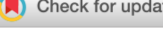

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RESEARCH ARTICLE

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Gastrulation and hatch as critical thermal windows for salmonid embryo development

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Abstract

Climate change and impoundment increase river temperatures, shifting the bioclimatic envelope in which freshwater biota have evolved and increasing salmonid egg mortality. To mitigate this, conservation flows from reservoirs are often implemented to maintain favourable water temperatures downstream from impoundments throughout salmonid embryo development. However, as water to maintain conservation flows becomes scarcer, there is a need to understand the requirements of salmonid embryos and balance these with anthropogenic demands. This study combines a laboratory-based and a modelling approach to test the effect of different temperatures on the survival from fertilisation to hatch of a model salmonid species. Further, the effect of dropping temperatures from high to optimal conditions at hatch—a perceived period of greater sensitivity to high temperatures—is tested. The study shows embryo mortality increases with temperature and is greatest during gastrulation and hatch. Also, embryos that experienced high temperatures during gastrulation had high mortality rates at hatch, even when hatch conditions were optimal. This indicates sublethal developmental abnormalities caused by high temperatures during gastrulation increase mortality at hatch. Therefore, to maintain high rates of salmonid embryo survival, cold water resources from reservoirs ideally will target both gastrulation and hatch developmental stages.

KEYWORDS

critical window, dams, egg, gastrulation, hatch, salmonid, temperature

1 | INTRODUCTION

A warming climate and a growing global population increase the likelihood of conflict in the allocation of freshwater resources between human and ecosystem needs (Falkenmark & Wang-Erlandsson, 2021). To address concerns associated with human demand, 58,500 reservoirs worldwide impound an equivalent of 17% of the annual global river flow to support 12–16% of global annual food production and 24% of electricity demand (Mulligan, van Soesbergen, & Sáenz, 2020). As fears associated with water and energy security increase, so will reservoir construction (Zarfl, Lumsdon, Berlekamp, Tydecks, & Tockner, 2015). However, this growing anthropogenic impact has negative consequences for the freshwater environments and human and socio-ecological communities that depend upon them (Falkenmark, Wang-Erlandsson, & Rockström, 2019).

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Ecological communities within river systems evolve in response to the specific environmental conditions they face (Araújo & Peterson, 2012). However, dam construction can shift this bioclimatic envelope by raising downstream water temperatures (Daniels & Danner, 2020). Cold-blooded organisms like salmonid fishes are particularly vulnerable to such changes because their physiological processes are strongly affected by temperature (McCullough, 1999). In particular, their metabolic rate, and therefore oxygen demand, increases with temperature (Hamor & Garside, 1976). While juvenile and adult salmonids can respond to such changes by seeking thermal refugia (Breau, Cunjak, & Peake, 2011), embryos contained within an egg membrane cannot. Further, their underdeveloped respiratory system (Rombough, 1988), an egg membrane that impedes oxygen diffusion (Bloomer, Sear, & Kemp, 2019), and immobility mean they cannot modify oxygen uptake in response to increasing demand. Therefore, the embryo is likely the stage of salmonid development most vulnerable to mortality caused by elevated temperatures (Dahlke, Wohlrab, Butzin, & Pörtner, 2020).

While embryos are particularly vulnerable to mortality caused by high temperatures, their responses to thermal perturbations changes substantially from fertilisation to hatch (Anderson, Beer, Israel, & Greene, 2022; Dahlke et al., 2020; Del Rio et al., 2021; Martin et al., 2020). Salmonid embryo development begins with rapid cellular cleavage (C) in which the newly fertilised egg, through multiple divisions, forms two layers of cells that cover the interior membrane of the egg. This is followed by gastrulation (G), where the two layers of cells migrate to form the longitudinal axis of the fish along which differentiated cell types begin to form the organs during organogenesis (O). The final stage is designated hatch (H) (Danner, 2008). While C and O appear to be less affected by thermal conditions (Koss, 1994; Mueller et al., 2015), elevated temperatures in the G stage can affect gene expression and increase the frequency of mutations (Solnica-Krezel et al., 1996). This can cause immediate mortality (Uchida, Uesaka, Yamamoto, Takeda, & Irie, 2018), carry-over effects that increase mortality during hatching (Irvine, 2020) and longer-term impacts on muscle development in juveniles and adults (Macqueen et al., 2008). Mortality due to thermal stress during H is also frequently observed but usually associated with higher oxygen demand that cannot be met by supply, resulting in anoxia (Del Rio, Davis, Fangue, & Todgham, 2019; Greig, Sear, & Carling, 2007; Martin et al., 2020; Rombough, 1988; D. A. Sear et al., 2017; Wood, Clark, Elliott, Frappell, & Andrewartha, 2019). The oxygen supply requirements of hatched embryos are lower than the embryo stage due to the absence of the egg membrane and the switch to more efficient branchial respiration (Rombough, 1988). Therefore, embryo sensitivity to high temperatures could be limited to just G and H.

To mitigate the impact of temperature elevation caused by impoundment on embryo survival, cold water resources can be released from reservoirs to sustain favourable downstream thermal conditions. In some cases, this action is demanded by endangered species protection to ensure water temperatures meet the environmental requirements of target species (Anderson et al., 2022). Often, these conservation flows are designed to provide consistent, favourable temperatures throughout embryo development. However, the recognition of two critical thermal stages in embryo development (G and H) presents an opportunity to conserve water while safeguarding protected species by only implementing conservation flows for cold water temperature management during the period when embryos are most sensitive to thermal stress. This is particularly important as water scarcity, driven by a changing climate, demands innovative approaches to water management. However, there is insufficient data to support the theory that optimal temperatures during only these critical windows will maintain similar levels of survival compared with continuous favourable temperatures, and it is unclear how wide the critical window needs to be.

This paper addresses these knowledge gaps using two laboratory experiments supported by statistical analysis of changing mortality rates throughout development. Study A tested the impact of a range of incubation temperatures on the survival of rainbow trout (*Oncorhynchus mykiss*) embryos from fertilisation to hatch. These data supported Study B, in which embryos were exposed to high temperatures through to either the G or O stage, after which temperatures were reduced to favourable conditions prior to the H stage. The aim of the temperature reduction was to mitigate the anticipated high rates of hatch mortality. Statistical analysis of the datasets identified significant and unexpected differences in stage-dependent mortalities under the temperature schedules.

2 | METHODOLOGY

Study A ran from 18th June 2020 to 21st July 2020 and determined the effect of different continuous temperature regimes on the survival of rainbow trout embryos to hatch. Study B ran from 30th July 2020 to 6th September 2020 and tested the hypothesis that pre-hatch temperature reduction during rainbow trout embryo development maintains higher levels of survival than continuous high temperature exposure. While eggs and milt for studies A and B were obtained from the same hatchery, they were taken from different broodstock due to their different timings (see Supplementary Information [Tables S1 and S2]).

3 | EXPERIMENTAL PROTOCOL

Research was conducted in a recirculating hatchery system (Figure 1). Water of dissolved oxygen concentration 8 ± 0.03 mg/L flowed past the eggs at a rate of 0.04 cm/s. DO concentration was representative of conditions found in natural salmonid spawning habitat (e.g., Greig et al., 2007) and the flow rate was determined by the median of all published field data of intra-gravel flow through salmonid redds (e.g., David A Sear, DeVries, & Greig, 2008).

Temperature control was maintained by two independent heating and cooling systems. The cooling system maintained a constant supply of 10.5°C water to exposure treatments from a 570 L main sump. The chilled water was then distributed to four individual test treatment

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FIGURE 1 Systematic diagram of recirculating hatchery system. Arrows indicate direction of water flow. Demonstrated set-up is for Study B, but same methodology was employed for Study A, with one additional treatment [Color figure can be viewed at wileyonlinelibrary.com]

TABLE 1 Summary of treatments used

Study A					
Treatment label	**A1**	**A2**	**A3**	**A4**	**A5**
Temperature (°C)	12.5	14.5	16.5	18.5	20.5
Number of replicates	2	2	2	2	2
Study B					
Treatment label	**B1**	**B2**	**B3**	**B4**	
Temperature (°C)	12.5	17 from fertilisation to 150 *D*; 12.5 from 150 *D* to hatch	17 from fertilisation to 250 *D*; 12.5 from 250 *D* to hatch	17	
Number of replicates	3	3	3	3	

baths and heated to the required temperature for each treatment. Temperature measurements for each treatment were collected and recorded every 15 min and used to calculate the embryonic development state in accumulated degree days (*D*) (Bloomer, Sear, Dutey-Magni, & Kemp, 2016).

For both studies, each replicate contained 200 eggs that were exposed to the temperature treatments shown in Table 1 from fertilisation to hatch. The replicates were combined for the analysis, resulting in 400 eggs for each temperature treatment in study A and 600 eggs for each treatment in study B. Daily checks for the presence of dead embryos or hatched alevin were conducted. Dead embryos were removed with a sterile large-diameter pipette, separated according to treatment and replicate number, and preserved in 10% formalin. Alevin were removed, euthanised and preserved in a similar way.

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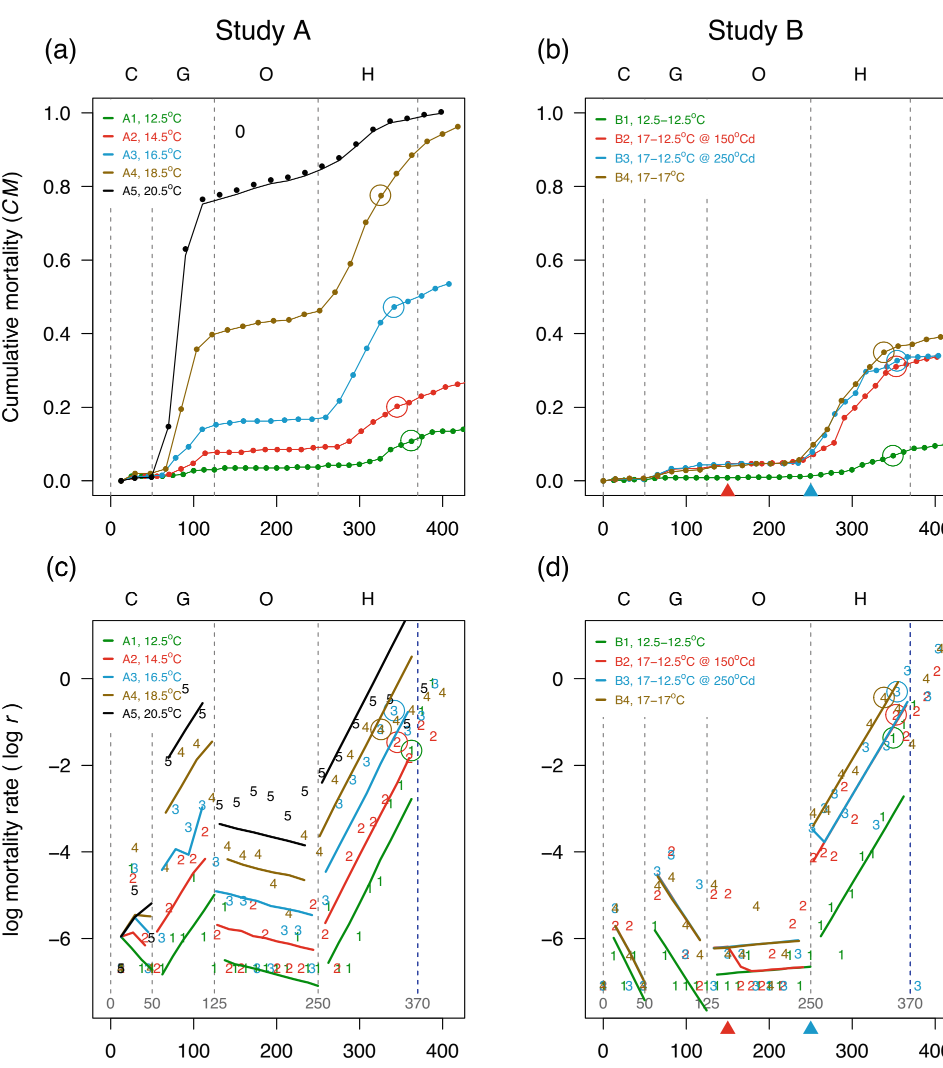

FIGURE 2 Mortality patterns of embryos incubated under constant or time-varying temperatures. (a) Study A cumulative mortality (Equation (1)) (*CM*) versus degree day (*D*) for treatments (A1–A5) using constant temperatures between 12.5 and 20.5°C at 2°C increments. (b) Study B *CM* versus *D* for treatments with varying temperature. Treatments B1 and B4 incubated at constant 12.5 or 17°C, respectively. Treatments B2 and B3 began incubation at 17°C and dropped to 12.5°C at *D* of 150 °Cd (▲) and 250 °Cd (▲) respectively. Dots (●) depict daily *CM* and *D* measurements. (c) Study A log mortality rate (Equation (2)) (log *r*) versus *D* for treatments A1–A5 depicted by numbers 1–5. Lines show fits of log *r* versus *D* (Equation (3)) for each treatment and developmental stage (C, G, O, H). (d) Study B log *r* versus *D*. Lines show log *r* versus *D* fits for treatments B1-B4 and incubation stages (C, G, O, H). Circles (○) denote *D* on last day of hatch and dashed lines depict incubation stage boundaries: cleavage (C), gastrulation (G), organogenesis (O) and hatch (H). [Color figure can be viewed at wileyonlinelibrary.com]

4 | DATA ANALYSIS

The goal of the analysis was to characterise the cumulative mortality, the mortality rate and number hatched over each day i of the study. To track these measures the fate of embryos in each day were partitioned into three groups: alive (a_i), mortality (m_i) and hatched (h_i). The number of embryos beginning day i is then $n_i = a_i + m_i + h_i$. The cumulative daily mortality rate is

$$CM_i = \sum_{j=1}^{i} m_j. \tag{1}$$

The daily mortality rate, also known as the hazard rate (r), for day i is

$$r_i = \frac{dn}{\bar{n}dt} = \frac{m_i}{a_i + m_i/2 + h_i/2} \tag{2}$$

where $\bar{n}$ is the average number of embryos alive on day i that have not hatched.

The cumulative mortality and mortality rate exhibited distinct transitions which correspond to transitions in embryo development. Measured by accumulated degree days, D with units °Cd, these transitions were defined: C to G transition at $D = 50$°Cd, G to O transition at $D = 125$°Cd and O to H transition at $D = 250$ °Cd. The C stage started at $D = 0$ and the H stage end was set at $D = 370$°Cd, which was the D past which no egg hatching occurred.

The relationship of the mortality rate in each stage to temperature and state of development was determined by a linear regression of the natural log of the mortality rate, $\log r_i$, against the temperature and accumulated degree day for each observation day. The equation assumes mortality rates in stages depend on temperature in stages plus allows for carryover effects of G-stage temperature to H-stage mortality, giving

$$\log(r_i) = \begin{cases} a_s + b_s D_i + c_s T_i & \text{if } i \in s = 1,2,3 \quad \text{(a)} \\ a_4 + b_4 D_i + c_4' T_i + c_2' \bar{T}_2 & \text{if } i \in s = 4 \quad \text{(b)} \end{cases} \tag{3}$$

where D_i and T_i are the embryo's accumulated degree days and temperature on day i and s = 1, 2, 3, 4 correspond to C, G, O, H stages.

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TABLE 2 Coefficients for mortality rate model from regression of Equation (3)

Stage	Coef.	Estimate	*SE*	Pr(>|*t*|)	R^2
Study A					
1. Cleavage	a_1	−8.17	1.664	0.00028	0.008
	b_1	−0.020	0.024	0.41455	
	c_1	0.198	0.136	0.16889	
2. Gastrulation	a_2	−16.10	1.426	<0.00001	0.790
	b_2	0.029	0.009	0.00686	
	c_2	0.601	0.070	<0.00001	
3. Organogenesis	a_3	−10.75	0.998	<0.00001	0.659
	b_3	−0.005	0.003	0.15381	
	c_3	0.390	0.047	<0.00001	
4. Hatch	a_4	−23.31	1.555	<0.00001	0.836
	b_4	0.037	0.004	<0.00001	
	c_4	0.555	0.051	<0.00001	
Study B					
1. Cleavage	a_1	−6.644	1.072	0.00010	0.539
	b_1	−0.038	0.010	0.00296	
	c_1	0.091	0.068	0.20944	
2. Gastrulation	a_2	−7.761	1.481	0.00010	0.614
	b_2	−0.030	0.008	0.00302	
	c_2	0.304	0.078	0.00147	
3. Organogenesis	a_3	−8.773	1.336	<0.00001	0.083
	b_3	0.002	0.004	0.67593	
	c_3	0.137	0.062	0.03507	
4. Hatch	a_4	−23.40	1.511	<0.00001	0.829
	b_4	0.032	0.003	<0.00001	
	c'_4	0.191	0.058	0.00200	
	c'_2	0.446	0.053	<0.00001	

Note: Coefficients a_i, are intercepts, b_i expresses the contribution of degree day (D) on the rate with positive (negative) values indicating increasing (decreasing) rate with D, c_i indicate effect of temperature on long mortality rate. Note, temperature increases rate in all stages. Stages designated: cleavage, $i = 1$, gastrulation, $i = 2$, organogenesis, $i = 3$, and hatch, $i = 4$. In study A effect of temperature on mortality rate is partitioned into effect of gastrulation temperature, c'_2, and hatch temperature, c'_2.

The mortality rate in the H stage includes the carryover effects of the average temperature $\overline{T}_2$ experienced in the G stage. Because Study A temperature was constant across all stages then $\overline{T}_2 = T_i$ and Equation (3b) reduces to Equation (3a) where $c_4 = c'_4 + c'_2$.

The model coefficients in Equation (3) were fit using a least-squares linear regression (lm) in the R statistical software (R Core Team, 2018).

5 | RESULTS

Figure 2 illustrates the results of the two studies in terms of patterns of cumulative mortality (*CM*) and log mortality rate (log *r*) versus stage of development depicted by degree day (*D*). Variation among replicates for Studies A and B was very low, so, to improve comprehension, mortality rates for all replicates were combined. To justify this conclusion, we compared the effect of degree day on the hatch stage mortality rate calculated for individual replicates of treatment 3 in Study B to b_4 (Table 2), which is derived from all treatments and replicates of Study B. The mean and *SD* from the individual replicates gives $b_4 = 0.031 \pm 0.005$, which is within the *SE* in Table 2, $b_4 = 0.032 \pm 0.003$.

In Study A, treatments were run at constant temperatures in 2°C increments, while in Study B, in two treatments temperature was held constant (B1 @ 12.5°C and B4 @ 17°C) and in two treatments temperatures dropped (17 to 12.5°C) at the beginning or end of the O stage (Table 1). At the stage boundaries, the slopes of the *CM* versus *D* curves changed (vertical lines in Figure 2a, b). All Study A treatments exhibited large increases in *CM* in the G and H stages and small increases in the C and O stages. Also, the increases were proportional to treatment temperature. In the corresponding Study B treatments, *CM* increases were much smaller in the C, G and O stages. However, in the H stage, the constant temperature treatments exhibited *CM* increases similar to their Study A counterparts, that is, A1 versus B1

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and A3 versus B4. Unexpectedly, in treatments B2 and B3, in which temperature dropped from high to low prior to entering the H stage, the *CM* increase followed the high-temperature B4 pattern, not the low-temperature B1 pattern corresponding to the stage temperature. This disconnect between the *CM* increase and stage temperature in Study B suggests that stage mortality depends on other factors. We explored these factors in terms of a temperature carryover effect.

Figure 2c, d depicts how the mortality rate changes over stage and temperature in terms of the regression of log r versus D and T (Equation (3)). As with the *CM* curves, the H stage exhibits the highest mortality rate, increasing with both T and D. Notably, under constant temperatures, 12.5°C for treatments A1 and B1 and 16.5–17°C for treatments A3 and B4, the two studies had similar H-stage mortality rate coefficients (Table 2). However, the G-stage temperature sensitivity (c_2) for study A was twice that for Study B. Thus, the higher *CM* in Study A can be attributed to the greater thermal sensitivity in the G stage.

The second important feature is the effect of G-stage temperature exposure on H stage thermal sensitivity. This is expressed by the additional coefficient c_2' (Equation (3b)) which characterises the effect of G-stage temperature on the H-stage thermal mortality. The effect is significant, with the carryover coefficient, c_2', being over twice the direct mortality coefficient, c_4' (Table 2). In study B, the effect of this G-stage temperature exposure essentially negated the benefits of lowering temperature in the H stage in treatments B2 and B3. Furthermore, the absence of correlation in plots of coefficients of one life-stage against another suggests the G-stage temperature exposure was also a major determinant of H-stage mortality in Study A. The analysis suggests that different biological mechanisms could be responsible for immediate mortality in G compared to the carryover effects causing mortality in H. Note that only in the H stage were the b_4 coefficients statistically equivalent in both A and B studies. Thus, in both studies, the mortality rate increased with D in the same manner. For other stages, the mortality rate slope with D was variable and of lower significance, with $Pr > 0.003$ compared to $Pr < 0.00001$ in the H stage. Also note that in the O stage the mortality rate exhibited no significant change with D in either study (Table 2). This pattern comports with the well documented increase in oxygen stress prior to egg hatching (Del Rio et al., 2019; Greig et al., 2007; Martin et al., 2020; Rombough, 1988; D. A. Sear et al., 2017; Wood et al., 2019).

6 | DISCUSSION

As the climate warms and reservoir construction increases, river temperatures will rise, with negative consequences for freshwater fish populations. While this study supports many others (e.g., Koss, 1994; Geist et al. 2006; Martin et al., 2020) by demonstrating rising temperatures increase salmonid embryo mortality, we provide two additional insights into the mechanisms behind these observations. First, we show and quantify the sensitivity of salmonid embryos to thermal stress by developmental stage. Second, thermal stress during gastrulation can cause immediate mortality or embryonic damage that gives rise to carryover effects that reduce survival in later stages. These findings have relevance to understanding population responses to a warming environment and offer vital information for water resource managers seeking to develop models to balance the needs of people and the environment.

While development from a fertilised egg to a complete alevin has many transitions (Danner, 2008), this study shows levels in embryonic sensitivity to high temperatures can be described in terms of four generalised developmental stages: cleavage (C), gastrulation (G), organogenesis (O) and hatch (H). In particular, the sensitivity of rainbow trout embryos to high temperatures was greatest during G and H and lowest during C and O stages. Sensitivity to thermal perturbation during G has been demonstrated in other species (Hosseini, Brenig, Tetens, & Sharifi, 2019; Solnica-Krezel et al., 1996; Uchida et al., 2018), where embryos are shown to suffer a greater frequency of mutations during cellular arrangement when exposed to high temperatures (Solnica-Krezel et al., 1996). This can result in immediate mortality, as shown at higher temperatures in Study A, or sublethal effects that become evident as mortality, as shown in Study B, or reduced fitness in later life-stages (Del Rio et al., 2019; Irvine, 2020; Macqueen et al., 2008). The importance of G as a critical thermal window for salmonid embryo development is a valuable contribution to supporting water resource managers.

In agreement with previous research (Del Rio et al., 2021; Hamor & Garside, 1976; Martin et al., 2020), embryos exposed to higher temperatures exhibited the greatest mortality rates during the H stage. These previous studies attributed this observation to the mismatch between embryonic oxygen demand and supply in higher temperatures (Del Rio et al., 2021; Martin et al., 2020; Rombough, 1988). While this mechanism was evident here, it was less significant than the effect of temperature during G, which accounted for two thirds of the mortality observed at H in Study B. This finding mirrors similar observations for zebrafish (Icoglu Aksakal & Ciltas, 2018; Hosseini et al., 2019; Uchida et al., 2018) and suggests that high temperatures during G give rise to embryonic damage and carryover effects that result in mortality at H. This finding adds to other models (e.g., Anderson et al., 2022; Martin et al., 2020) that suggest the thermal sensitivity of salmonid embryos is controlled by temperature mediated increase in oxygen demand exceeding supply. In combination with ours, these models demonstrate that mortality at H is influenced by the imbalance between oxygen supply and demand in high temperatures at this life stage and embryonic damage caused by pre-hatch exposure to thermal stress.

Unfortunately, it is not possible to define the mechanism behind the observed carryover effect in this study, but there are several possibilities. For example, high temperatures reduce embryo growth efficiency by increasing catabolism (Beer & Anderson, 1997), and this could lead to a greater oxygen imbalance prior to hatching. Alternatively, the carryover effect could involve mutations during G that produce distinct effects on cell fates and morphogenesis (Solnica-Krezel et al., 1996) that reduce the activity of the hatching enzyme or the embryo's ability to escape the egg membrane at hatch (Icoglu Aksakal & Ciltas, 2018; Hosseini et al., 2019). While more work is

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required to understand the mechanisms of the carryover effect, this study demonstrates and models the effects of an additional critical thermal window of salmonid embryo development with implications for water management strategies seeking to cool incubating fish populations (Anderson et al., 2022; Del Rio et al., 2021; Hamor & Garside, 1976; Martin et al., 2020).

7 | MANAGEMENT IMPLICATIONS

Salmonid embryos suffer greater mortality rates in higher temperatures, so the release of cold-water resources from reservoirs to maintain favourable thermal conditions is an essential conservation tool. However, C and O appear relatively unaffected by thermal perturbation, and research on other salmonids shows post-hatch embryos have a greater tolerance to high temperatures (Myrick & Cech, 2004; USFWS, 1999). Therefore, conservation flows targeted to the G and H stages could maintain high levels of survival while retaining reservoir resources at times of water scarcity.

While the recommendation to target conservation flows at specific developmental stages has strong theoretical foundations, water resource managers face a challenge in determining precisely when to carry them out for several reasons. First, our study shows the effective critical thermal window of G and H is larger than previously assumed when H alone was assumed to be the focus of such flows. Second, salmonid populations spawn over several weeks or months, so defining the timing of conservation flows to match these critical windows of development is challenging. Third, the thermal benefits of conservation flows are greatest near the impoundment and decrease downstream where atmospheric warming and dilution from tributaries play a greater role. Therefore, especially during drought conditions, it may be necessary for water resource managers to target conservation flows towards a limited number of eggs within a river system according to their timing or location of spawning. To maximise the benefit of this approach, understanding of water availability, the spatial–temporal pattern of spawning and the stage-dependent thermal sensitivity of the fishes will be required (Anderson et al., 2022).

It is also important to note that, although the theory presented here is robust, the temperature regimes used are not suitable to apply to real world situations. This is because embryo survival in laboratory conditions is typically greater than in the field (Thorn & Morbey, 2019), and the sensitivity of different species to the same environmental conditions varies substantially. Furthermore, as demonstrated by differences in survival between studies A and B, within species variation in response to similar thermal stress is observed. In short, the data from this study is not suitable for designing a specific thermal management strategy. However, we believe the primary value of the work is in highlighting a simplified framework in which to view embryogenesis in river management. The framework, based on biological and mathematical principles, should be useful for developing field and laboratory studies for the design and evaluation of conservation flows tuned to the developmental stages of the target species.

8 | CONCLUSIONS

This study provides further evidence that warming conditions pose a risk for salmonid embryo survival but refines our understanding of the critical importance of timing of exposure to thermal stress. In contrast to previous studies, the data suggest that gastrulation, as well as hatch, represent critical windows of salmonid embryo sensitivity to warm conditions. In particular, the damage experienced by embryos during gastrulation has carryover effects that limit survival at hatch. This stage-specific sensitivity to thermal stress provides an opportunity for a mechanistic approach to water resource management that simultaneously conserves water resources and protects aquatic species. However, further research is required to understand the mechanisms behind these observations and improve our understanding of species and population specific sensitivity to thermal regimes in association with other stressors.

ACKNOWLEDGEMENTS

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ETHICS STATEMENT

The experiments were conducted in accordance with recommendations put forward in the use of fishes in research (2014) and methods were reviewed and approved by the State of California and US EPA auditors.

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REFERENCES

Anderson, J. J., Beer, W. N., Israel, J. A., & Greene, S. (2022). Targeting river operations to the critical thermal window of fish incubation: Model and case study on Sacramento River winter-run Chinook salmon. *River Research and Applications*, *38*, 1–11. https://doi.org/10.1002/rra.3965

Araújo, M. B., & Peterson, A. T. (2012). Uses and misuses of bioclimatic envelope modeling. *Ecology*, *93*(7), 1527–1539.

Beer, W. N., & Anderson, J. J. (1997). Modelling the growth of salmonid embryos. *Journal of Theoretical Biology*, *189*(3), 297–306. https://doi.org/10.1006/jtbi.1997.0515

Bloomer, J., Sear, D., Dutey-Magni, P., & Kemp, P. (2016). The effects of oxygen depletion due to upwelling groundwater on the posthatch fitness of Atlantic salmon (Salmo salar). *Canadian Journal of Fisheries and Aquatic Sciences*, *73*(12), 1830–1840. https://doi.org/10.1139/cjfas-2016-0189

Bloomer, J., Sear, D., & Kemp, P. (2019). Does variation in egg structure among five populations of Atlantic salmon (<i>Salmo salar</i>) influence their survival in low oxygen conditions? *Royal Society Open Science*, *6*(1), 181020. https://doi.org/10.1098/rsos.181020

Breau, C., Cunjak, R. A., & Peake, S. J. (2011). Behaviour during elevated water temperatures: Can physiology explain movement of juvenile Atlantic salmon to cool water? *Journal of Animal Ecology*, *80*(4), 844–853. https://doi.org/10.1111/j.1365-2656.2011.01828.x

Dahlke, F. T., Wohlrab, S., Butzin, M., & Pörtner, H.-O. (2020). Thermal bottlenecks in the life cycle define climate vulnerability of fish. *Science*, *369*(6499), 65–70. https://doi.org/10.1126/science.aaz3658

15351467, 2023, 1, Downloaded from https://onlinelibrary.wiley.com/doi/10.1002/rra.4066 by Test, Wiley Online Library on [24/05/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

Daniels, M. E., & Danner, E. M. (2020). The drivers of river temperatures below a large dam. *Water Resources Research*, *56*(5), e2019WR026751. https://doi.org/10.1029/2019WR026751

Danner, G. R. (2008). *Salmonid embryo development and pathology*. Paper presented at the American Fisheries Society Symposium (Vol. 65).

Del Rio, A. M., Davis, B. E., Fangue, N. A., & Todgham, A. E. (2019). Combined effects of warming and hypoxia on early life stage Chinook salmon physiology and development. *Conservation Physiology*, *7*(1). https://doi.org/10.1093/conphys/coy078

Del Rio, A. M., Mukai, G. N., Martin, B. T., Johnson, R. C., Fangue, N. A., Israel, J. A., & Todgham, A. E. (2021). Differential sensitivity to warming and hypoxia during development and long-term effects of developmental exposure in early life stage Chinook salmon. *Conservation Physiology*, *9*(1). https://doi.org/10.1093/conphys/coab054

Falkenmark, M., & Wang-Erlandsson, L. (2021). A water-function-based framework for understanding and governing water resilience in the Anthropocene. *One Earth*, *4*(2), 213–225. https://doi.org/10.1016/j.oneear.2021.01.009

Falkenmark, M., Wang-Erlandsson, L., & Rockström, J. (2019). Understanding of water resilience in the Anthropocene. *Journal of Hydrology X*, *2*, 100009. https://doi.org/10.1016/j.hydroa.2018.100009

Geist, D. R., Abernethy, C. S., Hand, K. D., Cullinan, V. I., Chandler, J. A., & Groves, P. A. (2006). Survival, Development, and Growth of Fall Chinook Salmon Embryos, Alevins, and Fry Exposed to Variable Thermal and Dissolved Oxygen Regimes. *Transactions of the American Fisheries Society*, *135*(6), 1462–1477. https://doi.org/10.1577/T05-294.1

Greig, S. M., Sear, D. A., & Carling, P. A. (2007). A review of factors influencing the availability of dissolved oxygen to incubating salmonid embryos. *Hydrological Processes*, *21*(3), 323–334. https://doi.org/10.1002/hyp.6188

Hamor, T., & Garside, E. T. (1976). Developmental rates of embryos of Atlantic salmon, *Salmo salar* L., in response to various levels of temperature, dissolved oxygen, and water exchange. *Canadian Journal of Zoology*, *54*(11), 1912–1917. https://doi.org/10.1139/z76-221

Hosseini, S., Brenig, B., Tetens, J., & Sharifi, A. R. (2019). Phenotypic plasticity induced using high ambient temperature during embryogenesis in domesticated zebrafish, Danio rerio. *Reproduction in Domestic Animals*, *54*(3), 435–444. https://doi.org/10.1111/rda.13382

Icoglu Aksakal, F., & Ciltas, A. (2018). The impact of ultraviolet B (UV-B) radiation in combination with different temperatures in the early life stage of zebrafish (Danio rerio). *Photochemical & Photobiological Sciences*, *17*(1), 35–41. https://doi.org/10.1039/c7pp00236j

Irvine, S. Q. (2020). Embryonic canalization and its limits—A view from temperature. *Journal of Experimental Zoology Part B: Molecular and Developmental Evolution*, *334*(2), 128–144. https://doi.org/10.1002/jez.b.22930

Koss, D. R. (1994). The effects of temperature and time of first feeding on egg and fry development in Atlantic salmon, salmo salar L. (Ph.D.), Stirling, Retrieved from http://citeseerx.ist.psu.edu/viewdoc/download?doi=10.1.1.900.1385&rep=rep1&type=pdf (2577)

Macqueen, D. J., Robb, D. H. F., Olsen, T., Melstveit, L., Paxton, C. G. M., & Johnston, I. A. (2008). Temperature until the 'eyed stage' of embryogenesis programmes the growth trajectory and muscle phenotype of adult Atlantic salmon. *Biology Letters*, *4*(3), 294–298. https://doi.org/10.1098/rsbl.2007.0620

Martin, B. T., Dudley, P. N., Kashef, N. S., Stafford, D. M., Reeder, W. J., Tonina, D., … Danner, E. M. (2020). The biophysical basis of thermal tolerance in fish eggs. *Proceedings of the Royal Society B: Biological Sciences*, *287*(1937), 20201550. https://doi.org/10.1098/rspb.2020.1550

McCullough, D. A. (1999). *A review and synthesis of effects of alterations to the water temperature regime on freshwater life stages of salmonids, with special reference to Chinook salmon* (p. 10). Washington, DC: US Environmental Protection Agency, Region.

Mueller, C. A., Eme, J., Manzon, R. G., Somers, C. M., Boreham, D. R., & Wilson, J. Y. (2015). Embryonic critical windows: Changes in incubation temperature alter survival, hatchling phenotype, and cost of development in lake whitefish (Coregonus clupeaformis). *Journal of Comparative Physiology B*, *185*(3), 315–331. https://doi.org/10.1007/s00360-015-0886-8

Mulligan, M., van Soesbergen, A., & Sáenz, L. (2020). GOODD, a global dataset of more than 38,000 georeferenced dams. *Scientific Data*, *7*(1), 31. https://doi.org/10.1038/s41597-020-0362-5

Myrick, C. A., & Cech, J. J. (2004). Temperature effects on juvenile anadromous salmonids in California's central valley: What don't we know? *Reviews in Fish Biology and Fisheries*, *14*(1), 113–123. https://doi.org/10.1007/s11160-004-2739-5

R Core Team. (2018). *R: A language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing Retrieved from https://www.R-project.org/

Rombough, P. J. (1988). Respiratory gas exchange, aerobic metabolism, and effects of hypoxia during early life. In W. S. Hoar & D. J. Randall (Eds.), *Fish physiology (vol. 11, Part A)* (pp. 59–161). New York, NY: Academic Press.

Sear, D. A., DeVries, P., & Greig, S. M. (2008, Oct 2008). *Science and practice of salmonid spawning habitat remediation*. Paper presented at the Salmonid Spawning Habitat in Rivers: Physical Controls, Biological Responses, and Approaches to Remediation. Bethseda, MD: American Fisheries Society Press.

Sear, D. A., Pattison, I., Collins, A. L., Smallman, D. J., Jones, J. I., & Naden, P. S. (2017). The magnitude and significance of sediment oxygen demand in gravel spawning beds for the incubation of salmonid embryos. *River Research and Applications*, *33*(10), 1642–1654. https://doi.org/10.1002/rra.3212

Solnica-Krezel, L., Stemple, D. L., Mountcastle-Shah, E., Rangini, Z., Neuhauss, S. C., Malicki, J., … Driever, W. (1996). Mutations affecting cell fates and cellular rearrangements during gastrulation in zebrafish. *Development*, *123*(1), 67–80.

Thorn, M. W., & Morbey, Y. E. (2019). Survival under the gravel: The effects of egg size and parental identity on the In situ hatching success of Chinook Salmon. *Transactions of the American Fisheries Society*, *148*(5), 991–1003. https://doi.org/10.1002/tafs.10192

Uchida, Y., Uesaka, M., Yamamoto, T., Takeda, H., & Irie, N. (2018). Embryonic lethality is not sufficient to explain hourglass-like conservation of vertebrate embryos. *EvoDevo*, *9*, 7. https://doi.org/10.1186/s13227-018-0095-0

USFWS. (1999). Effects of temperature on early-life survival of Sacramento River fall-run and winter-run Chinook salmon. US Fish and Wildlire Service Final Report, January 1999.

Wood, A. T., Clark, T. D., Elliott, N. G., Frappell, P. B., & Andrewartha, S. J. (2019). Physiological effects of dissolved oxygen are stage-specific in incubating Atlantic salmon (*Salmo salar*). *Journal of Comparative Physiology B*, *189*(1), 109–120. https://doi.org/10.1007/s00360-018-1199-5

Zarfl, C., Lumsdon, A. E., Berlekamp, J., Tydecks, L., & Tockner, K. (2015). A global boom in hydropower dam construction. *Aquatic Sciences*, *77*(1), 161–170. https://doi.org/10.1007/s00027-014-0377-0

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