LETTER

Phenomenological vs. biophysical models of thermal stress in aquatic eggs

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Abstract

Predicting species responses to climate change is a central challenge in ecology. These predictions are often based on lab-derived phenomenological relationships between temperature and fitness metrics. We tested one of these relationships using the embryonic stage of a Chinook salmon population. We parameterised the model with laboratory data, applied it to predict survival in the field, and found that it significantly underestimated field-derived estimates of thermal mortality. We used a biophysical model based on mass transfer theory to show that the discrepancy was due to the differences in water flow velocities between the lab and the field. This mechanistic approach provides testable predictions for how the thermal tolerance of embryos depends on egg size and flow velocity of the surrounding water. We found support for these predictions across more than 180 fish species, suggesting that flow and temperature mediated oxygen limitation is a general mechanism underlying the thermal tolerance of embryos.

Keywords

Climate change, egg, embryo, mass transfer theory, oxygen, salmon, survival, temperature, thermal performance curve, thermal tolerance.

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INTRODUCTION

Predicting how species will respond to the world's increasing air, ocean and freshwater temperatures is a central challenge for ecology. Excluding approaches that do not explicitly consider thermal stress (e.g. climate envelope modelling), the most common forecasting approaches are based on phenomenological models such as thermal performance curves (Angilletta 2006; Schulte *et al.* 2011) that describe the relationship between a component of fitness (mortality rate, growth rate, etc.) and temperature. These relationships, typically derived from laboratory data, are used to forecast species' responses to future temperature regimes in nature (Deutsch *et al.* 2008; Kingsolver *et al.* 2013). As phenomenological models of thermal stress, these curves are not tied to any particular mechanism, and as a result they are flexible tools that can be used to model any species or fitness component. However, this flexibility comes with a cost: without a mechanistic understanding of the underlying causes of thermal tolerance, the range of applicable conditions is not known. If the mechanism underlying thermal tolerance operates independently of other factors, for example by temperature-dependent protein denaturation (Fields 2001), then lab-derived models of thermal tolerance may be useful predictors in the field. However, other mechanisms of thermal sensitivity, such as oxygen limitation (Pörtner & Knust 2007), depend not only on temperature, but also on other environmental and physiological variables that influence the demand or supply of oxygen to developing tissues. In such cases, thermal response forecasts that have been parameterised using laboratory response curves may mischaracterise the sensitivity of species' responses to warming in the field.

In this paper, we test whether such phenomenological approaches can predict the impacts of elevated water temperatures on endangered Sacramento River winter-run Chinook salmon (*Oncorhynchus tshawytscha*) in the Central Valley of California. Since the construction of large dams on the Sacramento River in the mid-1900s, winter-run Chinook salmon have been blocked from reaching their native spawning grounds in cold, spring-fed mountain tributaries, and are now forced to spawn in the low-elevation mainstem river below the lowest dam. In this new location, the thermal quality of their habitat is controlled by water releases from the dams. Because Chinook salmon embryos are the most sensitive life-stage to elevated temperatures (McCullough 1999), a set of temperature-related regulations governing dam operations was established to protect endangered winter-run salmon eggs (National Marine Fisheries Service 2009). Importantly, these regulations are based on thermal tolerance estimates from controlled laboratory experiments.

Here, we fit a phenomenological model of thermal tolerance to laboratory data on survival as a function of temperature and test whether it can retrospectively predict interannual variation in salmon survival through the embryonic stage in the Sacramento River. Although the phenomenological model successfully described temperature-dependent survival in the lab, the model failed entirely at predicting the effects of temperature on survival in the field, due to a ~3 °C reduction in

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thermal tolerance in the field compared to the lab. We hypothesised the observed reduction in thermal tolerance in the field was caused by differences in water flow velocities between the lab and the field, affecting the ability of embryos to balance temperature-dependent oxygen demand with supply. To test this hypothesis, we developed a biophysical model of oxygen supply and demand based on mass transfer theory to predict the thermal tolerance of Chinook salmon embryos from first principles. Finally, we tested the ability of our biophysical model to explain broad patterns of thermal tolerance across ~180 oviparous fish species.

METHODS

Temperature-dependent mortality model

We developed a phenomenological temperature-dependent mortality model for Chinook salmon embryos and fit to the model laboratory data to quantify the effect of temperature on survival. The model relates the temperature experienced by an embryo during the ith day of its development (T_i) to its instantaneous mortality rate (h_i; d^{-1}) with two parameters: T_{crit}, the temperature below which there is no mortality due to temperature, and b_T, the slope at which mortality rate increases with temperature above T_{crit}:

$$h_i = b_T \max(T_i - T_{crit}, 0) \tag{1}$$

We also analysed an alternative functional form for the temperature-dependent mortality rate where, $h_i = c_0 \exp(c_1 T_i)$, however, because the results were qualitatively unaffected by the choice of hazard model, and the exponential model has less easily interpretable parameters, we focus on the threshold model (eqn 1) although results for the exponential model are briefly summarised.

The length of the development period (n, days) was modelled using a temperature-dependent maturation function (Zueg $et\ al.$ 2012), where the relative developmental state at fertilisation equals 0 and increases at rate, 0.001044 ($°C^{-1}$ d^{-1})$\times$ $T_i + 0.00056$ (d^{-1}). Chinook embryos emerge when the relative developmental state exceeds 1.

Temperature-dependent mortality throughout the entire embryonic period (M_T) is the product of the daily temperature-dependent survival probabilities from hatching to emergence:

$$M_T = 1 - \prod_{i=1}^{n} \exp(-h_i) \tag{2}$$

Application to the lab

We estimated thermal tolerance parameters by fitting the phenomenological model to data from laboratory experiments conducted at constant temperatures ranging from 10 to 20 °C that recorded percent survival from fertilisation to emergence (Jensen & Groot 1991; USFWS 1999). To account for all sources of mortality not related to temperature we included a background survival parameter, S_{B_Lab} that represents the expected survival in the absence of temperature-dependent mortality. Thus, survival through the entire embryonic period,

S, is given by:

$$S = S_{B\_Lab}(1 - M_T) \tag{3}$$

Application to the field

We tested the ability of the laboratory-based thermal tolerance models to predict interannual variation in field-derived estimates for egg-to-fry survival of winter-run Chinook salmon in the Sacramento River from 1996 to 2015. Female Chinook salmon dig pits in the bottom of gravel bed rivers, lay eggs in the pit, and following fertilisation, bury the eggs with gravel, forming redds. Embryos develop for several weeks in the redd before hatching as alevins. After a further period of development within the gravel, alevins emerge as fry (Quinn 2005). Winter-run Chinook salmon spawn in summer, a unique behaviour among Chinook salmon populations.

We linked existing datasets on the date and location of winter Chinook salmon spawning determined from aerial redd surveys (Bergman $et\ al.$ 2012) with a one-dimensional temperature model of the Sacramento River with 1 km spatial resolution (Pike $et\ al.$ 2013) to generate daily temperature exposure profiles for all known redds within years. We applied the mortality model with lab-derived thermal tolerance parameters using daily temperature exposure profiles for all known redds from 1996 to 2015. For each year we calculated the percent population loss due to temperature-dependent mortality by averaging the mortality of all redds within that year. To account for other sources of mortality in the field we included a temperature-independent background survival probability, S_{B_Field}, which we treated as a free parameter. In the field, background survival probability represents the expected egg-to-fry survival for a year in the absence of temperature-dependent mortality, and includes survival through the embryonic period as well as an outmigration period from redds to Red Bluff Diversion Dam, 95 km below Keswick Dam. We assumed survival is temperature-independent during the outmigration period for two reasons. First, the thermal tolerance of fry is over 5 °C greater than for embryos (McCullough 1999), and second because the timing of fry passage at Red Bluff Diversion Dam closely mirrors the predicted emergence timing of fry. These observations suggest that most fry only spend a few days between emergence and Red Bluff Diversion Dam passage, a brief period compared to the roughly 80 day embryonic period.

Lastly, we hypothesised that, due to limited optimal habitat for spawning, mean redd quality decreases, and the probability of redds being superimposed by later spawners increases, with female spawner abundance, A (Essington $et\ al.$ 2000). Thus, we evaluated whether female spawner density affected egg-to-fry survival by evaluating a model including a Beverton–Holt density-dependence term (Beverton & Holt 1959) in the background survival probability:

$$S_{B\_Field} = S_0/(1 + A/K) \tag{4}$$

where S_0 is the expected egg to fry survival probability in the absence of temperature or density-dependent survival and K (# spawners) is a capacity parameter.

We treated background survival as an aggregate survival probability rather than a daily rate because many sources of mortality (e.g. superimposition, mortality during the outmigration period) are likely not dependent on the duration of the embryonic period, and because within non-lethal temperature ranges, natural mortality rates and development rates generally scale with temperature similarly, such that survival to a given developmental stage is time-independent (McCoy & Gillooly 2008).

Parameter estimation

The model was parameterized with data from lab experiments. Each lab study used replicate groups of embryos (~30-90 individuals per group), that were exposed to a given temperature treatment and the number of individuals in a group surviving through the embryonic stage was recorded. Thus, for the laboratory data we estimated model parameters using a mixed logistic model with two error terms: a logit-normal error term to account for random group effects where the predicted survival, S, for the ith group is:

$$\text{logit}(S_i) = \text{logit}(\bar{S}) + \epsilon_i \text{ where } \epsilon_i \sim N(0, \sigma)^2$$

where $\bar{S}$ is the expected survival at a given temperature and ε_i is the random effect of the ith group, and a binomial error term where the probability of observing x out of N embryos surviving in the ith group given S_i is:

$$(x_i | N_i, S_i) \sim B(N_i, S_i))$$

We estimated model parameters using maximum likelihood, where the likelihood function is the product of the likelihoods of the logit-normal and binomial error terms.

In the field we used winter-run egg-to-fry survival data to the Red Bluff Diversion Dam, estimated from the United States Fish and Wildlife Service (USFWS) rotary screw trap programme for the time period 1996–2015 (although data were not collected in 2001 and 2002). USFWS calculates egg-to-fry survival rates by estimating the number of out-migrating winter Chinook fry from rotary screw trap collections and dividing by the estimated number of eggs in-river based on carcass survey female estimates (Poytress 2016). Average female winter Chinook fecundity data were obtained from the Livingston Stone National Fish Hatchery annual spawning records (Poytress 2016). Unlike the laboratory data, the field data were not true binomial data as both the number of embryos produced and the number of survivors were estimates rather than known values. Because using logistic regression would have required arbitrary assumptions about the observational error, we analysed the logit-transformed egg-to-fry survival data (fraction survival) using Ordinary Least Squares (Warton & Hui 2011). This ensured predictions cannot exceed possible values (e.g. negative survival), and normalised residual error. Due to the large number of embryos produced each year (~5–50 000 000), binomial error is negligibly small. Thus, our analysis using OLS on logit transformed proportional data is functionally equivalent to a two-level mixed model with a random logit-normal year effect and a binomial error term.

In both the lab and field analysis, we generated confidence intervals for parameters and predictions via bootstrapping with replacement on 1000 simulated data sets (Efron & Tibshirani 1986).

RESULTS

Testing the lab-derived model

For the laboratory data, the phenomenological model captured much of the variation in survival through the embryonic period as a function of temperature (Fig. 1). The estimates of T_{crit} and b_T were 15.4 °C and 0.034 $°C^{-1}d^{-1}$ respectively. When applied to field data, the lab-based model predicted negligible temperature-dependent mortality (< 1%) in the Sacramento River in any year 1996–2015 (Fig. 2). Because effectively no temperature-dependent mortality was predicted, the model explained virtually no additional variation in egg-to-fry survival in the field compared to a null model assuming no temperature-dependent mortality ($r^2 < 0.01$). Similar results were found when the null and lab models included a density-dependence term (null model with density-dependent background survival $r^2 = 0.02$ vs. lab-based thermal tolerance model with density-dependent background survival $r^2 = 0.03$). The ability of the lab-derived model to predict interannual variation in egg-to-fry survival was similarly poor using the exponential hazard model (r^2 of 0.05 and 0.02 for models with and without density-dependent background survival).

The two most likely explanations for the lab-derived model of thermal tolerance being a poor predictor of annual variation in survival are (1) survival was primarily driven by factors other than temperature, or (2) thermal tolerance of Chinook salmon embryos in the field differs from their
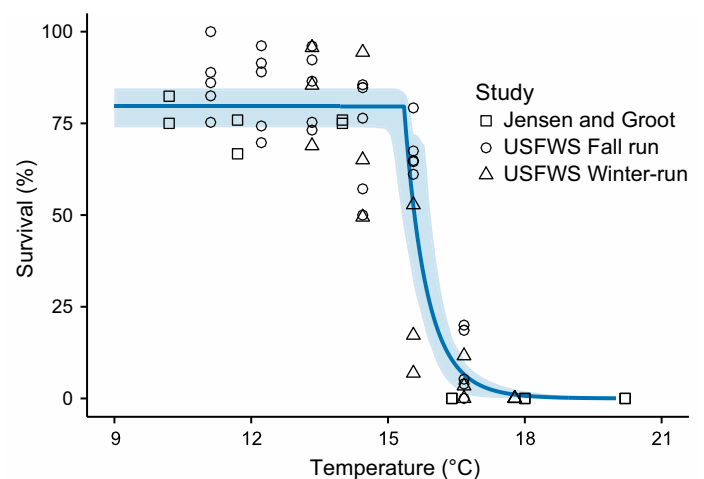

Figure 1 Fit of the phenomenological temperature-dependent mortality to laboratory data on survival through the embryonic period as a function of temperature (shaded region 95% CI). Data are from three populations reported in two studies (Jensen and Groot 1991; USFWS 1999). Jensen and Groot (1991) conducted experiments with Chinook salmon embryos from Big Qualicum River, British Columbia, and USFWS (1999) conducted experiments with both fall and winter Sacramento River populations. All three populations exhibited similar thermal response curves.
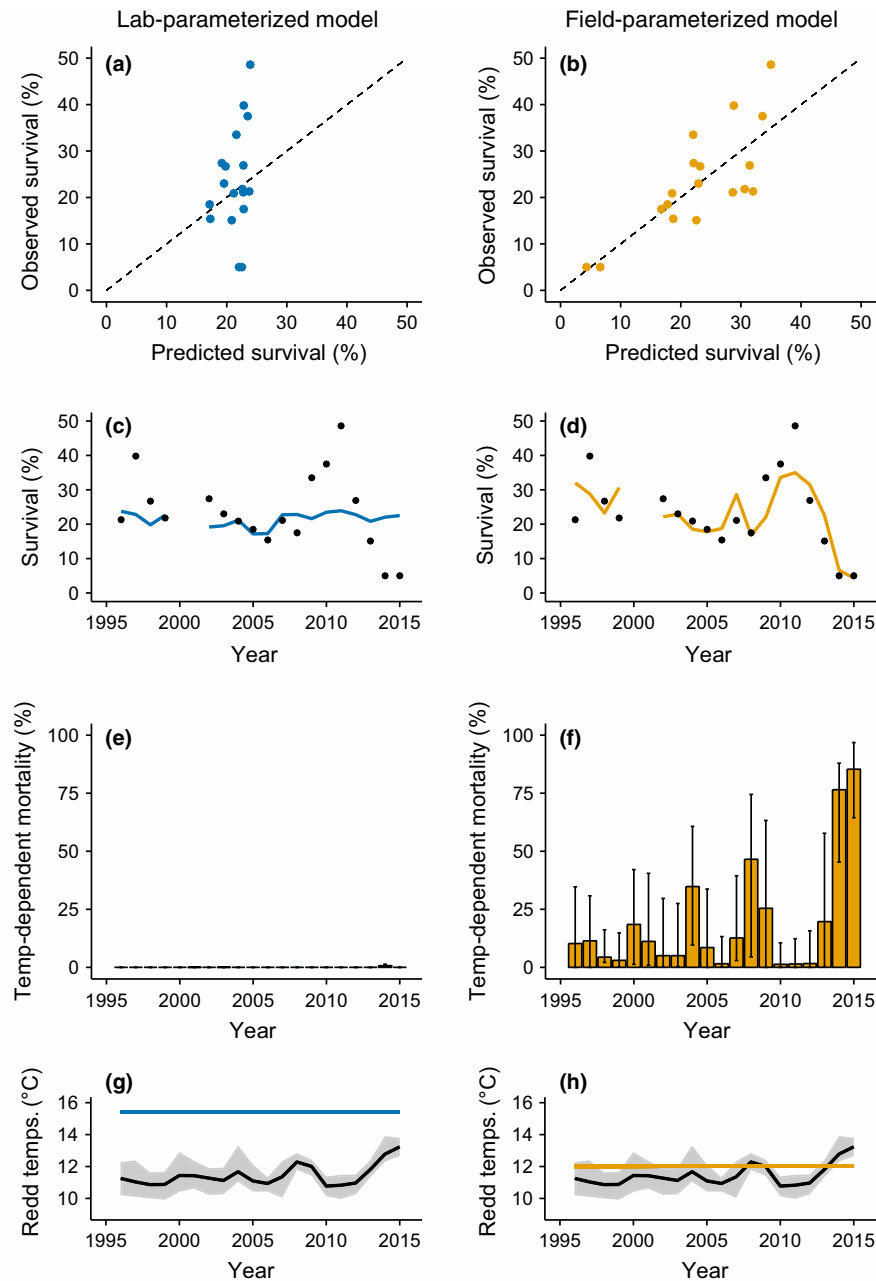
Figure 2 Model predictions for egg-to-fry survival (%) and temperature-dependent mortality of Sacramento River winter-run Chinook from 1996 to 2015 using thermal tolerance parameters estimated from laboratory (left column) or field data (right column). The lab-derived thermal tolerance model, when applied to the Sacramento River, failed to explain significant annual variation in the percent of embryos surviving through to the fry stage (a and c), where panel (a) shows observed vs. predicted and panel (c) shows observed and predicted (line) survival over time. Using the same model, but with thermal tolerance parameters treated as free parameters estimated directly from the field data, substantially improved model predictions (b and d). Differences in model predictions were due to the much higher thermal mortality predicted in given years when thermal tolerance parameters were estimated using field data (f) compared to lab-parameterised model (e). Bar height represents the predicted percent of embryos lost in years due to temperature-dependent mortality (error bars show 95% CI). Panels (g). and (h) show median (black solid line) and 5 and 95% quantiles (shaded region) of daily temperatures experienced by all redds within years relative to T_{crit} estimated using lab (blue line) or field (yellow line) data.

thermal tolerance in the lab. To distinguish between these two hypotheses, we repeated our analysis of the field data treating T_{crit} and b_T as free parameters rather than assuming their lab-derived estimates are applicable to field conditions.

In contrast to the lab parameterisation, fitting the model to field data revealed strong evidence of temperature-dependent mortality (Table 1; Fig. 2). Most notably, in 2014 and 2015,

when temperatures reached the highest levels over the 20-year period (Fig. 2g and h), the field-derived model estimated 78 and 86% temperature-dependent embryo mortality. The field-parameterised temperature-dependent mortality model vastly outperformed the null model and lab model (ΔAIC = 15.7 for both) when assuming constant background mortality ($r^2 = 0.66$). When density-dependent background survival was

Table 1 Comparison of phenomenological models performance at predicting interannual variation in egg-to-fry survival of winter-run Chinook in the Sacramento River

Model	Parameters	SSQ	r^2	ΔAIC	w(AIC)
Field model + density dep.	4	2.21	0.77	0	0.91
Field model	3	3.18	0.66	4.58	0.09
Lab model + density dep.	2	9.20	0.03	21.70	< 0.001
Null + density dep.	2	9.23	0.02	21.76	< 0.001
Lab model	1	9.44	< 0.01	20.17	< 0.001
Null	1	9.47		20.23	< 0.001

included, the field-derived model performed even better ($r^2 = 0.77$) against the null and lab models (ΔAIC = 20.2 for both). Results using the exponential hazard model were essentially identical as those with the threshold model (r^2 was 0.77 and 0.66 for the models with and without density-dependence).

For the field-parameterised model without density-dependent background survival, the background survival probability was ~28%. When the density-dependent term was included ($S_0 = 0.366$; $K = 9107$; Fig. S1), egg-to-fry survival in the absence of temperature-dependent mortality ranged from ~35% at the lowest female spawner densities observed (~500 spawners) to ~19% at the highest spawner densities observed (~8500 spawners).

The differences in survival predicted by the lab- and field-parameterised models were driven primarily by a > 3 °C reduction in T_{crit} in the field, 12.0 °C (95% CI 10.8–13.7) compared to the lab 15.4 °C (95% CI 15.0–15.8). Estimates of

b_T were similar between field (0.024 °C$^{-1}d^{-1}$, 95% CI 0.007–4.21) and lab (0.034 °C$^{-1}d^{-1}$, 95% CI 0.025–0.060) parameterisations. Due to covariation between T_{crit} and b_T in the field analysis, the region of likely parameter space is much smaller than the individual confidence intervals would suggest (Fig. 3). In general, most of the likely parameter sets for the field corresponded to T_{crit} values in the range of ~11–12.5 °C and b_T values in the same range as those estimated from laboratory data. A second, but smaller group (~5%) of parameter sets had T_{crit} values around 13.0–13.8 °C and extremely high values of b_T. These parameter sets corresponded to the scenario where embryos are unaffected by temperature up to a relatively high temperature, but exceeding this temperature by small amounts and for short durations results in near-complete mortality. Estimates of thermal tolerance parameters in the field were not affected by the inclusion of the density-dependent term in the background survival rate (T_{crit} 12.0 °C vs. 12.1 °C and b_T 0.024 vs. 0.023 in parameterisations with and without a density-dependent term in the background survival rate).

Accounting for the discrepancy between lab and field-derived thermal tolerance

A growing body of experimental and observational studies point to oxygen limitation as a general mechanism setting the thermal tolerance of aquatic ectotherms (Pörtner & Knust 2007; Eliason *et al.* 2011; Forster *et al.* 2012; Deutsch *et al.* 2015). Because developing embryos lack circulatory and ventilation systems, flow velocity and dissolved
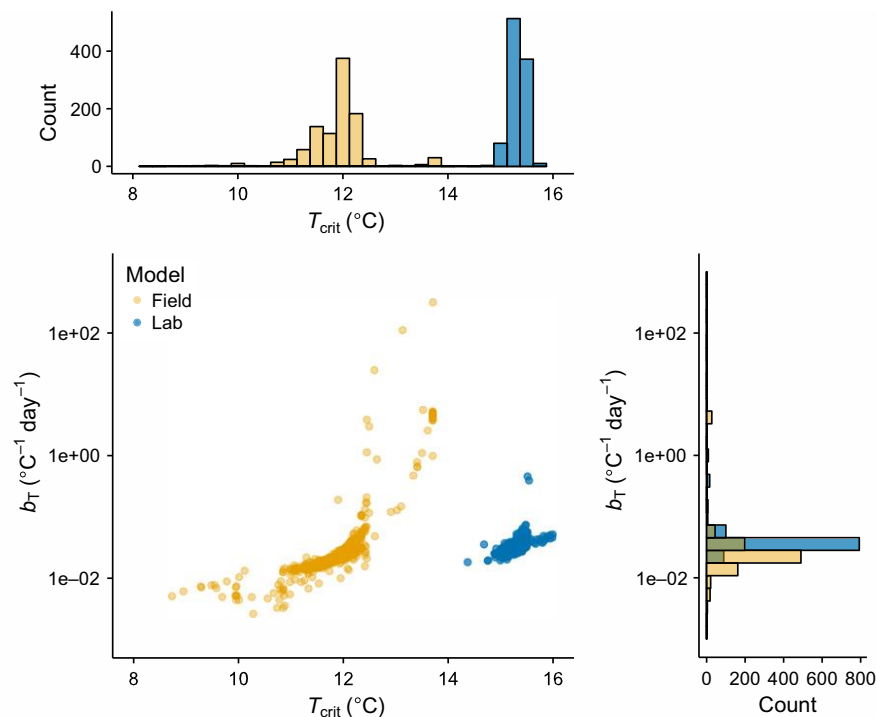
Figure 3 Comparison of field (gold) and lab (blue) estimates of thermal tolerance parameters. The top and right histograms show distribution of likely parameter estimates for T_{crit} and b_T from the bootstrap analysis. The scatter plot shows the covariance between the thermal tolerance parameter estimates in the lab and field parameterisations.

oxygen strongly influence the oxygen supply rate. In laboratory environments, Chinook salmon embryos are typically allowed to develop in highly oxygenated, fast flowing water (~0.15 cm s^{-1}; USFWS 1999, Murray and McPhail 1988; Jenson and Groot 1991), while in nature, embryos are embedded in gravel redds where flow velocities are lower (~0.04 cm s^{-1}; Zimmermann & LaPointe 2005). We therefore developed a biophysical model of oxygen supply and demand for embryos to evaluate whether this mechanism can account for differences in observed thermal tolerances in the lab and field.

Like all obligate aerobes, fish embryos must acquire enough oxygen to meet their metabolic demands. For a spherical embryo, the supply of oxygen (J) to the embryo can be calculated from mass transfer theory (Daykin 1965; Kranenbarg et al. 2001):

$$J = 4\pi R^2 (P_s - P_i)k_e, \tag{5}$$

where R is the radius of the approximately spherical embryo, P_s and P_i are the oxygen partial pressures (kPa) at the sphere surface and inside the embryo, and k_e (µgO$_2$/[cm^2 s kPa]) is the mass transfer coefficient for oxygen transfer to the embryo. Oxygen consumption by the embryo creates an area of local oxygen depletion such that the oxygen concentration at the sphere surface is less than the ambient oxygen concentration P_e (Daykin 1965):

$$P_s = P_e - \frac{N\delta}{4\pi R^2 k_w} \tag{6}$$

where N (µgO$_2$ s^{-1}) is the oxygen consumption rate of the embryo, δ (kPa/[µgO$_2$ cm^{-3}]) is a conversion factor from oxygen concentration to pressure and k_w (cm s^{-1}) is the mass transfer coefficient of oxygen in water. The mass transfer coefficient for oxygen in water depends on the combined influence of convective and diffusive transport processes in the water surrounding the developing embryo. In flowing water, a velocity boundary layer forms around the embryo; at the embryo's surface, velocity is zero and it approaches its asymptotic value with increasing distance away from the sphere. Because diffusion is relatively slow in water, a solute boundary forms within the velocity boundary layer. At the egg's surface, oxygen is transported entirely by diffusion and with increasing distance away from the egg convection plays an increasingly larger role in oxygen transfer. The combined effect of these processes on oxygen transport from the surrounding water can be estimated by dimensionless number analysis (Daykin 1965; Kranenbarg et al. 2001):

$$k_w = S_h D/2R \tag{7}$$

where D (cm^2 s^{-1}) is the diffusion coefficient for oxygen in water and S_h is the dimensionless Sherwood number reflecting the ratio of total mass transfer to diffusive mass transfer. For a given geometry, S_h can be estimated from semi-empirical functions of two other dimensionless numbers: the Reynolds number, $R_e = 2RU/v$ a ratio of inertial to viscous forces, where U and v are the velocity (cm s^{-1}) and kinematic viscosity (cm^2 s^{-1}) of the surrounding water,

respectively, and the Schmidt number $S_c = v/D$ a ratio of viscosity and mass diffusivity. For mass transfer to a sphere in flow, the semi-empirical relationship for the Sherwood number is (Daykin 1965):

$$S_h = 2 + 0.8R_e^{1/2}S_c^{1/3} \tag{8}$$

Substituting eqns 6–8 into eqn 5 gives oxygen supply to the embryo as a function of its size, its metabolic demand for oxygen, and the velocity and oxygen tension of the surrounding water:

$$J = 4\pi R^2 k_e \left(P_e - P_i - \frac{N\delta}{2DR\pi \left(2 + 0.8\left(\frac{v}{D}\right)^{1/3}\left(\frac{2RU}{v}\right)^{1/2} \right)} \right) \tag{9}$$

At steady state, oxygen demand and supply must balance ($J = N$). When demand for oxygen is low, there is only a small reduction in internal oxygen tension. As demand for oxygen increases, P_i drops, increasing the concentration gradient until supply again matches demand. Eventually, with increasing demand, P_i will drop to a level that limits aerobic metabolism, P_i^*. At this critical point, metabolism switches from being demand driven to being supply constrained. Rearranging eqn 9 and solving for the flow velocity where oxygen supply equals oxygen demand yields:

$$U_{crit} = \frac{v \left(\frac{5N\delta RK_e}{2D\left(N - 4\pi R^2 k_e \left(P_e - P_i^* \right) \right)} + \frac{5}{2} \right)^2}{2R \left(\frac{v}{D} \right)^{2/3}} \tag{10}$$

where U_{crit} is the velocity below which oxygen supply to the embryo is not sufficient to meet demand.

D, v and δ are well-established physical parameters that depend on temperature (see Appendix S1). The remaining two supply parameters, P_i^* and k_e, have been estimated empirically for the embryos of Chinook salmon and other salmonids (Rombough 1989). Because the estimate of the mass transfer coefficient of the embryo, k_e, by Rombough (1989) did not explicitly account for the depletion of oxygen at the egg surface, he overestimated the oxygen gradient at the egg's surface ($P_s - P_i$) and therefore his estimate of k_e is biased lower than its true value (eqn 5). To account for boundary layer effects, we used mass transfer theory (eqn 6) to estimate the oxygen tension at the surface of the egg (see Appendix S1). We then fit the same relationship as Rombough 1989 but using the critical oxygen tension corrected for boundary layer effects. This resulted in a k_e of 3.65×10^{-4} µgO$_2$/(cm^2 s kPa) compared to 2.02×10^{-4} µgO$_2$/(cm^2 s kPa) estimated by Rombough (1989). The estimate of the critical oxygen tension, P_i^*, was negligibly affected by accounting for boundary layer effects (6.1 kPa vs. 6.2 kPa from Rombough 1989).

Like most ectotherms, the metabolic demand for oxygen in developing Chinook salmon embryos increases exponentially with temperature (Rombough 1994). Furthermore, as Chinook salmon embryos grow their demand for oxygen increases in proportion to their live tissue mass. We therefore fit a model for oxygen demand as a function of tissue

mass, M (g), and temperature, T, to data from Rombough (1994):

$$N = b_0 M \exp(b_1 T) \qquad (11)$$

where $b_0 = 81.8 \ \mu gO_2 \ g^{-1} \ h^{-1}$ and $b_1 = 0.0945 \ °C^{-1}$ ($r^2 = 0.95$). eqn 10 predicts that embryos are most vulnerable to oxygen limitation when total oxygen demand, N, is highest, which will occur right before hatching. We therefore evaluated the oxygen limitation temperature above which oxygen supply is not sufficient to meet demand for Chinook salmon embryos at their mass immediately before hatching (0.05 g).

In general, the temperature at which oxygen becomes limiting is strongly dependent on flow velocity (Fig. 4). At the flow velocities typical for lab experiments ($> 0.1 \ cm \ s^{-1}$), the oxygen limitation temperature approaches its asymptotic value where the solute boundary layer becomes infinitely thin, and oxygen supply is constrained only by mass transfer through and within the sphere. At flows below $0.1 \ cm \ s^{-1}$, the thickness of the boundary layer expands rapidly with decreasing flow velocity, reducing the rate of oxygen supply. Consequently, the oxygen limitation temperature drops rapidly with decreasing flow below $0.1 \ cm \ s^{-1}$ such that flow velocities typically observed within gravel redds result in thermal tolerance 2–5 °C lower than in the lab, consistent with the discrepancy in thermal tolerance we observed between lab and field data in our statistical analysis (Fig. 4). Not only were the relative differences in thermal tolerance between lab and field well accounted for by the biophysical model, but also the absolute thermal tolerances predicted in the lab and field regimes corresponded closely with the values estimated from our statistical analysis. This supports our hypothesis that oxygen supply and demand is the mechanism driving thermal tolerance of Chinook embryos, and consequently that thermal tolerance is not fixed but is highly dependent on flow.

Generality of oxygen-limited thermal tolerance in aquatic eggs

Among oviparous aquatic eggs, Chinook eggs are atypically large and thus may be more vulnerable to oxygen limitation due to their low surface area-to-volume ratios. We therefore addressed whether oxygen limitation broadly underlies the thermal tolerance of developing fish embryos or if it is only relevant for the few species with larger egg sizes. We used eqn 10 to predict the oxygen limitation temperatures of fish embryos, where the upper thermal threshold for a species is determined by its embryonic characteristics, R, N, k_e and P_i^*, and environmental characteristic P_e and U. Given the little species-specific variation in P_i^* and k_e observed among salmonids with marked different egg capsule characteristics (Rombough 1989), we fixed these values to those used for Chinook salmon. However, further experimental work is needed to assess the degree to which these parameters vary over a wider phylogenetic and ecological range of species.

To determine N, we compiled respiration data for developing embryos using the last observed metabolic rate before hatching (data archived at Dryad DOI:10.5061/dryad.8f14k). We fit an allometric relationship between embryo radius and temperature corrected metabolic rate before hatching (Fig. S2), where $N_0 = a_1 R^{a2}$ ($a_1 = 140.0 \ \mu gO_2 \ embryo^{-1} \ h^{-1} \ cm^{-a2}$ and $a_2 = 2.94$, $r^2 = 0.91$) and $N = N_0 e^{dT}$. The allometric relationship explained most of the variance in embryonic oxygen demand, however, given the vast differences in embryo sizes, any particular observation could vary by a factor of 2–3.

Using the parameter values specified above, we compared the predicted oxygen limitation temperatures to observed development temperatures of ~180 fish species varying broadly in egg size (~0.04–0.4 cm in radius) and ecology (e.g. marine and freshwater; pelagic, benthic, and buried) compiled from three sources (Ware 1975; Pauly & Pullin 1988; and the
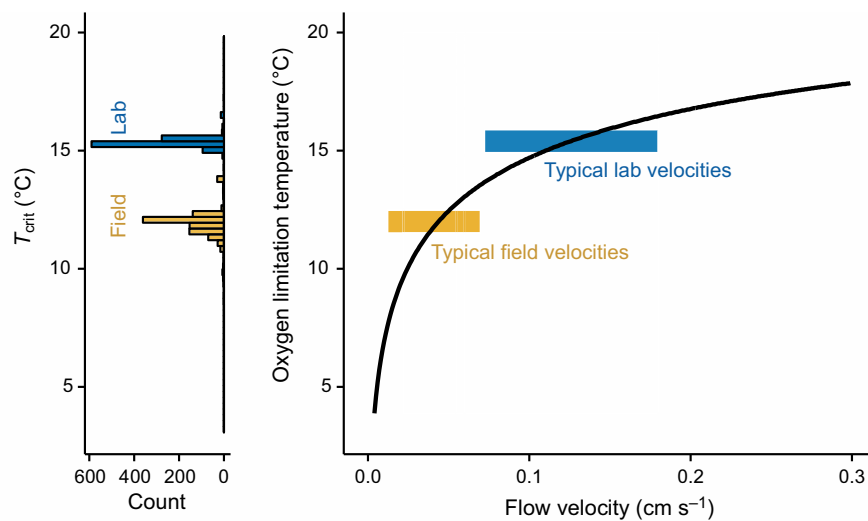

Figure 4 Comparison of statistically (left) and mechanistically (right) determined thermal tolerances in lab (blue) and field (gold) contexts. The left panel shows the distribution of parameter estimates for T_{crit} (the temperature above which embryos experience elevated thermal mortality) when fit using lab (blue) and field (gold) data. The right panel shows the mechanistically determined oxygen limitation temperature (the temperature above which oxygen supply is insufficient to meet demand) for a Chinook embryo at a size near hatching (0.05 g) as a function of the velocity of the surrounding water at 100% oxygen saturation. Ranges of flow velocities typically found in lab (shaded blue region, from USFWS 1999, Murray and McPhail 1988; Jenson and Groot 1991) and field (shaded gold region, from Zimmerman and LaPointe 2005) contexts respectively.

STOREFISH database [Teletchea *et al.* 2007, 2009]). The values used reflect temperatures in which the embryos have been observed to develop, but not necessarily their upper limit. Our simple model, based on the physics of oxygen diffusion, captured several salient patterns of variation: (1) observed developmental temperatures of species were negatively related to their radius (ANCOVA $P < 0.0001$; see Appendix S1 for details on statistical test); (2) for a given size, species that undergo embryonic development in high flow environments (e.g. spawn in rivers or exhibit parental nest fanning) tended to have a higher thermal tolerance than species in low flow environments (ANCOVA; $P = 0.0003$; see Appendix S1 for details on statistical test); (3) the observed developmental temperatures of species were clustered around the predicted thermal limits for a given egg size and developmental flow regime (Fig. 5). These findings suggest that oxygen-mediated temperature limitation may be a common problem in oviparous fishes.

DISCUSSION

Our analysis underscores the potential pitfalls of extrapolating phenomenological models of thermal stress from the lab to the field. Our lab-parameterised model of thermal tolerance failed to predict the substantial temperature-dependent mortality revealed by our field analysis. In the case of Sacramento River Chinook salmon, field-derived estimates of thermal tolerance can be used to re-evaluate temperature management actions to protect this endangered salmon population. However, for most species, the necessary field survival data are not available. For these species, it is necessary to understand the mechanisms that underlie thermal tolerance to translate measures in laboratory settings to variable environmental conditions.

We found strong support for the role of oxygen limitation as the mechanism underlying the thermal tolerance of fish embryos. While the physics of mass transfer to a sphere have been worked out for a half a century (Daykin 1965), the theory has not been extensively applied to predict the thermal tolerance of embryos. The model is elegantly simple, requiring only four biological parameters to predict thermal tolerance as a function of egg size, dissolved oxygen, and flow velocity: two to specify temperature-dependent oxygen demand (b_0 and b_1 in eqn 11), as well as the critical oxygen tension (P_i^*) and mass transfer coefficient of the embryo (k_e). All four of these parameters can be measured using simple respirometry techniques (Rombough 1989). Furthermore, these four parameters vary little enough across species such that we were able to predict thermal tolerance from egg size alone. Our model explained 91% of the variation in embryonic oxygen demand across species (Fig. S2) using fixed oxygen demand parameters
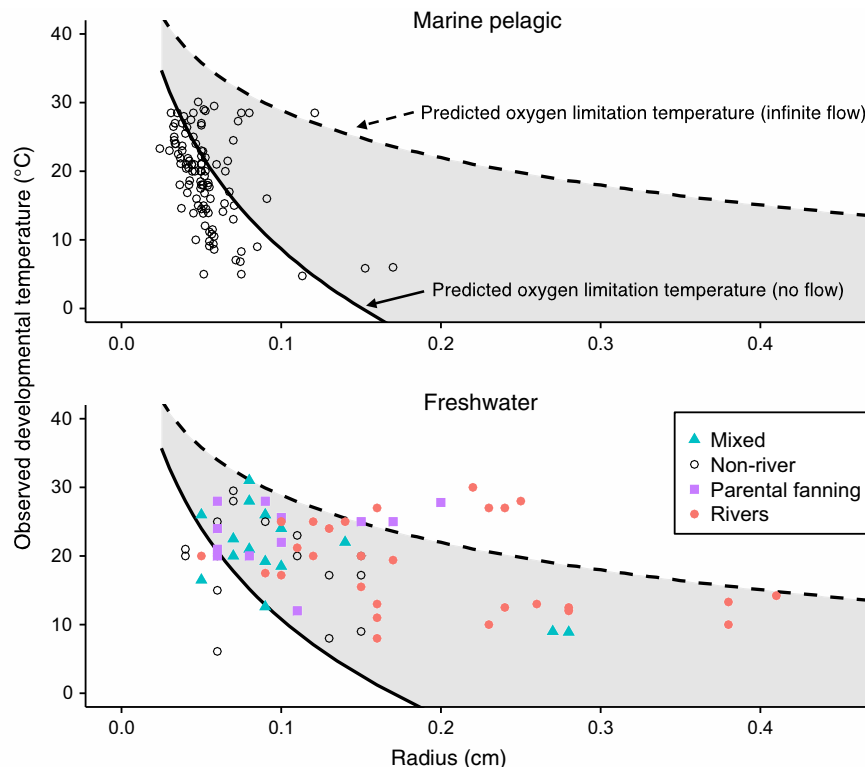

Figure 5 Predictions of oxygen limitation temperature as a function of egg size (radius) and flow regime. The solid black line is the predicted temperature at which oxygen becomes limiting in stagnant water and dashed black line is the oxygen limitation temperature in infinitely fast flowing water. The top panel compares predicted oxygen limitation temperatures with observed developmental temperatures of pelagic marine embryos (Ware 1975; and Pauly & Pullin 1988). Bottom panel are freshwater spawning species separated by groups (river spawners, non-river spawners, mixed (spawns both in river and non-river habitats) spawners, and parental fanners) from the STOREFISH database (Teletchea *et al.* 2007, 2009). Observed development temperatures do not necessarily reflect the upper thermal limits of species. When multiple developmental temperatures or egg sizes were recorded for a species, we used the maximum non-lethal developmental temperature recorded and the mean egg size.

(b_0 and b_1 in eqn 11). The remaining two parameters, P_i^* and k_e, were relatively constant for different species of salmonids (Rombough 1989). Although further work is needed to evaluate these parameters for a wider subset of species, we were able to capture multiple patterns related to the thermal tolerance of embryos by fixing these parameters to the estimates for Chinook salmon. Our results add theoretical support to a growing body of empirical evidence of the importance of oxygen limitation in determining the thermal tolerance of aquatic species. More importantly, 97% of the world's fish species and many other aquatic taxa are oviparous (Avise 2013), and our model allows for testable quantitative predictions for how thermal tolerance will scale with embryo size and environmental conditions for this critical developmental stage.

The physics of diffusion and flow set an upper limit on the metabolic rate an embryo of a given size can sustain. Our analysis of observed developmental temperatures suggests that the metabolic demand of most embryos frequently approach this limit (Fig. 5). This is likely due to the significant tradeoffs required to increase thermal resilience. Species could increase k_e (the mass transfer coefficient for oxygen transfer to the embryo) or decrease P_i^* (oxygen partial pressure at which aerobic metabolism is limited), however, the consistency of these parameters across species suggest their limited capacity for change to improve oxygen supply. For example, k_e has likely evolved to be as high as possible while still maintaining structural integrity. P_i^* is obviously constrained to positive values and dropping P_i^* from ~5–7 kPa to 0 would only boost thermal tolerance by a 3–4 °C. Otherwise, fish only have two options to increase embryonic thermal tolerance. They could produce smaller eggs, resulting in smaller, less developed larvae. Or, they could produce eggs with a lower volume-specific metabolic rate, resulting in longer development time during which they are vulnerable to predation (McGurk 1986). Considering the drawbacks associated with either option, it is perhaps not surprising that when thermal stress is relaxed in cooler waters, species have responded by producing larger eggs (Ware 1975; Pauly & Pullin 1988). Moreover, many species with embryonic stages occurring in environments with high flow velocities (such as rivers), take advantage of the improved capacity for oxygen supply by producing larger eggs. In other cases, species that produce larger eggs in warm environments can compensate by fanning their eggs, with potentially large costs in terms of energy, time and predation risk. To the degree that oxygen-mediated temperature limitation has played an important selective role in the evolution of egg size in fishes, the concurrent warming and declining oxygen content of water bodies may increase selective pressures on fishes that favour smaller eggs in the future, with likely negative consequences on productivity.

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DATA ACCESSIBILITY

Should the manuscript be accepted, the data supporting the results will be archived in Dryad.

AUTHOR CONTRIBUTION

BTM designed the study, developed the models, performed the analysis and wrote the first draft of the manuscript. EMD, JR and STL contributed to project design. AP and SJ produced the river temperature model output. NH assisted with model development, SJ assisted with analysis and EMD assisted with manuscript development. BTM, EMD, JR, STL and AP contributed to editing the manuscript.

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