25 | 65.10 |
| 22.5 | 25 | 86.63 | 30.4 | 25 | 54.29 |
| 25.2 | 25 | 57.99 | 31.4 | 25 | 59.82 |
| 27.3 | 25 | 51.67 | 32.4 | 25 | 67.18 |
| 29.4 | 25 | 63.28 | 33.3 | 25 | 80.11 |
| 32.0 | 25 | 77.97 | 34.5 | 25 | – |
| 35.6 | 25 | – | 35.8 | 25 | – |
| 39.3 | 25 | – | 37.1 | 25 | – |
| 15.7 | 50 | – | 27.8 | 50 | 63.19 |
| 18.1 | 50 | 279.83 | 28.4 | 50 | 52.48 |
| 20.3 | 50 | 200.39 | 29.5 | 50 | 44.95 |
| 22.5 | 50 | 104.56 | 30.4 | 50 | 46.76 |
| 25.2 | 50 | 81.87 | 31.4 | 50 | 51.40 |

| | | | | | |
|---|---|---|---|---|---|
| 27.3 | 50 | 56.04 | 32.4 | 50 | 46.83 |
| 29.4 | 50 | 53.02 | 33.3 | 50 | 53.41 |
| 32.0 | 50 | 45.98 | 34.5 | 50 | 46.43 |
| 35.6 | 50 | 84.18 | 35.8 | 50 | – |
| 39.3 | 50 | – | 37.1 | 50 | – |
| 15.7 | 100 | – | 27.8 | 100 | 58.26 |
| 18.1 | 100 | 224.71 | 28.4 | 100 | 61.82 |
| 20.3 | 100 | 167.61 | 29.5 | 100 | 49.61 |
| 22.5 | 100 | 106.94 | 30.4 | 100 | 55.21 |
| 25.2 | 100 | 70.12 | 31.4 | 100 | 47.86 |
| 27.3 | 100 | 76.72 | 32.4 | 100 | 41.46 |
| 29.4 | 100 | 66.49 | 33.3 | 100 | 47.93 |
| 32.0 | 100 | 41.52 | 34.5 | 100 | 53.05 |
| 35.6 | 100 | 42.07 | 35.8 | 100 | 50.17 |
| 39.3 | 100 | – | 37.1 | 100 | – |
| 15.7 | 200 | – | 27.8 | 200 | 61.55 |
| 18.1 | 200 | 279.83 | 28.4 | 200 | 60.80 |
| 20.3 | 200 | 146.73 | 29.5 | 200 | 52.45 |
| 22.5 | 200 | 120.45 | 30.4 | 200 | 54.19 |
| 25.2 | 200 | 87.88 | 31.4 | 200 | 43.08 |
| 27.3 | 200 | 79.30 | 32.4 | 200 | 53.46 |
| 29.4 | 200 | 83.68 | 33.3 | 200 | 53.61 |
| 32.0 | 200 | 47.52 | 34.5 | 200 | 60.43 |
| 35.6 | 200 | 91.64 | 35.8 | 200 | 75.88 |
| 39.3 | 200 | – | 37.1 | 200 | – |

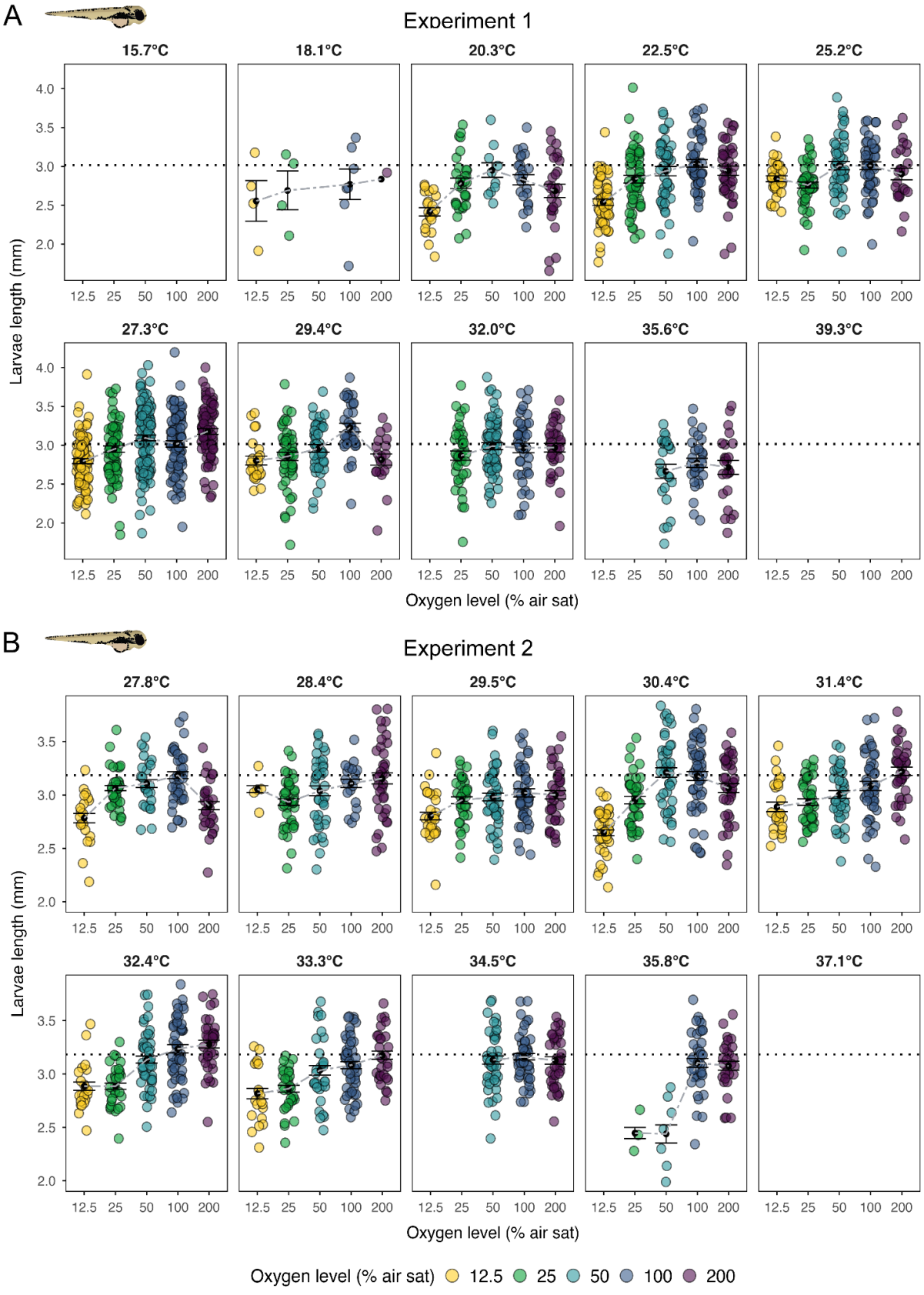


**Fig. S10: Larval length (mm) of zebrafish (*Danio rerio*) across oxygen levels (colors) and temperatures (panel columns) in *Experiment 1*** ($n$ = 2–15; A) **and *Experiment 2*** ($n$ = 2–24; B). Points show individual larvae (jittered); black circles and dashed lines indicate group

means ± S.E. Dotted lines mark control means (*Exp 1*: 27.3 °C, normoxia = 3.0 mm; *Exp 2*: 27.8 °C, normoxia = 3.2 mm).

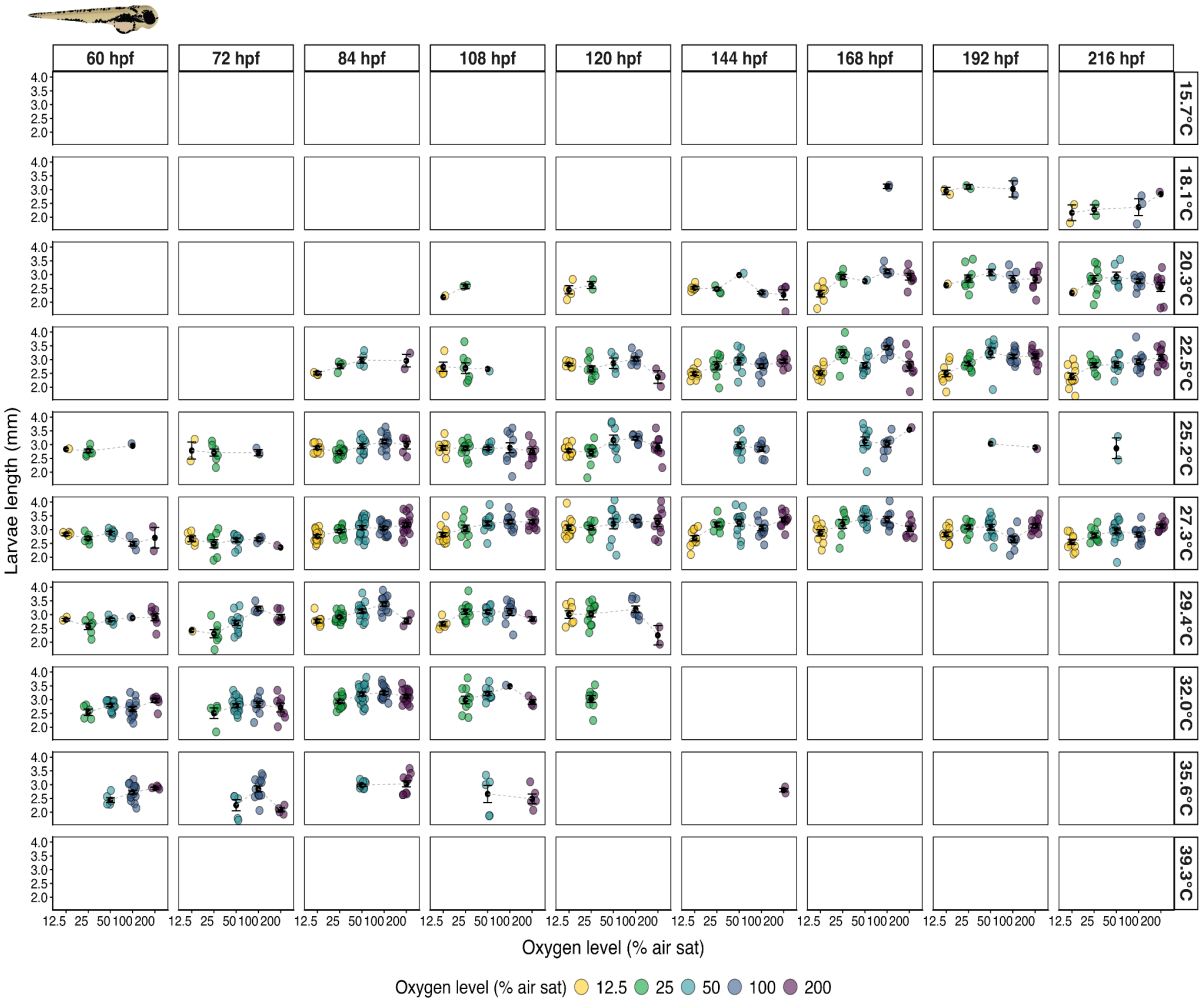


**Figure S11 | Larval length of zebrafish across temperature, oxygen, and developmental time in *Experiment 1*.** Larval length (mm) of *Danio rerio* across oxygen levels (colors) and temperatures (vertical panels) over time post-fertilization (60, 72, 84, 120, 144, 168, 192, and 216 hpf; horizontal panels). Points represent individual larvae; black circles indicate group means ± s.e., and grey dashed lines connect mean values across developmental time.

**Table S9: Larval length of zebrafish across temperature, oxygen, and developmental time in *Experiment 1*.** Anova (type III) and linear model (LM) includes oxygen level, temperature centered to 15.7 °C ($Temp_1$), and its quadratic term ($Temp_2$), their interaction, and developmental time (hpf): Length ~ $O_2$ + $Temp_1$ + $Temp_2$ + $O_2$×$Temp_1$ + $O_2$×$Temp_2$ + hpf. Significance was assessed relative to 15.7 °C and 100% air saturation. Estimates (β), standard errors (SE), t-values, and p-values are shown for each predictor.

| *Parameters (Anova, type III)* | *Sum Squares* | *Df* | *F-value* | *p-value* |
|---|---|---|---|---|
| (Intercept) | 96.714 | 1 | 747.933 | < 0.0001 *** |
| $O_2$ | 1.662 | 4 | 3.212 | 0.0123* |

| | | | | |
|---|---|---|---|---|
| $Temp_1$ | 3.114 | 1 | 24.081 | < 0.0001 *** |
| $Temp_2$ | 3.004 | 1 | 23.232 | < 0.0001 *** |
| $O_2$:$Temp_1$ | 1.129 | 4 | 2.183 | 0.0687 |
| $O_2$:$Temp_2$ | 1.047 | 4 | 2.025 | 0.0886 |
| hpf | 1.888 | 1 | 14.601 | 0.00014*** |
| Residuals | 185.041 | 1431 | | |

| ***Parameters (LM)*** | ***Estimate (B)*** | ***Std. Error*** | ***t-value*** | ***p-value*** |
|---|---|---|---|---|
| (Intercept) | 2.533 | 0.093 | 27.348 | < 0.0001 *** |
| $O_2$(12.5%) | -0.423 | 0.139 | -3.042 | 0.0024** |
| $O_2$(25%) | -0.023 | 0.116 | -0.203 | 0.8394 |
| $O_2$(50%) | -0.074 | 0.135 | -0.550 | 0.5822 |
| $O_2$(200%) | -0.250 | 0.117 | -2.140 | 0.0325* |
| $Temp_1$ | 0.085 | 0.017 | 4.907 | < 0.0001 *** |
| $Temp_2$ | -0.004 | 0.001 | -4.820 | < 0.0001 *** |
| hpf | 0.001 | 0.000 | 3.821 | 0.0001*** |
| $O_2$(12.5%):$Temp_1$ | 0.007 | 0.043 | 0.161 | 0.8717 |
| $O_2$(25%):$Temp_1$ | -0.037 | 0.029 | -1.273 | 0.2032 |
| $O_2$(50%):$Temp_1$ | 0.017 | 0.028 | 0.605 | 0.5452 |
| $O_2$(200%):$Temp_1$ | 0.052 | 0.026 | 1.963 | 0.0498* |
| $O_2$(12.5%):$Temp_2$ | 0.001 | 0.003 | 0.305 | 0.7603 |
| $O_2$(25%):$Temp_2$ | 0.002 | 0.002 | 1.273 | 0.2033 |
| $O_2$(50%):$Temp_2$ | -0.001 | 0.001 | -0.771 | 0.4406 |
| $O_2$(200%):$Temp_2$ | -0.003 | 0.001 | -1.889 | 0.0591 |

| | |
|---|---|
| Residual standard error: | 0.3596 on 1431 degrees of freedom |
| Multiple R-squared: | 0.154 |
| F-statistic: | 17.37 on 15 and 1431 DF, p-value: < 0.0001 *** |
| Adjusted R-squared: | 0.1452 |

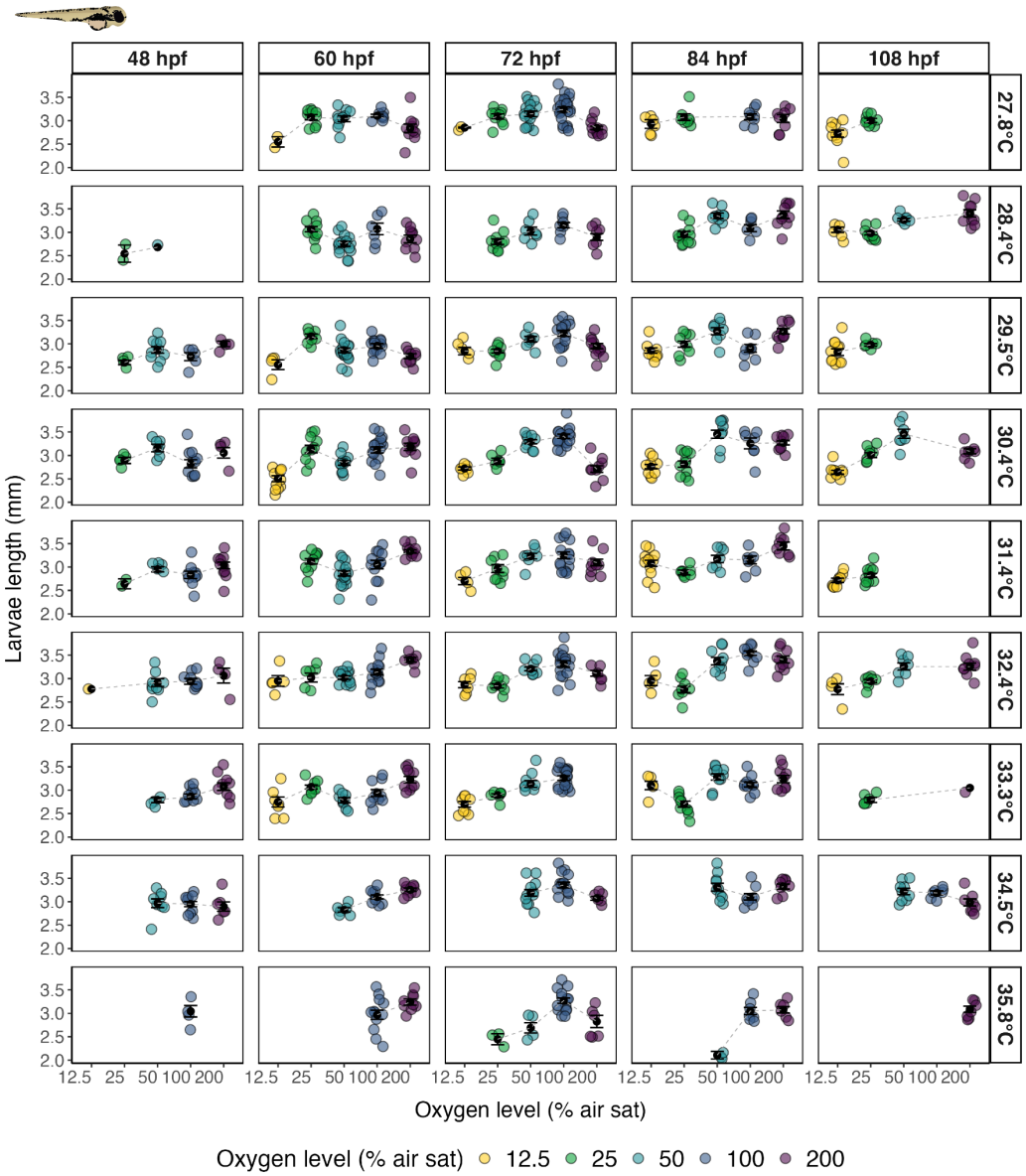


**Fig. S12: Larval length of zebrafish across temperature, oxygen, and developmental time in *Experiment 2.*** Larval length (mm) of *Danio rerio* across oxygen levels (colors) and temperatures (vertical panels) over time post-fertilization (48, 60, 72, 84, and 108 hpf; horizontal panels). Points represent individual larvae; black circles indicate group means ± s.e., and grey dashed lines connect mean values across developmental time.

**Table S10: Larval length of zebrafish across temperature, oxygen, and developmental time in *Experiment 2.*** Anova (type III) and linear model (LM) includes oxygen level, temperature centered to 27.8 °C ($Temp_1$), and its quadratic term ($Temp_2$), their interaction, and developmental time (hpf): Length ~ $O_2$ + $Temp_1$ + $Temp_2$ + $O_2$×$Temp_1$ + $O_2$×$Temp_2$ + hpf. Significance was assessed relative to 27.8 °C and 100% air saturation. Estimates (β), standard errors (SE), t-values, and p-values are shown for each predictor.

| *Parameters (Anova, type III)* | *Sum Squares* | *Df* | *F-value* | *p-value* |
|---|---|---|---|---|
| (Intercept) | 262.798 | 1 | 3991.617 | < 0.0001 *** |

| | | | | |
|---|---|---|---|---|
| $O_2$ | 3.127 | 4 | 11.874 | < 0.0001 *** |
| $Temp_1$ | 0.039 | 1 | 0.597 | 0.4398 |
| $Temp_2$ | 0.037 | 1 | 0.556 | 0.4560 |
| $O_2$:$Temp_1$ | 1.466 | 4 | 5.566 | 0.0002*** |
| $O_2$:$Temp_2$ | 1.258 | 4 | 4.776 | 0.0008*** |
| hpf | 7.414 | 1 | 112.605 | < 0.0001 *** |
| Residuals | 92.699 | 1408 | | |

| ***Parameters (LM)*** | ***Estimate (B)*** | ***Std. Error*** | ***t-value*** | ***p-value*** |
|---|---|---|---|---|
| (Intercept) | 2.809 | 0.044 | 63.179 | < 0.0001 *** |
| $O_2$(12.5%) | -0.410 | 0.062 | -6.577 | < 0.0001 *** |
| $O_2$(25%) | -0.166 | 0.046 | -3.586 | 0.0003*** |
| $O_2$(50%) | -0.112 | 0.048 | -2.332 | 0.0198* |
| $O_2$(200%) | -0.193 | 0.047 | -4.077 | < 0.0001 *** |
| $Temp_1$ | 0.014 | 0.019 | 0.773 | 0.4398 |
| $Temp_2$ | -0.002 | 0.002 | -0.746 | 0.4560 |
| hpf | 0.004 | 0.000 | 10.612 | < 0.0001 *** |
| $O_2$(12.5%):$Temp_1$ | -0.051 | 0.044 | -1.148 | 0.2512 |
| $O_2$(25%):$Temp_1$ | -0.013 | 0.031 | -0.429 | 0.6682 |
| $O_2$(50%):$Temp_1$ | 0.056 | 0.029 | 1.916 | 0.0555 |
| $O_2$(200%):$Temp_1$ | 0.099 | 0.028 | 3.579 | 0.0004*** |
| $O_2$(12.5%):$Temp_2$ | 0.013 | 0.007 | 1.835 | 0.0667 |
| $O_2$(25%):$Temp_2$ | -0.004 | 0.005 | -0.857 | 0.3917 |
| $O_2$(50%):$Temp_2$ | -0.009 | 0.004 | -2.460 | 0.0139* |
| $O_2$(200%):$Temp_2$ | -0.011 | 0.003 | -3.154 | 0.0016** |
| Residual standard error: | 0.2566 on 1408 degrees of freedom | | | |
| Multiple R-squared: | 0.2336 | | | |
| F-statistic: | 28.61 on 15 and 1408 DF, p-value: < 0.0001 *** | | | |
| Adjusted R-squared: | 0.2255 | | | |

**Table S11: Survival to first feeding of zebrafish across temperature, oxygen, and developmental time in *Experiment 1 and 2.*** GLM-TMB includes oxygen level, temperature

centered to 18.1 °C ($Temp_1$, lowest temperature at which hatching occurred), and its quadratic term ($Temp_2$), their interaction, log-transformed developmental time (hpf), and Experiment as random effect: Survival to First Feeding ~ $O_2$ + $Temp_1$ + $Temp_2$ + $O_2$×$Temp_1$ + $O_2$×$Temp_2$ + log(hpf) + (1 | Experiment). Significance is shown first with oxygen as numeric relative to 18.1 °C and subsequently relative to 100% air saturation. Estimates (β), standard errors (SE), z-values, and p-values are shown for each predictor.

| ***Parameters (GLM-TMB)*** | ***Estimate (B)*** | ***Std. Error*** | ***z-value*** | ***p-value*** |
|---|---|---|---|---|
| (Intercept) | -26.1100 | 0.5732 | -45.550 | < 0.0001 *** |
| $O_2$ | -0.0114 | 0.0022 | -5.180 | < 0.0001 *** |
| $Temp_1$ | 0.9085 | 0.0391 | 23.230 | < 0.0001 *** |
| $Temp_2$ | -0.0433 | 0.0019 | -22.370 | < 0.0001 *** |
| $O_2$:$Temp_1$ | 0.0028 | 0.0004 | 7.020 | < 0.0001 *** |
| $O_2$:$Temp_2$ | -0.0001 | 0.0000 | -5.500 | < 0.0001 *** |
| log(hpf) | 4.6060 | 0.0990 | 46.510 | < 0.0001 *** |
| Experiment (Intercept): | 0.0598 (Var.) | 0.2446 (St. Dev.) | | |
| Number of obs: | 973 | groups: | Experiment 1, 2 | |
| AIC: | 6960.7 | logLik: | -3472.3 | |

| ***Parameters (GLM-TMB)*** | ***Estimate (B)*** | ***Std. Error*** | ***z-value*** | ***p-value*** |
|---|---|---|---|---|
| (Intercept) | -38.6488 | 0.8895 | -43.450 | < 0.0001 *** |
| $O_2$(12.5%) | -0.1135 | 0.4524 | -0.250 | 0.8019 |
| $O_2$(25%) | -1.8757 | 0.4954 | -3.790 | 0.0001 *** |
| $O_2$(50%) | -4.9287 | 0.6807 | -7.240 | < 0.0001 *** |
| $O_2$(200%) | -2.4898 | 0.5497 | -4.530 | < 0.0001 *** |
| $Temp_1$ | 1.2624 | 0.0596 | 21.170 | < 0.0001 *** |
| $Temp_2$ | -0.0442 | 0.0026 | -17.000 | < 0.0001 *** |
| $O_2$(12.5%):$Temp_1$ | 0.1149 | 0.1301 | 0.880 | 0.3775 |
| $O_2$(25%):$Temp_1$ | 1.1954 | 0.1088 | 10.980 | < 0.0001 *** |
| $O_2$(50%):$Temp_1$ | 1.1965 | 0.1247 | 9.590 | < 0.0001 *** |
| $O_2$(200%):$Temp_1$ | 0.6411 | 0.0995 | 6.440 | < 0.0001 *** |
| $O_2$(12.5%):$Temp_2$ | -0.0483 | 0.0091 | -5.290 | < 0.0001 *** |
| $O_2$(25%):$Temp_2$ | -0.0919 | 0.0058 | -15.870 | < 0.0001 *** |
| $O_2$(50%):$Temp_2$ | -0.0608 | 0.0055 | -10.990 | < 0.0001 *** |

| | | | | |
|---|---|---|---|---|
| $O_2$(200%):$Temp_2$ | -0.0394 | 0.0044 | -9.000 | < 0.0001 *** |
| log(hpf) | 6.8743 | 0.1509 | 45.560 | < 0.0001 *** |
| Experiment (Intercept): | 0.08689 (Var.) | 0.2948 (St. Dev.) | | |
| Number of obs: | 973 | groups: | Experiment 1, 2 | |
| AIC: | 4348.5 | logLik: | -2157.2 | |

**Table S12: Larval count and survival of zebrafish transferred to control conditions.** Number of larvae transferred to control conditions (28.0 ± 0.5 °C, normoxia) from *Experiment 1* and (27.0 ± 0.5 °C, normoxia) from *Experiment 2.* Hatched larvae that died during treatment exposure are indicated by †. Zero values denote treatments in which no hatching occurred.

| | | **Oxygen level (% air sat)** | | | | |
|---|---|---|---|---|---|---|
| | **Temperature (°C)** | **12.5** | **25** | **50** | **100** | **200** |
| **Experiment 1** | **20.3** | 8 | 10 | 4 | 9 | 9 |
| | **25.2** | 10 | 11 | 10 | 10 | 10 |
| | **29.4** | 2 | 11 | 9 | 10 | 8 |
| | **32** | 0 | 9 | 10 | 9 | 7 |
| | **35.6** | 0 | 0 | † | 13 | 1 |
| **Experiment 2** | **27.8** | † | 21 | 16 | 21 | 22 |
| | **29.5** | † | 13 | 20 | 20 | 22 |
| | **31.4** | † | 12 | 17 | 19 | 21 |
| | **33.3** | † | 3 | 15 | 19 | 19 |
| | **35.8** | 0 | 0 | † | 11 | 2 |

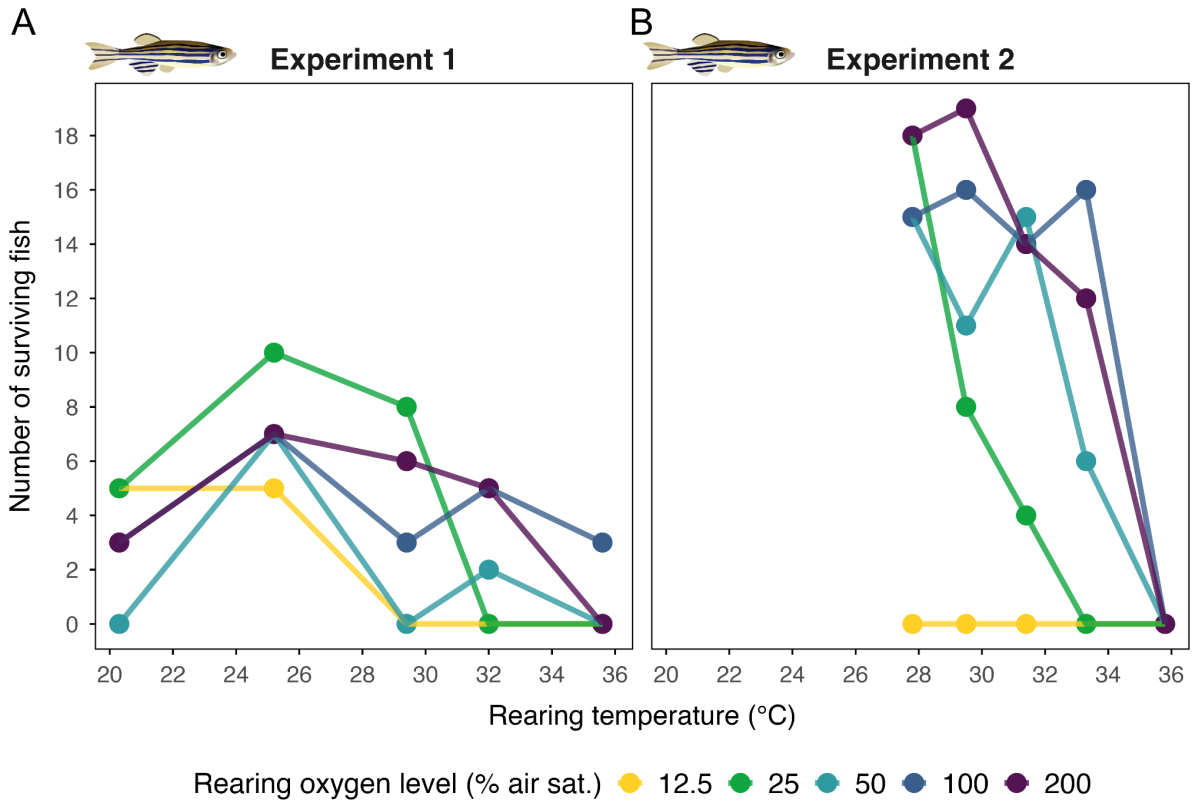


**Fig. S13: Survival number of zebrafish reared across temperature and oxygen treatments.** (A) Numbers represent individuals that survived to the juvenile stage after being transferred to control conditions from *Experiment 1* (normoxia; 28 °C) for $CT_{max}$ testing during the juvenile stage. (B) equivalent data for *Experiment 2* (normoxia; 27 °C).

**Table S13: Critical thermal maximum ($CT_{max}$) of zebrafish across rearing temperature and oxygen treatments in *Experiment 1.*** Anova (type III) and linear model (LM) includes oxygen level ($O_2$), temperature (Temp), and their interaction: $CT_{max} \sim O_2 \times Temp$. Significance was assessed relative to 20.3 °C and 100% air saturation. Estimates (β), standard errors (SE), t-values, and p-values are shown for each predictor.

| Parameters (Anova, type III) | Sum Squares | Df | F-value | P-value |
|---|---|---|---|---|
| $O_2$ | 0.747 | 4 | 1.717 | 0.1566 |
| Temp | 1.863 | 4 | 4.283 | 0.0038** |
| $O_2$:Temp | 0.849 | 7 | 1.115 | 0.3644 |
| Residuals | 7.288 | 67 | | |
| **Parameters (LM)** | **Estimate (B)** | **Std. Error** | **t value** | **P-value** |
| (Intercept) | 41.669 | 0.113 | 370.304 | < 0.0001*** |
| $O_2$(12.5%) | -0.304 | 0.138 | -2.197 | 0.0311* |
| $O_2$(25%) | -0.022 | 0.109 | -0.198 | 0.8439 |

| | | | | |
|---|---|---|---|---|
| $O_2$(50%) | -0.165 | 0.144 | -1.147 | 0.2551 |
| $O_2$(200%) | -0.134 | 0.107 | -1.248 | 0.2160 |
| Temp(20.5°C) | -0.324 | 0.122 | -2.656 | 0.00967** |
| Temp(25.5°C) | -0.107 | 0.103 | -1.042 | 0.3009 |
| Temp(32°C) | 0.156 | 0.133 | 1.170 | 0.2457 |
| Temp(36°C) | -0.436 | 0.222 | -1.962 | 0.05347· |
| Residual standard error: | 0.3316 on 74 degrees of freedom | | | |
| Multiple R-squared: | 0.2794 | | | |
| F-statistic: | 3.587 on 8 and 74 DF, p-value: 0.00144 | | | |
| Adjusted R-squared: | 0.2015 | | | |

**Table S14: Critical thermal maximum ($CT_{max}$) of zebrafish across rearing temperature and oxygen treatments in *Experiment 2.*** Anova (type III) and linear model (LM) includes oxygen level ($O_2$), temperature (Temp), and their interaction: $CT_{max} \sim O_2 \times Temp$. Significance was assessed relative to 27.8 °C and 100% air saturation. Estimates (β), standard errors (SE), t-values, and p-values are shown for each predictor.

| **Parameters (Anova, type III)** | **Sum Squares** | **Df** | **F-value** | **P-value** |
|---|---|---|---|---|
| $O_2$ | 4.265 | 3 | 10.797 | < 0.0001*** |
| Temp | 1.588 | 3 | 4.020 | 0.0084** |
| $O_2$:Temp | 2.049 | 8 | 1.945 | 0.0559· |
| Residuals | 24.097 | 183 | | |
| **Parameters (LM)** | **Estimate (B)** | **Std. Error** | **t value** | **P-value** |
| (Intercept) | 40.808 | 0.065 | 629.348 | < 0.0001*** |
| $O_2$(25%) | -0.483 | 0.088 | -5.490 | < 0.0001*** |
| $O_2$(50%) | -0.158 | 0.073 | -2.163 | 0.0318* |
| $O_2$(200%) | -0.094 | 0.067 | -1.407 | 0.1611 |
| Temp(29.5°C) | 0.115 | 0.069 | 1.657 | 0.0991 |
| Temp(31.4°C) | 0.025 | 0.072 | 0.340 | 0.7344 |
| Temp(33.3°C) | -0.164 | 0.082 | -2.014 | 0.0455* |
| Residual standard error: | 0.37 on 191 degrees of freedom | | | |
| Multiple R-squared: | 0.1714 | | | |

| | |
|---|---|
| F-statistic: | 6.583 on 6 and 191 DF, p-value: < 0.0001*** |
| Adjusted R-squared: | 0.1453 |

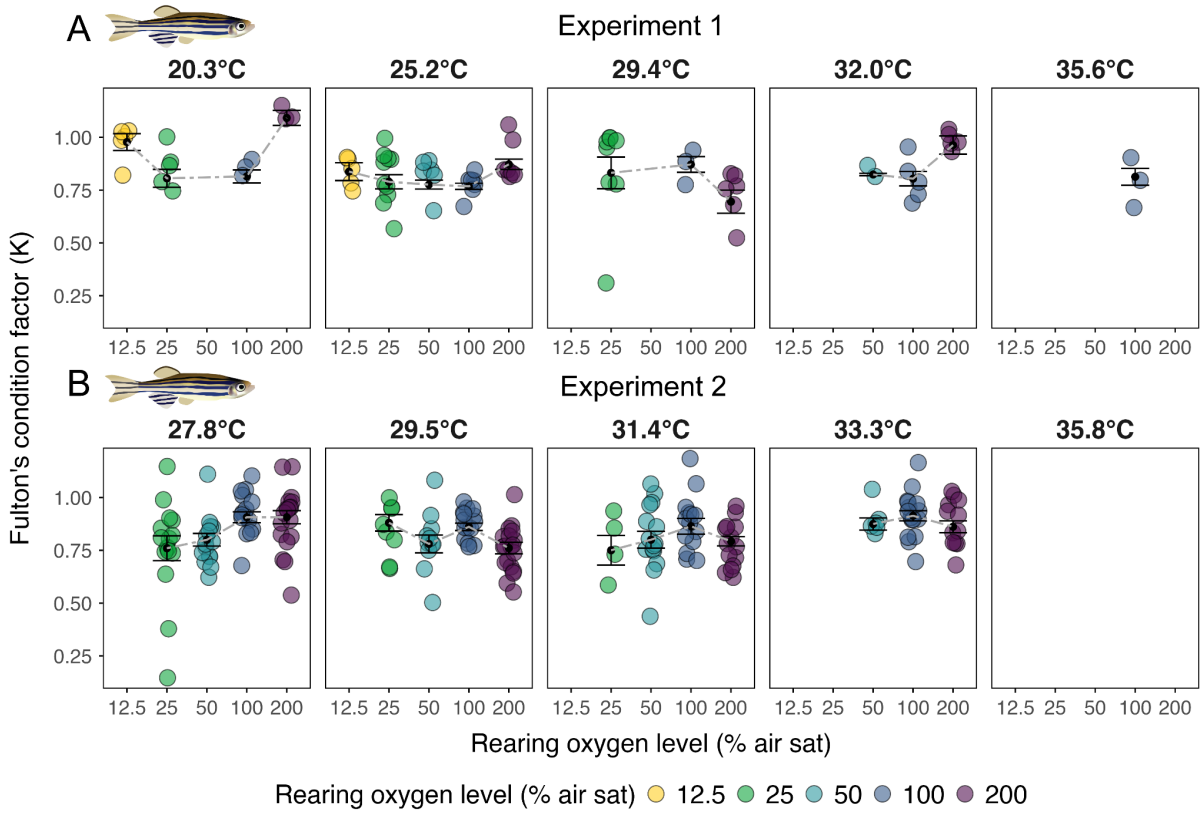


**Fig. S14: Fulton's condition factor (K) of juvenile zebrafish across rearing temperature and oxygen treatments.** Fulton's condition factor (K) of juvenile *Danio rerio* reared during the embryonic stage under five oxygen levels (colors) and temperature treatments (horizontal panels) in (A) *Experiment 1* and (B) *Experiment 2.* Points represent individual fish (jittered), with color indicating oxygen treatment.

**Table S15: Fulton's condition factor (K) of juvenile zebrafish across rearing temperature and oxygen treatments for *Experiment 1* and *2*.** Anova (type III) includes oxygen level ($O_2$) and temperature (Temp): K ~ $O_2$ + Temp. P-values are shown for each predictor.

**Experiment 1**

| *Parameters (Anova, type III)* | *Sum Squares* | *Df* | *F-value* | *P-value* |
|---|---|---|---|---|
| (Intercept) | 1.818 | 1 | 113.872 | < 0.0001*** |
| $O_2$ | 0.034 | 1 | 2.111 | 0.1502 |
| Temp | 0.052 | 1 | 3.237 | 0.0758 |
| Residuals | 1.277 | 80 | | |

**Experiment 2**

| *Parameters (Anova, type III)* | *Sum Squares* | *Df* | *F-value* | *P-value* |
|---|---|---|---|---|
| (Intercept) | 0.417 | 1 | 22.120 | < 0.0001*** |
| $O_2$ | 0.012 | 1 | 0.635 | 0.4264 |
| Temp | 0.014 | 1 | 0.753 | 0.3868 |
| Residuals | 3.672 | 195 | | |

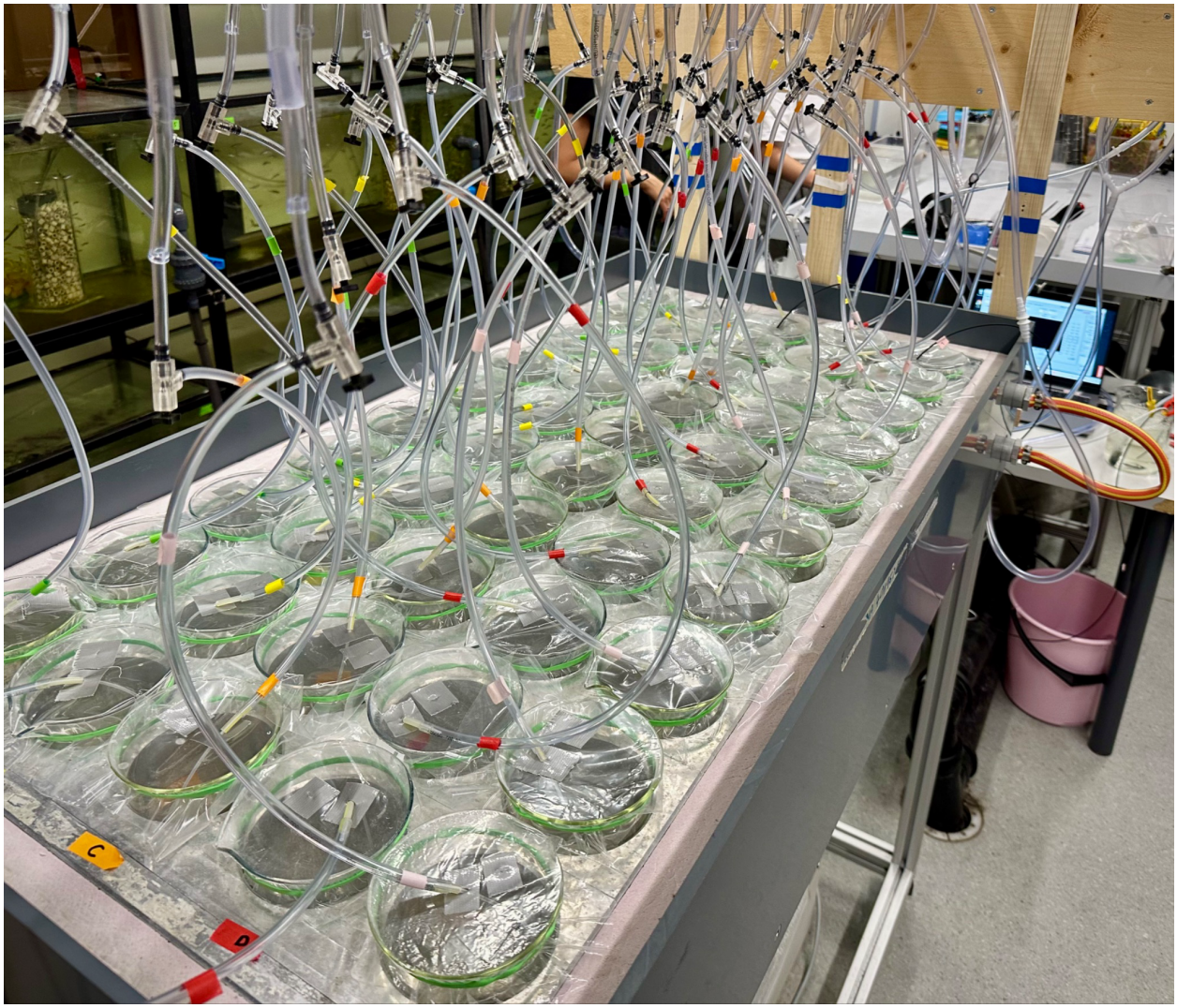


**Fig. S15: Gradient table set up depicting a factorial design of 10 temperatures and 5 oxygen levels yielding 50 unique temperature-oxygen combinations.** Color coded tubes supplied with air to each beaker, where different color lines represent an oxygen level. Beakers were hermetically covered to keep a constant $DO_2$ concentration per treatment. The mixed gas was delivered to the respective column of 10 beakers via a 10 mm diameter outflow tube. Each beaker was aerated through an individual air tube tightly inserted through the lid and fitted with an air valve, providing gentle bubbling.


**Fig. S16: Beaker (800 mL) filled with 300 mL treated fresh water held the embryos and early larvae during the rearing period.** The aluminium wells holding a beaker had a 17 cm depth.

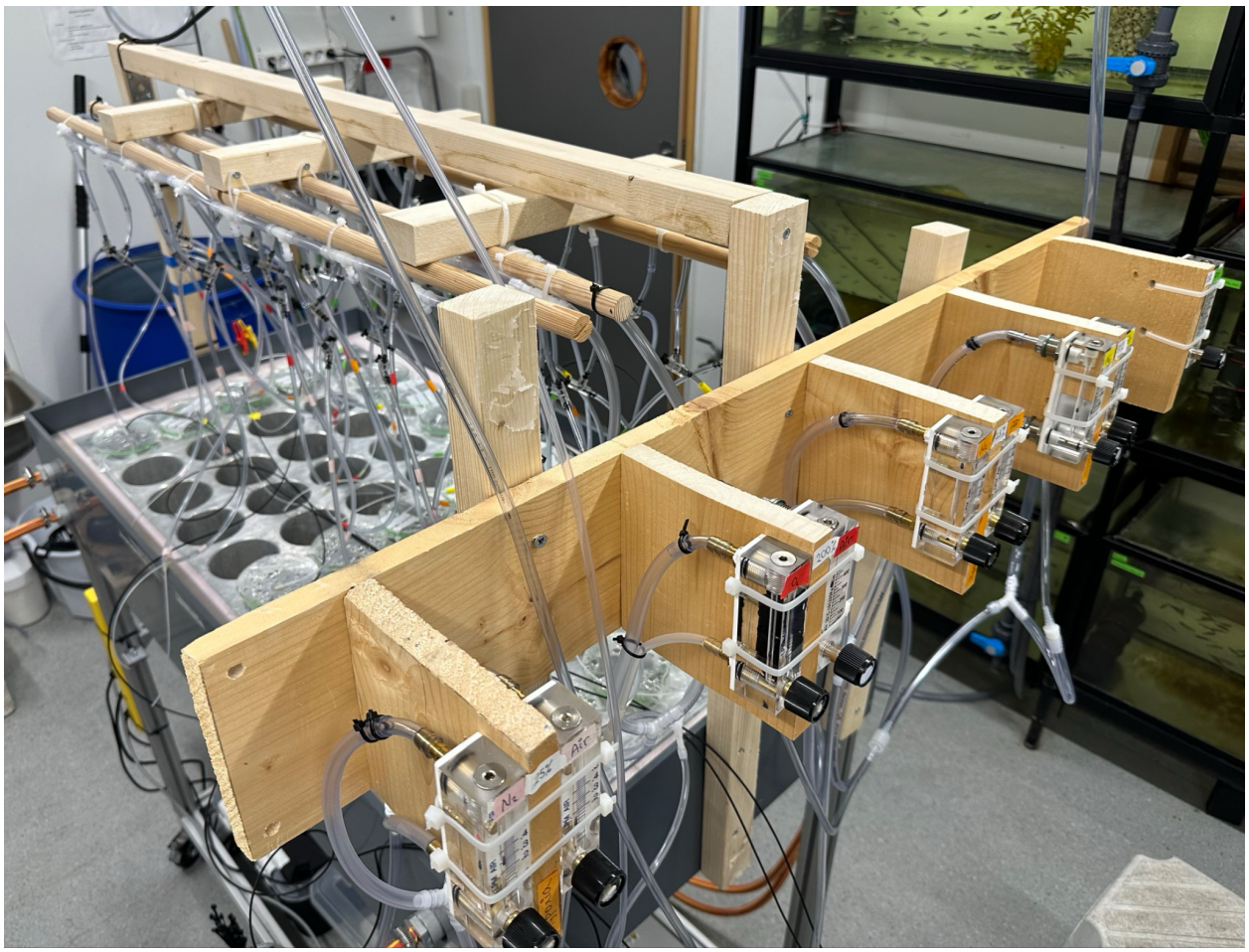

**Fig. S17: Set up with five gas mixing stations per oxygen level (12.5, 25, 50, 100, and 200% air saturation).** Oxygen concentrations were regulated using manual gas flow controllers (RS Pro, 500 ml/min) that mixed air with either nitrogen (50 L, 99.6% N2) or oxygen (50 L, 99.5% O2, Linde Co.) to create hypoxia or hyperoxia, respectively. Normoxia was achieved using ambient air. The two flow controllers supplied with the target oxygen concentration via a 10 mm diameter outflow tube to each column (10 beakers) of the thermal gradient table Each beaker was aerated through an individual air tube tightly inserted through the lid and fitted with an air valve, providing gentle bubbling.

**Fig. S18: Aluminium gradient table connected to a cool and warm temperature water bath at each extreme.** The system consisted of a cooler (Titan 200, Aqua Medic, Germany) and a heater circulator (Grant Instruments, GD100), each with a built-in thermostats that maintain their respective water baths at a set temperature. Water from each bath was pumped to the respective edges of the table using an Eheim Universal 1000 pump (Germany), generating a consistent thermal gradient, from cold to warm, across the aluminium slab of the table.