Combined effects of warming and hypoxia on early life stage Chinook salmon physiology and development

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Key words: Chinook salmon, hypoxia, climate change, temperature, developmental physiology

Lay summary (50 words): Salmonids are particularly susceptible to warming and hypoxia during development in redds. We reared Chinook salmon embryos and larvae under chronic warming and hypoxia to evaluate the effects of each stressor individually and their interaction. Warming and hypoxia affected survival, physiological performance and development with management implications for salmon conservation.

Total word count: 6,234

Abstract

Early life stages of salmonids are particularly vulnerable to warming and hypoxia, which are common stressors in hyporheic, gravel bed, rearing habitat (i.e. a 'redd'). With the progression of global climate change, high temperatures and hypoxia may co-occur more frequently within redds, particularly for salmonid species at their southern range limit. Warming and hypoxia have competing effects on energy supply and demand, which can be detrimental to energy-limited early life stages. We examined how elevated temperature and hypoxia as individual and combined stressors affected the survival, physiological performance, growth, and development of Chinook salmon (*Oncorhynchus tshawytscha*). We reared late fall-run Chinook salmon from fertilization to the fry stage in a fully factorial design of two temperatures [10°C (ambient) and 14°C (warm)] and two oxygen levels [normoxia (100% air saturation, 10 mg O_2/l) and hypoxia (50% saturation, 5.5 mg O_2/l)]. Rearing in hypoxia significantly reduced hatching success, especially in combination with warming. Both warming and hypoxia improved acute thermal tolerance. While rearing in hypoxia improved tolerance to acute hypoxia stress, warming reduced hypoxia tolerance. Hypoxia-reared fish were smaller at hatch, but were able to reach similar sizes to the normoxia-reared fish by the fry stage. High temperature and normoxia resulted in the fastest rate of development while low temperature and hypoxia resulted in the slowest rate of development. Despite improved physiological tolerance to acute heat and hypoxia stress, hypoxia-reared embryos had reduced survival and growth, which could have larger population-level effects. These results suggest that both warming and hypoxia are important factors to address in conservation strategies for Chinook salmon.

Introduction

Increasing water temperatures resulting from climate change are predicted to be problematic for numerous species, particularly for fishes such as Pacific salmonids, which require cool, flowing, highly oxygenated water (Moyle, 2002). The Central Valley watershed of California supports the southernmost populations of Chinook salmon (*Oncorhynchus tshawytscha)*, and is projected to see large, consistent temperature increases nearing 5°C this century (Hayhoe *et al*., 2004; Dettinger, 2005). In addition to warming, hypoxia (low dissolved oxygen [DO] in the environment) is rapidly becoming more prevalent globally because of climate change and anthropogenic influences, such as eutrophication from agriculture and sewage runoff (Diaz, 2001; Diaz and Rosenberg, 2008; Breitburg *et al.,* 2018). Warming and hypoxia are likely to co-occur, and oxygen is less soluble in warmer water (Keeling *et al.,* 2010; Helm *et al.,* 2011). In California, the effects of climate change have been exacerbated by prolonged drought as warming and low water flows increase water temperatures and thus the potential for hypoxia to occur (Hanak *et al.,* 2015). While the effects of each stressor on animal physiology have been studied in depth individually, there is a greater need to study the interaction between the two stressors in environmentally relevant scenarios (Crain *et al.,* 2008; Todgham and Stillman, 2013; Gunderson *et al.,* 2016).

Both warming and hypoxia are common stressors within the microhabitat of salmon redds, the gravel nests where embryos and larvae develop within the streambed. Temperature and DO within redds are influenced by numerous abiotic and biotic factors including intragravel flow velocity, sedimentation, gravel size, groundwater upwelling, and oxygen consumption by developing embryos or other organic matter present (Acornley, 1999; Greig *et al.,* 2007a; Sear *et al.,* 2014). Hypoxia within redds has been correlated with detrimental effects on survival and

66 growth of developing salmonids in natural streams (Rubin and Glimsäter, 1996; Youngson *et al.,*
67 2004; Greig *et al.,* 2007b). The combination of warming and low DO as a result of low water
68 flows is thought to have reduced the thermal tolerance, and thus survival, of Chinook salmon
69 embryos in the Sacramento River (Martin *et al.,* 2017).

70 From a physiological perspective, warming and hypoxia are likely to interact through
71 contrasting effects on energy metabolism. Temperature is a controlling factor that determines
72 metabolic rates in ectotherms, whereas oxygen is a limiting factor that restricts metabolic rate
73 (Fry, 1971). Therefore, as warming increases metabolism, hypoxia limits the oxygen supply
74 available to support increased metabolic demand (McBryan *et al.,* 2013). The concept of oxygen
75 and capacity limitation of thermal tolerance (OCLTT) hypothesizes that the mismatch in oxygen
76 supply and demand can reduce thermal tolerance and affect the physiology and ecology of many
77 species (Pörtner, 2001; Pörtner, 2002). The OCLTT hypothesis predicts temperature and oxygen
78 will interact negatively to influence stress tolerance such that exposure to high temperature is
79 expected to reduce hypoxia tolerance and hypoxia is expected to reduce thermal tolerance
80 (McBryan *et al*., 2013).

81 Early life stages of Chinook salmon are particularly sensitive to both warming and
82 hypoxia, as embryos and alevins are the least thermally tolerant life stages and have little to no
83 mobility to avoid suboptimal habitat conditions (McCullough, 1999; Myrick and Cech, 2004).
84 Embryos of oviparous fish such as salmonids have stronger energy constraints than older
85 organisms because they possess a finite amount of energy in the form of yolk to support their
86 development (Rombough, 2006). Under optimal conditions during development, the majority of
87 energy is allocated towards growth. When energy supply or demand is altered, as with warming
88 or hypoxia, there is increased competition for energy between coping with stress and continued

89 growth and development (Sokolova, 2013). With a limited ability to increase aerobic metabolic
90 rate above routine levels, compensatory energy partitioning may detract energy from processes
91 necessary for development (Rombough, 2011). Therefore, the metabolic interactions between
92 warming and hypoxia may be especially detrimental during early development.

93 Developing salmon are known to be sensitive to warming and hypoxia individually but
94 are likely to experience both stressors simultaneously in their rearing environment, especially as
95 climate change progresses and local anthropogenic impacts (e.g. drought) persist. In this study,
96 we assessed the effects of chronic warming and hypoxia, on developing late fall-run Chinook
97 salmon, as individual and combined stressors. We reared salmon from fertilization through the
98 fry stage in a fully factorial design of two temperatures (10 and 14°C) and two oxygen levels
99 (100 and 50% air saturation). Throughout development we measured hatching success, growth,
100 and developmental rate as well as tolerance to acute thermal and hypoxic stress to examine the
101 lethal and sublethal responses to rearing in each treatment. We predicted that there would be
102 detrimental effects of warming and hypoxia as individual stressors that would be amplified
103 through synergistic interactions in the multiple stressor treatment due to competing effects on
104 balancing energy supply and demand. Examining the effects of two key stressors across salmon
105 development will further our understanding of the capacity of early life stage salmonids to cope
106 with multiple stressors in their natural environment and how we can better promote their survival
107 in a complex environment through water management.

108

109 **Materials and Methods**

110 Fish acquisition and care

111 Freshly fertilized late fall-run Chinook salmon embryos were obtained from four separate
112 breeding pairs spawned at the Coleman National Fish Hatchery (US Fish and Wildlife Service,
113 Anderson, CA). Embryos were transported to the University of California Davis Center for
114 Aquatic Biology and Aquaculture in January 2017. Embryos were immediately transferred to
115 their rearing treatments in one of four replicate 19l square culture buckets. Embryos were held in
116 floating mesh baskets affixed with plastic dividers creating individual wells to keep embryos
117 separated in an even layer. Embryos from all four families were evenly distributed across each
118 replicate bucket. Once alevins could sustain swimming, the baskets were removed from the
119 culture buckets. Since early developmental stages rely on endogenous yolk reserves (Kamler,
120 2008), fish were not fed during the experiment. The experiment ended when fish reached the fry
121 stage and nearly all of the yolk sac was absorbed. All fish care and protocols were reviewed and
122 approved by the UC Davis Institutional Animal Care and Use Committee (protocol no. 19593).

123 Experimental Design

124 To assess the effects of elevated temperature and decreased oxygen as individual and
125 combined stressors, we reared developing Chinook salmon from fertilization to the fry stage in
126 four treatments in a fully factorial design of two temperatures [10°C (ambient) and 14°C
127 (warm)] and two oxygen (O_2) saturation levels [normoxia (100% air saturation, 10 mg O_2/l) and
128 hypoxia (50% saturation, 5.5 mg O_2/l)]. Ambient temperature of 10°C was chosen as this is
129 within the average range of temperatures in the Sacramento River when late fall-run salmon
130 embryos are present (Bureau of Reclamation, Central Valley Operations, Sacramento River
131 Temperature Report). The warm temperature of 14°C was chosen to represent a 4°C increase of
132 water temperatures projected with climate change and is a potentially stressful, but not lethal,
133 temperature because embryo mortality increases around 16°C in Chinook salmon (Myrick and

Cech, 2004; Williams, 2006). Dissolved oxygen within natural redds can fluctuate widely between 2-11 mg O_2/l (Coble, 1961; Peterson and Quinn, 1996). Normoxia was maintained at 100% saturation to represent optimal habitat conditions and 50% was chosen as a moderate level of hypoxia that is potentially stressful, but not lethal (Silver *et al*., 1963). Two different temperature treatments were maintained by placing culture and reservoir buckets in four large water bath tanks (1.2 m in diameter) containing flow through water at the corresponding treatment temperature. Each water bath (at 10ºC or 14ºC) held two culture buckets from the normoxia and hypoxia treatments, with two replicate water bath tanks for each temperature (n=4 culture buckets per temperature and O_2 treatment combination). Oxygen saturation was manipulated using mass flow controller valves (Sierra Instruments, Monterey, CA, USA) to mix N_2 gas and air to maintain low DO in hypoxic treatments or air alone for normoxic treatments. The gas mixture was bubbled into reservoir buckets using venturi injectors (one reservoir bucket for each temperature × oxygen treatment). Equilibrated treatment water from each reservoir was then dripped into the culture buckets holding salmon at 16 l/h to ensure high turnover. Gas mixtures were also bubbled directly into culture buckets through air stones for further mixing and adjustment of DO levels within each individual bucket. Temperature and DO were measured in each culture bucket, reservoir bucket, and water bath tank daily using a handheld meter (OxyGuard Handy Polaris 2, OxyGuard International, Farum, Denmark), summarized in Table 1.

Physiological testing occurred four times during the study period for each treatment. A stage-based sampling design was chosen to account for differences in developmental rate caused by the varying temperatures and oxygen saturation levels between treatments. Sampling took place when 50% or more of embryos in a treatment reached 1) eyed stage, when dark pigmented eyes were clearly visible, 2) silver eyed stage, when silver pigment in eyes was visible, 3) alevin

stage larvae, one day after hatching, and lastly 4) fry stage, when the yolk sac was almost completely absorbed. Development of salmon was monitored daily with visual inspections of each culture bucket. Stage was assessed at the treatment level because families were equally distributed among replicates, contributing to minimal variation in developmental timing between replicates. Hatching success was calculated as the ratio between the number of alevins one-day post-hatch and the initial number of embryos per treatment. Upper thermal tolerance was assessed at each stage (eyed, silver-eyed, alevin, and fry) as critical thermal maximum (CTMax), and hypoxia tolerance (time to loss of equilibrium) was tested for fry only. At the alevin and fry stages total length and mass were recorded.

Determination of Upper Thermal Tolerance

Acute upper thermal tolerance was measured using critical thermal maximum (CTMax) methodology (Beitinger *et al*., 2000; Fangue *et al*., 2006). The endpoint used to indicate CTMax differed between embryos and larvae due to the inability of embryos to exhibit loss of equilibrium, a common endpoint for fishes after hatch (Zebral *et al*., 2018).

Embryos

Critical thermal maximum for embryos at the eyed and silver eyed stages was defined as the temperature at which the heart stopped beating, similar to Angilletta *et al.,* (2013). CTMax was determined in four embryos per replicate per treatment (16 embryos total). Embryos were placed in individual wells of a divided plastic dish with water at their corresponding rearing temperature. The plastic dish was held in a well of an aluminum block and treatment water was circulated through the aluminum block to maintain treatment temperature. Embryos were given 1h in the dishes before CTMax trials began. Circulating water was then heated using a

submersible heater and YSI Thermistemp Temperature Controller (YSI Incorporated, Yellow Springs, OH, USA) such that the water temperature in the dish increased at a rate of 0.3°C/min. Water was aerated using a pipette to ensure full oxygenation and circulation. Embryos were continuously monitored under a dissecting microscope and CTMax was recorded as the temperature when the heart was observed to stop beating for more than 30 s.

Larvae and Fry

For larvae and fry CTMax was determined for four fish per replicate per treatment (16 fish total per treatment). The apparatus consisted of a 37l aquarium containing a water heater connected to a YSI Thermistemp Temperature Controller (YSI Incorporated), a submersible pump for circulation, and eight glass chambers suspended in the aquaria. Individual fish were placed in the jars for 1h prior to the start of each trial with water at the corresponding rearing temperature. Eight fish were run at a time and jars were each continuously aerated throughout the CTMax protocol to ensure full oxygenation. After the 1h acclimation the heater was turned on and the water temperature increased at a rate of 0.3°C/min. Fish were closely monitored until they reached loss of equilibrium (LOE), defined as the point at which a fish could no longer swim upright or respond to a gentle physical stimulus. Temperature at LOE was recorded with a calibrated immersion thermometer (0.1 °C precision, Fisher Scientific), after which individuals were immediately transferred to a fully oxygenated recovery tank with water at their rearing temperature. Temperature at LOE was included in the final dataset if the individual survived a 24h recovery period.

Fry Hypoxia Tolerance

Acute hypoxia tolerance of salmon fry was measured using time to loss of equilibrium methodology (Anttila *et al.,* 2015, McBryan *et al.,* 2016). Time to loss of equilibrium was determined for four fish per replicate per treatment (16 fish per treatment). Hypoxia tolerance trials were conducted in a 37l aquarium held in a temperature-controlled water bath. The aquarium contained eight floating plastic beakers with mesh sides for individual fry and a submersible pump for water circulation. The water surface within each beaker was covered with bubble wrap to prevent surface respiration during trials. The water surface surrounding the beakers was also covered with bubble wrap to prevent diffusion of oxygen into the water during trials. DO was monitored throughout the trial using two oxygen dipping probes (PreSens Precision Sensing, Regensburg, Germany). Individual fish were placed in each beaker 30 min prior to the start of the trial to recover from handling. Fish were tested in water at the same temperature and DO level as their rearing treatment. In each trial DO of the water was reduced at a rate of 1.5-2%/min from initial oxygen levels (i.e. 100 and 50%) by bubbling in N_2 gas until 8% air saturation was reached (0.9 mg O_2/l at 10°C and 0.8 mg O_2/l at 14°C). Oxygen was then held at 8% by manually adjusting the flow of N_2. This final oxygen concentration was chosen based on pilot studies where all fish could maintain equilibrium indefinitely at 10% and there was little variation in the rapid time to LOE at 6%. Time to LOE was defined as the time (min) after DO saturation reached 8% until the fish could no longer swim upright or respond to a gentle physical stimulus. Upon achieving LOE fish were immediately transferred to fully oxygenated recovery chambers at respective rearing temperatures. Time to LOE for fish that survived a 24h recovery period were included in the final dataset. Each trial was conducted with a maximum trial time of 2h. Fish that maintained equilibrium when the 2h trial ended were assigned a time to LOE of 120 minutes and transferred to recovery.

223 Body condition factor

224 Fish at the alevin and fry stages (n=5 per replicate tank, n=20 total per treatment) were
225 euthanized in tricaine methanesulfonate (MS-222, Western Chemical, Ferndale, WA, USA),
226 weighed, and measured for total length. Alevin mass measurements included the yolk sac. Body
227 condition was used to compare overall size differences between treatment. Fulton's condition
228 factor (K) was calculated as:

229 $$K = 100\, x\, \frac{W}{L^3}$$

230 where W is the wet mass in grams and L is the total length of the fish in cm.

231

232 Statistical analyses

233 Statistical analyses were performed using R Studio (v3.3.0, http:// www.R-project.org).
234 Datasets were visually inspected for assumptions of normality and homogeneity of variances
235 using Q-Q plots and residuals vs. fitted values. All data were normally distributed and met the
236 assumptions of the tests used unless otherwise noted. Data are reported as means ± SEM with α
237 set at 0.05. Hatching success, time to LOE under hypoxia, and condition factor were analyzed as
238 dependent variables using a two-way analysis of variance (ANOVA) with temperature and
239 oxygen saturation as fixed factors. Post hoc tests for two-way ANOVA were carried out using
240 TukeyHSD. CTMax was analyzed using a three-way ANOVA with temperature, oxygen
241 saturation, and developmental stage as fixed factors. Since different CTMax methodologies were
242 used for embryo stages (cardiac cessation [eyed and silver-eyed]) and post-hatch stages (LOE
243 [alevin and fry]), a separate ANOVA was conducted for each. Post hoc tests for three-way

ANOVA were carried out using a Tukey's test ('lsmeans' package, Lenth, 2016). Initial models nested fish within their corresponding replicate treatment buckets; however, with no significant effects, replicate was removed as a factor to reduce models to their simplest form. Condition factor of alevins did not meet assumptions of homogeneity of variance and was log transformed.

Results

Hatching success

Rearing under hypoxia significantly reduced the percentage hatched ($F_{1,12}$=37.3, $p<0.001$). Percentage hatched was highest for embryos reared in normoxia with ~40% (40.5 ± 2.6) hatching success at 10°C and ~35% (35.1 ± 4.4) hatching success at 14°C (Fig. 1). At 10°C, embryos reared in the hypoxia treatment had 50% lower hatching success compared to the normoxia treatment (19.8% ± 4.4 vs. 40.5%). Although temperature did not significantly affect hatching success ($F_{1,12}$=4.19, p=0.06) and there was no significant interaction between temperature and oxygen ($F_{1,12}$=0.36, p=0.56), hatching success was lowest in the multiple stressor treatment of hypoxia and 14°C with only ~10% hatched (9.9% ± 3.4).

Upper thermal tolerance

Embryos

Upper thermal tolerance was highly variable across treatments and development (Fig. 2A). There was a significant two-way interaction between temperature and oxygen ($F_{1, 120}$=8.36, p=0.005). In addition, a significant three-way interaction ($F_{1,120}$=36.30, $p<0.001$) between the

main effects of temperature ($F_{1, 120}$=12.05, p<0.001), oxygen saturation ($F_{1, 120}$=145.44, p<0.001), and developmental stage ($F_{1, 120}$=67.1, p<0.001) indicated salmon CTMax was dependent on the life stage and stressors. For example, eyed stage embryos reared under hypoxia at both temperatures had the highest thermal tolerance with a CTMax of 30.6°C ± 0.6 at 10°C and 30.7°C ± 0.2 at 14°C. Eyed embryos reared at 14°C in normoxia had the lowest CTMax (27.9°C ± 0.2) and 10°C normoxia reared embryos had an intermediate thermal tolerance (28.9°C ±0.2). Thermal tolerance significantly increased at the silver eyed stage for 10°C hypoxia and 14°C normoxia treatments. The 10°C hypoxia treatment had the highest CTMax (32.8°C ±0.1) with both hypoxia treatments again being the most thermally tolerant. Silver eyed embryos in the 10°C normoxia treatment had the lowest CTMax (29.6°C ± 0.2) and 14°C normoxia was intermediate (30.4°C ± 0.3).

Larvae and Fry

There were significant interactions between temperature, oxygen saturation, and developmental stage ($F_{1,111}$=7.68, p=0.007) in the thermal tolerance of the post-hatch alevin and fry stages (Fig. 2B). There were significant two-way interactions between temperature and oxygen ($F_{1,111}$=29.52, p<0.001), temperature and stage ($F_{1,111}$=15.71, p<0.001), and oxygen and stage ($F_{1,111}$=4.89, p=0.029). Temperature had a significant effect on CTMax ($F_{1, 111}$=11.45, p<0.001) but there was no effect of oxygen saturation ($F_{1,111}$=0.7, p=0.40) or developmental stage ($F_{1,111}$=0.39, p=0.53). At the alevin stage, the normoxia and hypoxia treatments at 14°C had the highest (29.7°C ± 0.1) and lowest CTMax (28.4°C ± 0.2), respectively, with the alevins reared at 10°C having intermediate CTMax (29.2°C ± 0.2 vs. 28.8°C ± 0.2 for hypoxia and normoxia treatments, respectively). Upon reaching the fry stage CTMax significantly increased in only the 14°C hypoxia treatment (increased to 29.3°C ± 0.1) such that it was no longer

significantly different from the 14°C normoxia treatment. Both 14°C treatments had the highest CTMax, while the 10°C normoxia treatment had the lowest (28.4°C ± 0.3) CTMax.

Fry Hypoxia Tolerance

Hypoxia tolerance was only measured at the fry stage, when the fish had absorbed nearly all of the yolk sac. Rearing in hypoxia significantly increased time to LOE ($F_{1,54}$=6.49, p=0.014) while rearing at 14°C significantly decreased time to LOE ($F_{1,54}$=91.74, p< 0.001) (Fig. 3). Oxygen and temperature did not significantly interact ($F_{1,54}$=0.35, p=0.56). Fish reared at 14°C in normoxia maintained equilibrium for ~20 minutes (20.4 ± 3.3) compared to ~36 minutes (36.3 ± 12) for fry reared at 14°C in hypoxia and ~94.5 minutes (± 11) for fry reared at 10°C in normoxia. Fry reared at 10°C in hypoxia were the most tolerant to hypoxia and all maintained equilibrium indefinitely during the 2-h trial period at 8% air saturation (120 min).

Growth

Alevins reared in hypoxia had a significantly higher Fulton's condition factor ($F_{1,75}$=37.51, p<0.001) compared to alevins reared under normoxic conditions (Fig. 4). There was no significant interaction between temperature and oxygen on alevin condition factor ($F_{1,75}$=0.39, p=0.53). Upon reaching the fry stage there were no significant differences in condition factor between treatments. Temperature ($F_{1,70}$=1.004, p=0.32) and oxygen saturation ($F_{1,70}$=0.44, p=0.51) did not significantly affect Fulton's condition factor, although there was a significant interaction between the two stressors ($F_{1,70}$=7.62, p=0.007) (Fig. 5), where warming decreased condition factor in hypoxia-reared fish.

310

311 Developmental Rate

312 Developmental rate was assessed at the treatment level because there was very little
313 variation between replicate buckets within a treatment. Fish developed faster at 14°C (Table 2).
314 Under normoxia, fish reared at 14°C reached each stage 7-10 days before fish reared at 10°C.
315 Rearing in hypoxia further delayed development within each temperature. At 14°C rearing in
316 hypoxia delayed development by 4-6 days depending on the stage, although hypoxia-reared fish
317 hatched just one day after normoxia-reared fish. At 10°C fish reared in hypoxia reached each
318 stage 4-10 days later than in normoxia, depending on the stage.

319 **Discussion**

320 This study investigated how Chinook salmon development is influenced by the
321 interaction between warming and hypoxia, two common stressors in salmonid rearing
322 environments that can co-occur. Acclimation to elevated temperature and hypoxia improved
323 acute thermal tolerance and hypoxia acclimation also improved tolerance to acute hypoxic stress.
324 Despite improved physiological performance with chronic rearing under elevated temperature
325 and hypoxia, hypoxia reduced early growth and hatching success, especially in combination with
326 warming. Reduced growth and hatching success could lead to detrimental effects at the
327 population level as climate change progresses.

328 ***Hatching success***

329 The hatching process in fish embryos is a critical period during development and is
330 strongly influenced by both temperature and oxygen (Yamagami, 1988; Korwin-Kossakowski,
331 2012). In the present study, warm temperature alone minimally reduced hatching compared to

controls, which is not surprising given that California Central Valley Chinook salmon embryos can tolerate temperatures up to 16°C (Myrick and Cech, 2004; Williams, 2006). Fish embryos are particularly susceptible to low DO in their environment during the critical period of hatching (Dudley and Eipper, 1975; Keckeis *et al.,* 1996). Here, rearing in hypoxia markedly reduced hatching success by ~50-75% at 10°C and 14°C, respectively (Fig. 1). The majority of this mortality occurred within a day or two of the mean hatch date for a given treatment, consistent with observations in hypoxia-reared lake trout (Garside, 1959; Carlson and Siefert, 1974) and largemouth bass (Dudley and Eipper, 1975). The mortality observed at hatch often occurred in partially hatched embryos where individuals were able to free their heads from the chorion but were unable to fully escape, suggesting the physical process of hatching was more challenging in hypoxia.

Hatching is an energetically costly process due to increased movement and oxygen consumption (Hamor and Garside, 1959; Ninness *et al.*, 2006). With a limited capacity for anaerobic metabolism in embryos (Rombough, 2011), hatching may increase aerobic energy demand to a level that cannot be matched by energy supply under hypoxic conditions (Polymeropoulos *et al.,* 2016). Warmer water temperatures increase the metabolic rate, and thus oxygen demand, of embryos. Combined with the additional energy required for hatching, the mismatch between energy supply and demand may have been greatest in the multiple stressor treatment of 14°C hypoxia, which had the lowest hatching success (Fig. 1). Of note, embryos reared in normoxia at 10°C had an unexpectedly low hatching success rate for control conditions (~40%). The overall low percentage hatched was likely influenced by unusually high mortality observed in one family of embryos, possibly due to poor embryo quality.

Upper thermal tolerance

Many fish species have some degree of plasticity in thermal tolerance (Beitinger *et al.,* 2000), such that upper thermal tolerance commonly increases with acclimation to warmer temperatures (e.g. Healy and Schulte, 2012; Anttila *et al*., 2015). In the present study, the effects of warming were largely dependent on developmental stage. Consistent with results from other studies of warm acclimation in fishes, alevins and fry reared at 14°C under normoxia had the highest CTMax. In contrast to what would be predicted, eyed embryos (the first stage measured) reared at 14°C in normoxia had a lower CTMax than the equivalent stage reared at 10°C in normoxia (Fig. 2). The 10°C normoxia treatment consistently had the lowest or second lowest CTMax in both the embryonic and post-hatch stages when comparing all the treatment groups, as expected with acclimation to a lower rearing temperature. Thermal tolerance is often life stage specific (Komoroske *et al.,*2014), particularly in fishes that occupy different habitats throughout development such as Pacific salmon (McCullough, 1999; Richter and Kolmes, 2005). Salmon embryos develop in cold streams and are therefore likely to be more sensitive to warming at this stage. Embryos at 14°C may have been near their thermal limit, such that they were less able to allocate energy to stress tolerance mechanisms to the extent that other treatments could.

Oxygen limitation of thermal tolerance hypothesizes that CTMax will be lower when exposed to environmental hypoxia. Consistent with OCLTT, reduced upper thermal tolerance following acclimation to hypoxia has been observed in many studies (e.g. Rutledge and Beitinger, 1989; Healy and Schulte, 2012; Ellis *et al.*, 2013). In contrast, the CTMax of 10°C hypoxia-reared embryos and larvae in the present study were consistently higher than the CTMax of 10°C normoxia-reared fish at all developmental stages. The 10°C hypoxia-reared embryos were surprisingly thermally tolerant with the highest CTMax. Alevins and fry reared at 10°C in hypoxia maintained a higher CTMax compared to 10°C normoxia reared fish, but had a

lower CTMax than fry reared at 14°C in either oxygen treatment suggesting a stronger effect of acclimation temperature on the thermal tolerance of post-hatch stages. There is mixed support of the OCLTT hypothesis. In some cases, CTMax is independent of oxygen availability, unaffected by chronic hypoxia, or species-specific in relation to hypoxia (e.g. Ern *et al.*, 2016; Motyka *et al.*, 2017; Verberk *et al.*, 2018). CTMax can be maintained in moderate levels of hypoxia, such as those maintained in this study, even in stenothermal species (Ern *et al*., 2017); however, the improvement of CTMax with acclimation to hypoxia as observed in the present study is unexpected.

The multiple stressor treatment of 14°C hypoxia had a relatively high CTMax throughout development with the exception of the alevin stage. Alevins reared under conditions of elevated temperature and reduced oxygen levels had the lowest CTMax at that stage. Low CTMax might reflect recovery from hatching one day prior to the upper thermal tolerance trials because the lowest hatching success was observed in this multiple stressor treatment. The improved upper thermal tolerance observed with hypoxic acclimation at 10°C appeared to be limited at 14°C in the silver-eyed, alevin, and fry stages. While the multiple stressor treatment maintained a high thermal tolerance, it did not consistently exceed the CTMax of 14°C normoxia-reared fish at these stages in the way 10°C hypoxia-reared fish had a higher CTMax than 10°C normoxia-reared fish at each stage. Additionally, CTMax did not increase from the eyed to silver-eyed stages at 14°C under hypoxia as all other treatments did, suggesting this treatment was near the fish's thermal limit. Mechanisms to cope with hypoxia include adjustments to increase oxygen uptake at the gills and improve transport to increase the supply of oxygen to tissues, as well as reductions in metabolic rate to decrease oxygen demand (Miller *et al.,* 2008; Richards, 2009; Polymeropoulos *et al.,* 2016). Since upper thermal tolerance can benefit from improved oxygen

delivery, the mechanisms underlying long-term acclimation to hypoxia can also maintain or improve thermal tolerance (Burleson and Silva, 2011; Motyka *et al*., 2017). It should be noted that all CTMax trials were conducted in normoxic conditions, so embryos acclimated to hypoxia may have been more thermally tolerant in part because of an increased availability of oxygen during the CTMax trials, compared to acclimation conditions. Although the exact mechanisms leading to improved CTMax under acclimation to warming and hypoxia were not examined here, it is likely that physiological adjustments to rearing in hypoxia could be responsible for increased upper thermal tolerance.

Hypoxia tolerance in fry

Within the OCLTT framework elevated temperatures are predicted to decrease tolerance to acute hypoxia (McBryan *et al*., 2013). Consistent with the OCLTT, the time to loss of equilibrium in hypoxia was significantly shorter in fish reared at 14°C compared to 10°C, indicating reduced hypoxia tolerance with warming (Fig. 3). Lower hypoxia tolerance at warmer temperatures has been observed in many other studies (Nilsson *et al.,* 2010; Barnes *et al*., 2011; Remen *et al*., 2013; McDonnell and Chapman, 2015; Borowiec *et al*., 2016), although it varies by species (e.g. He *et al*., 2015). Higher temperatures are thought to reduce hypoxia tolerance by increasing metabolic rates and in turn, oxygen demand (Pörtner, 2010), and may also decrease the oxygen binding affinity of hemoglobin, thereby reducing oxygen supply (McBryan *et al*., 2013). Further study on early stages of Chinook salmon are needed to better understand mechanistically how warming is reducing hypoxia tolerance in fry and whether this is consistent at other developmental stages.

Rearing in hypoxia improved tolerance to acute hypoxia at both temperatures compared to the normoxia treatments. The combination of low temperature and hypoxia resulted in the

highest tolerance to hypoxia as the fish in the 10°C hypoxia treatment did not lose equilibrium within the 2-h trial period. In contrast, the 14°C normoxia treatment had the lowest tolerance to hypoxia suggesting that, although not statistically significant, acclimation to elevated temperature and hypoxia interact to influence hypoxia tolerance. Improvement of hypoxia tolerance following short-term (24-48 h) exposure to hypoxia has been observed in zebrafish (Rees *et al.,* 2001), spot and Atlantic menhaden (Shimps *et al.,* 2005), and goldfish (Fu *et al.,* 2011), while longer acclimation periods of 4-6 weeks improved hypoxia tolerance in sailfin molly (Timmerman and Chapman, 2004), but not Atlantic cod (Petersen and Gamperl, 2010) or Atlantic salmon (Remen *et al.,* 2013). Acclimation to hypoxia can involve a number of mechanisms such as improved oxygen uptake and transport through changes in gill morphology, concentration of red blood cells and hemoglobin, as well as alterations to cellular energy metabolism (Farrell and Richards, 2009; Borowiec *et al.* 2015). Our results suggest that Chinook salmon fry also have the capacity to acclimate to hypoxia during chronic exposure, although the degree of improved hypoxia tolerance is temperature dependent.

Growth and development

Reduced growth and delayed development in hypoxia are compensatory responses where metabolic demand is adjusted to match the oxygen supply available (Rombough, 1988a). Despite having higher condition factor, hypoxia-reared alevins were smaller due to less body tissue length and more yolk retained at the time of hatch (Fig. 4), similar to observations by Polymeropoulos *et al.,* (2017) in hypoxia-reared Atlantic salmon. A reduction in size of post-hatch hypoxia-reared larvae has been observed in many other studies (Alderdice *et al*., 1958; Garside, 1959; Shumway *et al*., 1964; Marks *et al*. 2012). Growth is the most energetically demanding activity in early embryonic development and is almost entirely dependent on aerobic

447 metabolism (Rombough, 2011). While the ecological significance of size at hatch is difficult to
448 determine, alevins that are smaller at hatch may have lower chances of survival due to size
449 selective predation pressure, decreased competitive ability, and slower swimming speeds
450 (Mason, 1969; Pepin, 1991). Given the challenges of predicting the effects of size on survival,
451 size is best considered alongside performance (Conover and Schultz, 1997; Green and Fisher,
452 2004). Although hypoxia reared alevins were smaller, they had higher or comparable CTMax
453 throughout development and higher hypoxia tolerance as fry. Despite their smaller size, the
454 physiological performance of hypoxia reared larvae was not hindered. Upon reaching the fry
455 stage there were no significant differences in condition factor between treatments (Fig. 5).
456 However, it took hypoxia reared fry 6-10 days longer to reach the fry stage and fully absorb the
457 yolk sac.

458 Developmental rate in fish embryos is highly dependent on both temperature and DO in
459 the rearing environment (Murray and McPhail, 1987; Beacham and Murray, 1990). Decreased
460 temperature leads to slower development in many fish species (Pepin, 1991; Green and Fisher,
461 2004). As expected in the present study, development was delayed by 7-10 days in 10°C
462 normoxia compared to 14°C normoxia (Table 2). Low oxygen is also known to further delay
463 development (Garside, 1966). Consequently, rearing in hypoxia delayed development at both
464 temperatures compared to rearing in normoxia. The developmental delay increased from 4-5
465 days during the embryonic stages to 6-10 days to reach the post-hatch stages, as in Geist *et al.,*
466 (2006), with the exception of the 14°C hypoxia treatment time to hatch.

467 Low oxygen can have two opposite effects on time to hatch (Carlson and Siefert, 1974;
468 Ciuhandu *et al.,* 2005; Hassell *et al.,* 2008), both of which appear to have occurred in this study,
469 dependent on acclimation temperature. Hypoxia slows the overall rate of development extending

the time to hatch as with the 10°C hypoxia treatment hatching 6 days after the 10°C normoxia treatment. However, hypoxia can also reduce the time to hatch. As embryonic development progresses, metabolic rate increases until ambient oxygen can no longer meet metabolic oxygen demand (Rombough, 1988b). Low oxygen is an important natural signal to hatch in fish embryos (Czerkies *et al*., 2001) and acute hypoxia can trigger hatching in mature embryos (Oppen-Berntsen *et al*., 1990). Thus, hypoxia can also trigger premature hatching when oxygen becomes limited before embryos are fully developed (DiMichele and Powers, 1984; Latham and Just, 1989). Given the increased metabolic demand at 14°C, early oxygen limitation may explain why embryos reared at 14°C in hypoxia hatched just one day after those in 14°C normoxia when the hypoxia treatment reached all other stages multiple days later. Similarly, precocious hatching resulting from acute hypoxia exposure was greatest at high temperature in whitefish embryos (Czerkies *et al*., 2001).

Conclusions

Late fall-run Chinook salmon in the Central Valley of California are listed as a Species of Concern under the federal Endangered Species Act and occupy some of the same river habitat as threatened and endangered Chinook salmon runs (i.e. threatened spring-run and endangered winter-run). Survival of wild Central Valley salmon embryos can be highly variable but is generally low, with average egg to fry survival likely in the tens of percent (Williams, 2006). A further decrease in hatching success resulting from hypoxia, as demonstrated in this study, could potentially have large impacts on population size as a whole if hypoxia is widespread throughout the rearing habitat. Martin *et al.* (2017) suggested interactions between high temperatures and low dissolved oxygen contributed to high embryo mortality in winter-run Chinook salmon, an

endangered run with a population of less than 1,000 estimated to be in the Sacramento River during the 2017 spawning season (Azat, 2018).

The delayed developmental rate in hypoxia may have larger phenological consequences as there may be selection against late emerging salmon (Einum and Fleming, 2000). For fish that do survive hatching in hypoxia there is a potential tradeoff between a smaller size at hatch and being more tolerant to acute thermal and hypoxic stressors. Smaller salmon may be more vulnerable to predation in the Sacramento-San Joaquin Delta where predation on juvenile Chinook salmon by abundant native and non-native fish predators is high (Grossman, 2016). Salmon reared at high temperature were more thermally tolerant, but less hypoxia tolerant, while hypoxia reared salmon were more tolerant to both temperature and hypoxia compared to normoxia reared fish suggesting a capacity to acclimate to warming and hypoxia during early life stages. The exact mechanisms underlying the acclimation capacity at these early stages, as well as the potential for persistent or latent physiological effects of exposure to warming and hypoxia during early development warrant further investigation.

Funding

This work was supported by the California Agricultural Experimental Station of the University of California Davis [grant numbers CA-D-ASC-2252-H and CA-D-ASC-2253-RR to AET and CA-D-ASC-2098 to NAF], National Science Foundation Graduate Research Fellowship [grant number 1650042 AMD] and a Fly Fishers of Davis scholarship to AMD.

Acknowledgements

We thank the Coleman National Fish Hatchery for providing salmon embryos. Additionally, we thank D. Cocherell for assistance with equipment and facilities maintenance, as well as undergraduates G. Mukai and L. Olano, and Todgham lab members for assistance with fish care and data collection.

References

Acornley RM (1999) Water temperatures within spawning beds in two chalk streams and implications for salmonid egg development. *Hydrol Process* 13: 439-446.

Alderdice DF, Wickett WP, Brett JR (1958) Some effects of temporary exposure to low dissolved oxygen levels on Pacific salmon eggs. *J Fish Res Bd Can* 15: 229-75.

Anttila K, Lewis M, Prokkola JM, Kanerva M, Seppänen E, Kolari I, Nikinmaa M (2015) Warm acclimation and oxygen depletion induce species-specific responses in salmonids. *J Exp Biol* 218: 1471-1477.

Azat, J (2018) GrandTab 2018.04.09 California Central Valley Chinook Population Database Report. California Department of Fish and Wildlife.

Barnes RK, King H, Carter CG (2011) Hypoxia tolerance and oxygen regulation in Atlantic salmon, *Salmo salar* from a Tasmanian population. *Aquaculture* 318: 397-401.

Beacham TD, Murray CB (1990) Temperature, egg size, and development of embryos and alevins of five species of Pacific salmon: a comparative analysis. *Trans Ameri Fish Soc* 119: 927-945.

Bureau of Reclamation (2018) Sacramento River temperature report. https://www.usbr.gov/mp/cvo/temperature.html. (last accessed 1 August 2018).

Beitinger TL, Bennett WA, McCauley RW (2000) Temperature tolerances of North American freshwater fishes exposed to dynamic changes in temperature. *Environ Biol Fish* 58: 237-75.

Borowiec BG, Darcy KL, Gillette DM, Scott GR (2015) Distinct physiological strategies are used to cope with constant hypoxia and intermittent hypoxia in killifish (*Fundulus heteroclitus*). *J Exp Biol* 218: 1198-1211.

Borowiec BG, Crans KD, Khajali F, Pranckevicius NA, Young A, Scott GR (2016) Interspecific and environment-induced variation in hypoxia tolerance. *Comp Biochem Physiol A Mol Integr Physiol* 198: 59-71.

Breitburg D, Levin LA, Oschiles A, Grégoire M, Chavez FP, Conley DJ, Garçon V, Gilbert D, Gutiérrez D, Isensee K, *et al*. (2018) Declining oxygen in the global ocean and coastal waters. *Science* 359: Issue 6371, eaam7240, DOI: 10.1126/science.aam7240

Burleson ML, Silva PE (2011) Cross tolerance to environmental stressors: Effects of hypoxic acclimation on cardiovascular responses of channel catfish (*Ictalurus punctatus*) to a thermal challenge. *J Therm Biol* 36: 350-254.

Carlson AR, Siefert RE (1974) Effects of reduced oxygen on the embryos and larvae of lake trout (*Salvelinus namaycush*) and largemouth bass (*Micropterus salmoides*). *J Fish Res Bd Can* 31: 1393-1396.

Ciuhandu CS, Stevens ED, Wright PA (2005) The effect of oxygen on the growth of *Oncorhynchus mykiss* embryos with and without a chorion. *J Fish Biol* 67: 1544-1551.

Conover DO, Schultz ET (1997) Natural selection and the evolution of growth rate in the early life history: what are the trade-offs? In: Chambers RC, Trippel EA, eds. Early Life History and Recruitment in Fish Populations. Chapman and Hall, London, pp 305-332.

Crain CM, Kroeker K, Halpern BS (2008) Interactive and cumulative effects of multiple human stressors in marine systems. *Ecol Lett* 11: 1304-1315.

Czerkies P, Brzuzan P, Kordalski K, Luczynski M (2001) Critical partial pressures of oxygen causing precocious hatching in *Coregonus lavaretus* and *C. albula* embryos. *Aquaculture* 196: 151-158.

Dettinger MD (2005) From climate-change spaghetti to climate-change distributions for 21st century California. *San Francisco Estuary and Watershed Sciences* 3: 1-14.

DiMichele L, Powers DA (1984) Developmental and oxygen consumption rate differences between lactate dehydrogenase-B genotypes of *Fundulus heteroclitus* and their effect on hatching time. *Physiol Zool* 57: 52-56.

Diaz RJ (2001) Overview of hypoxia around the world. *J Environ Qual* 30: 275-281.

Diaz RJ, Rosenberg R (2008) Spreading dead zones and consequences for marine ecosystems. *Science* 321: 926-929.

Dudley RG, Eipper AW (1975) Survival of largemouth bass embryos at low dissolved oxygen concentrations. *Trans Ameri Fish Soc* 104: 122-128.

Einum S, Fleming IA (2000) Selection against late emergence and small offspring in Atlantic salmon (*Salmo salar*). *Evolution* 54: 628-639.

Ellis LE, Sacobie CFD, Kieffer JD, Benfey TJ (2013) The effects of dissolved oxygen and triploidy on critical thermal maximum in brook charr (*Salvelinus fontinalis*). *Comp Biochem Physiol A Mol Integr Physiol* 166: 426-433.

Ern R, Norin T, Gamperl AK, Esbaugh AJ (2016) Oxygen dependence of upper thermal limits in fishes. *J Exp Biol* 219: 3376-3383.

Ern R, Johansen JL, Rummer JL, Esbaugh AJ (2017) Effects of hypoxia and ocean acidification on the upper thermal niche boundaries of coral reef fishes. *Biol Lett* 13: 20170135.

Fangue NA, Hofmeister M, Schulte PM (2006) Intraspecific variation in thermal tolerance and heat shock protein gene expression in common killifish, *Fundulus heteroclitus*. *J Exp Biol* 209: 2859-2872.

Fry FEJ (1971) The effect of environmental factors on the physiology of fish. In: Hoar WS, Randall DJ, eds. Fish Physiology. Vol. 6. Academic Press, New York, pp 1-98.

Fu S, Brauner CJ, Cao A, Richards JG, Peng J, Dhillon R, Wang Y (2011) The effect of acclimation to hypoxia and sustained exercise on subsequent hypoxia tolerance and swimming performance in goldfish (*Carassius auratus*). *J Exp Biol* 2011: 2080-2088.

Garside ET (1959) Some effects of oxygen in relation to temperature on the development of lake trout embryos. *Can J Zool* 37: 689-698.

Garside ET (1966) Effects of oxygen in relation to temperature on the development of embryos of brook trout and rainbow trout. *J Fish Res Bd Can* 23: 1121-1134.

Geist DR, Abernethy CS, Hand KD, Cullinan VI, Chandler JA, Groves PA (2006) Survival, development, and growth of fall Chinook salmon embryos, alevins, and fry exposed to variable thermal and dissolved oxygen regimes. *Trans Ameri Fish Soc* 135: 1462-1477.

Green BS, Fisher R (2004) Temperature influences swimming speed, growth, and larval duration in coral reef fish larvae. *J Exp Mar Bio Ecol* 299: 115-132.

Greig SM, Sear DA, Carling PA (2007a) A review of factors influencing the availability of dissolved oxygen to incubating salmonid embryos. *Hydrol Process* 21: 313-334.

Greig S, Sear D, Carling P (2007b) A field-based assessment of oxygen supply to incubating Atlantic salmon (*Salmo salar*) embryos. *Hydrol Process* 21: 3087-3100.

Grossman GD (2016) Predation on fishes in the Sacramento-San Joaquin Delta: Current knowledge and future directions. *San Francisco Estuary and Watershed Sciences* 14: doi: http://dx.doi.org/10.15447/sfews.2016v14iss2art8.

Gunderson AR, Armstrong EJ, Stillman JH (2016) Multiple stressors in a changing world: The need for an improved perspective on physiological responses to the dynamic marine environment. *Ann Rev Mar Sci* 8: 357-378.

Hamor T, Garside ET (1959) Developmental rates of embryos of Atlantic salmon, *Salmo salar* L., in response to various levels of temperature, dissolved oxygen, and water exchange. *Can J Zool* 54: 1912-1917.

Hanak E, Mount J, Chappelle C, Lund J, Medellín-Azuara J, Moyle P, Seavy N (2015) What if California's drought continues? Public Policy Institute of California: San Francisco, CA, USA.

Hassell KL, Coutin PC, Nugegoda D (2008) Hypoxia impairs embryo development and survival in black bream (*Acanthopagrus butcheri*). *Mar Pollut Bull* 57: 302-306

Hayhoe K, Cayan D, Field CB, Frumhoff PC, Maurer EP, Miller NL, Moser SC, Schneider SH, Cahill KN, Cleland EE, *et al.* (2004) Emissions pathways, climate change, and impacts on California. *Procs Natl Acad Sci* 101: 12422-12427.

He W, Cao Z, Fu S (2015) Effect of temperature on hypoxia tolerance and its underlying biochemical mechanism in two juvenile cyprinids exhibiting distinct hypoxia sensitivities. *Comp Biochem Physiol A Mol Integr Physiol* 187: 232-241.

Healy TM, Schulte PM (2012) Factors affecting plasticity in whole-organism thermal tolerance in common killifish (*Fundulus heteroclitus*). *Comp Biochem Physiol B Biochem Mol Biol* 182: 49-62.

Helm KP, Bindoff NL, Church JA (2011) Observed decreases in oxygen content of the global ocean. *Geophys Res Lett* 38: L23602.

Kamler, E (2008) Resource allocation in yolk-feeding fish. *Rev Fish Biol Fisher* 18: 143-200.

Keckeis H, Bauer-Nemeschkal E, Kamler E (1996) Effects of reduced oxygen level on the mortality and hatching rate of *Chondrostoma nasus* embryos. *J Fish Biol* 49: 430-440.

Keeling RF, Körtzinger A, Gruber N (2010) Ocean deoxygenation in a warming world. *Ann Rev Mar Sci* 2: 199-229.

Komoroske LM, Connon RE, Lindberg J, Cheng BS, Castillo G, Hasenbein M, Fangue NA (2014) Ontogeny influences sensitivity to climate change stressors in an endangered fish. *Cons Physiol* 2: doi:10.1093/conphys/cou008.

Korwin-Kossakowski M (2012) Fish hatching strategies: a review. *Rev Fish Biol Fisher* 22: 225-240.

Latham KE, Just JJ (1989) Oxygen availability provided a signal for hatching in the rainbow trout (*Salmo gairdneri*) embryo. *Can J Fish Aquat Sci* 46: 55-58.

Lenth RV (2016) Least-squares means: the R package lsmeans. *J Stat Softw* 69: 1–33.

Marks C, Kaut KP, Moore FBG, Bagatto B (2012) Ontogenetic oxygen changes alter zebra fish size, behavior, and blood glucose. *Physiol Biochem Zool* 85: 635-644.

Martin BT, Pike A, John SN, Hamda N, Roberts J, Lindley ST, Danner EM (2017) Phenomenological vs. biophysical models of thermal stress in aquatic eggs. *Ecol Lett* 20: 50-59.

Mason J (1969) Hypoxial stress prior to emergence and competition among Coho salmon fry. *J Fish Res Bd Can* 26: 63-91.

McBryan TL, Anttila K, Healy TM, Schulte PM (2013) Responses to temperature and hypoxia as interacting stressors in fish: Implications for adaptation to environmental change. *Integr Comp Biol* 53: 648-659.

McBryan TL, Healy TM, Haakons KL, Schulte PM (2016) Warm acclimation improves hypoxia tolerance in *Fundulus heteroclitus*. *J Exp Biol* 219: 474-484.

McCullough DA (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. EPA, Seattle. EPA 910-R-99-010. 279 pp.

McDonnell LH, Chapman LJ (2015) At the edge of the thermal window: effects of elevated temperature on the resting metabolism, hypoxia tolerance and upper critical thermal limit of a widespread African cichlid. *Conserv Physiol* 3: cov050; doi:10.1093/conphys/cov050.

Miller SC, Reeb SE, Wright PA, Gillis TE (2008) Oxygen concentration in the water boundary layer next to rainbow trout (*Oncorhynchus mykiss*) embryos is influenced by hypoxia exposure time, metabolic rate, and water flow. *Can J Fish Aquat Sci* 65: 2170-2177.

Motyka R, Norin T, Petersen LH, Huggett DB, Gamperl AK (2017) Long-term hypoxia exposure alters the cardiorespiratory physiology of steelhead trout (*Oncorhynchus mykiss*), but does not affect their upper thermal tolerance. *J Thermal Biol* 68: 149-161.

Moyle PB (2002) Inland fishes of California. Berkeley, CA: University of California Press.

Murray CB, McPhail JD (1987) Effect of incubation temperature on the development of five species of Pacific salmon (*Oncorhynchus*) embryos and alevins. *Can J Zool* 66: 266-273.

Myrick CA, Cech JJ (2004) Temperature effects on juvenile anadromous salmonids in California's central valley: what don't we know? *Rev Fish Biol Fisher* 14: 113-123.

Nilsson GE, Ostlund-Nilsson S, Munday PL (2010) Effects of elevated temperature on coral reef fishes: Loss of hypoxia tolerance and inability to acclimate. *Comp Biochem Physiol A Mol Integr Physiol* 156: 389-393.

Ninness MM, Stevens ED, Wright PA (2006) Energy expenditure during hatching in rainbow trout (*Oncorhynchus mykiss*) embryos. *Can J Fish Aquat Sci* 63: 1405-1413.

Oppen-Berntsen DO, Bogsnes A, Walther TH (1990) The effects of hypoxia alkalinity and neurochemicals on hatching of Atlantic salmon (*Salmo salar*) eggs. *Aquaculture* 86: 417-430.

Pepin P (1991) Effect of temperature and size on development, mortality, and survival rates of the pelagic early life history stages of marine fish. *Can J Fish Aquat Sci* 48: 503-518.

Petersen LH, Gamperl AK (2010) Effect of acute and chronic hypoxia on the swimming performance, metabolic capacity and cardiac function of Atlantic cod (*Gadus morhua*). *J Exp Biol* 213: 808-819.

Peterson NP, Quinn TP (1996) Spatial and temporal variation in dissolved oxygen in natural egg pockets of chum salmon, in Kennedy Creek, Washington. *J Fish Biol* 48: 131-143.

Polymeropoulos ET, Elliot NG, Frappell PB (2016) The maternal effect of differences in egg size influence metabolic rate and hypoxia induced hatching in Atlantic salmon eggs: implications for respiratory gas exchange across the egg capsule. *Can J Fish Aquat Sci* 73: 1173-1181.

Polymeropoulos ET, Elliot NG, Frappell PB (2017) Hypoxic acclimation leads to metabolic compensation after reoxygenation in Atlantic salmon yolk-sac alevins. *Comp Biochem Physiol A Mol Integr Physiol* 213: 28-35.

Pörtner HO (2001) Climate change and temperature-dependent biogeography: oxygen limitation of thermal tolerance in animals. *Naturwissenschaften* 88: 137-146.

Pörtner HO (2002) Climate variations and the physiological basis of temperature dependent biogeography: systemic to molecular hierarchy of thermal tolerance in animals. *Comp Biochem Physiol A Mol Integr Physiol* 132: 739-761.

Pörtner HO (2010) Oxygen- and capacity-limitation of thermal tolerance: a matrix for integrating climate-related stressor effects in marine ecosystems. *J Exp Biol* 213: 881-893.

R Development Core Team (2013) R: A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing. Retrieved from http://www.R-project.org/

Rees BB, Sudradjat FA, Love JW (2001) Acclimation to hypoxia increases survival time of zebrafish, *Danio rerio*, during lethal hypoxia. *J Exp Zool* 289: 266-272.

Remen M, Oppedal F, Imsland AK, Olsen RE, Torgersen T (2013) Hypoxia tolerance thresholds for post-smolt Atlantic salmon: Dependency of temperature and hypoxia acclimation. *Aquaculture* 416-417: 41-47.

Richards JG (2009) Metabolic and molecular responses of fish to hypoxia. In: Richards JG, Farrell AP, Brauner CJ eds. Fish physiology. Vol. 27. Elsevier, London, pp. 443-485.

Richter A, Kolmes SA (2005) Maximum temperature limits for Chinook, coho, and chum salmon, and steelhead trout in the Pacific Northwest. *Rev Fish Sci* 13: 23-49.

Rombough PJ (1988a) Growth, aerobic metabolism, and dissolved oxygen requirements of embryos and alevins of steelhead, *Salmo gairdneri*. *Can J Zool* 66: 651-660.

Rombough PJ (1988b) Respiratory gas exchange, aerobic metabolism, and effects of hypoxia during early life. In: Hoar WS, Randall DJ, eds. Fish Physiology, Vol. 11A. Academic Press, New York, pp 59-161.

Rombough PJ (2006) Developmental costs and partitioning of metabolic energy. In: Warburton SJ, Burggren WW, Pelster B, Reiber CL, Spicer J, eds, Comparative Developmental Physiology Contributions, Tools and Trends. Oxford University Press, Oxford, pp. 99–123.

Rombough P (2011) The energetics of embryonic growth. *Resp Physiol Neurobi* 178: 22-29.

Rubin JF, Glimsäter C (1996) Egg-to-fry survival of the sea trout in some streams of Gotland. *J Fish Biol* 48: 585-606.

Sear DA, Pattison I, Collins AL, Newson MD, Jones JI, Naden PS, Carling PA (2014) Factors controlling the temporal variability in dissolved oxygen regime of salmon spawning gravels. *Hydrol Process* 28: 86-103.

Shimps EL, Rice JA, Osborne JA (2005) Hypoxia tolerance in two juvenile estuary-dependent fishes. *J Exp Mar Biol Ecol* 325: 146-162.

Silver SJ, Warren CE, Doudoroff P (1963) Dissolved oxygen requirements of developing steelhead trout and Chinook salmon embryos at different water velocities. *Trans Ameri Fish Soc* 92: 327-343.

Shumway DL, Warren CE, Doudoroff P (1964) Influence of oxygen concentration and water movement on the growth of steelhead trout and coho salmon embryos. *Trans Am Fish Soc* 93: 342-356.

Sokolova IM (2013) Energy-limited tolerance to stress as a conceptual framework to integrate the effects of multiple stressors. *Integr Comp Biol* 53: 597–608.

Timmerman CM, Chapman LJ (2004) Behavioral and physiological compensation for chronic hypoxia in the sailfin molly (*Poecilia latipinna*). *Physiol Biochem Zool* 77: 601-610.

Todgham AE, Stillman JH (2013) Physiological responses to shifts in multiple environmental stressors: relevance in a changing world. *Integr Comp Biol* 53: 539-544.

Verberk WCEP, Leuven RSEW, van der Velde G, Gabel F (2018) Thermal limits in native and alien freshwater peracarid Crustacea: The role of habitat use and oxygen limitation. *Funct Ecol* 32: 926-936.

Williams JG (2006) Central Valley salmon: A perspective on Chinook and steelhead in the Central Valley of California. *San Francisco Estuary and Watershed Science* 4: 1-393.

Yamagami K (1988) Mechanisms of hatching in fish. In: Hoar WS, Randall DJ, eds. The Physiology of Developing Fish: Eggs and Larvae, Fish Physiology Vol. 11A, Academic Press, San Diego, pp 447-499.

Yang H, Cao Z, Fu S (2013) The effects of diel-cycling hypoxia acclimation on the hypoxia tolerance, swimming capacity and growth performance of southern catfish (*Silurus meridionalis*). *Comp Biochem Physiol A Mol Integr Physiol* 165: 131-138.

Youngson AF, Malcolm IA, Thorley JL, Bacon PJ, Soulsby C (2004) Long-residence groundwater effects on incubating salmonid eggs: low hyporheic oxygen impairs embryo development. *Can J Fish Aquat Sci* 61: 2278-2287.

Zebral YD, Lansini LR, Costa PG, Roza M, Bianchini A, Robaldo RB (2018) A glyphosate-based herbicide reduces fertility, embryonic upper thermal tolerance and alters embryonic diapause of the threatened annual fish *Austrolebias nigrofasciatus*. *Chemosphere* 196: 260-269.

Tables

Table 1. Water temperature (°C) was measured daily in each water bath tank and is reported as the average between the duplicate tanks for each temperature treatment (± SD). Dissolved oxygen (DO) (mg/l and % saturation) was measured daily in each culture bucket and is reported as the average of the four replicate culture buckets per treatment (± SD).

Treatment	Temperature (°C)	DO (mg/l)	DO % Saturation
14°C Normoxia	14.1 ± 0.7	10.1 ± 0.5	98.2 ± 4.2
14°C Hypoxia	14.1 ± 0.7	5.9 ± 3.1	55.3 ± 7.2
10°C Normoxia	10.6 ± 0.9	10.8 ± 0.4	97.5 ± 3.2
10°C Hypoxia	10.6 ± 0.9	5.5 ± 0.8	49.9 ± 7.6

Table 2. Time (days post fertilization) for 50% of individuals or more in each treatment to reach four developmental stages. Development was assessed daily in all replicate culture buckets per treatment.

	Time (days post fertilization) to reach stage			
Treatment	Eyed	Silver Eyed	Post-hatch	Fry
14°C Normoxia	17	26	35	64
14°C Hypoxia	21	30	36	70
10°C Normoxia	25	36	42	75
10°C Hypoxia	29	41	48	85

Figure legends

Figure 1. Hatching success measured as percentage hatched in each treatment (10°C Normoxia [green], 10°C Hypoxia [blue], 14°C Normoxia [yellow], and 14°C Hypoxia [red]). The center line of the boxplots represents the median, the box represents the inter-quartile range (IQR), the whiskers extend 1.5 times IQR, black points represent values outside 1.5 the IQR, and diamonds represent the mean. Letters indicate a significant ($p<0.05$) differences between dissolved oxygen treatments.

Figure 2. Critical thermal maximum (CTMax) throughout development in four rearing treatments: 10°C Normoxia (green circle), 10°C Hypoxia (blue square), 14°C Normoxia (yellow), and 14°C Hypoxia (red diamond). Average CTMax ± 95% confidence is given for n=16 individuals per treatment at each developmental stage. Within each panel CTMax is defined as A) the temperature at which the heart beat stopped (embryonic stages, eyed and silver eyed) and B) the temperature at which equilibrium was lost (larval stages, alevin and fry). Letters indicate significant ($p<0.05$) differences between treatments within a given developmental stage. Asterisks indicate significant ($p<0.05$) differences between developmental stages within a single treatment.

Figure 3. Acute hypoxia tolerance of fry was measured as the time (min) until fish lost equilibrium while held at 8% dissolved oxygen saturation. A total of n=16 individuals per treatment (10°C Normoxia [green], 10°C Hypoxia [blue], 14°C Normoxia [yellow], and 14°C Hypoxia [red]) were tested. Each test was conducted at the temperature fish were reared at and began at the dissolved oxygen saturation of the corresponding treatment. The center line of the boxplots represents the median, the box represents the inter-quartile range (IQR), the whiskers extend 1.5 times IQR, black points represent values outside 1.5 the IQR, and diamonds represent the mean. Letters indicate significant ($p<0.05$) differences between treatments.

Figure 4. Fulton's condition factor in post-hatch alevins was calculated as the relationship between mass and length in n=20 individuals per treatment (10°C Normoxia [green], 10°C Hypoxia [blue], 14°C Normoxia [yellow], and 14°C Hypoxia [red]). The center line of the boxplots represents the median, the box represents the inter-quartile range (IQR), the whiskers extend 1.5 times IQR, black points represent values outside 1.5 the IQR, and diamonds represent

the mean. Letters indicate a significant ($p<0.05$) difference between the main effects of dissolved oxygen (Normoxia, and Hypoxia).

Figure 5. Fulton's condition factor in fry was calculated as the relationship between mass and length in n=20 individuals per treatment. The center line of the boxplots represents the median, the box represents the inter-quartile range (IQR), the whiskers extend 1.5 times IQR, black points represent values outside 1.5 the IQR, and diamonds represent the mean. Letters indicate significant differences between treatments (10°C Normoxia [green], 10°C Hypoxia [blue], 14°C Normoxia [yellow], and 14°C Hypoxia [red]).

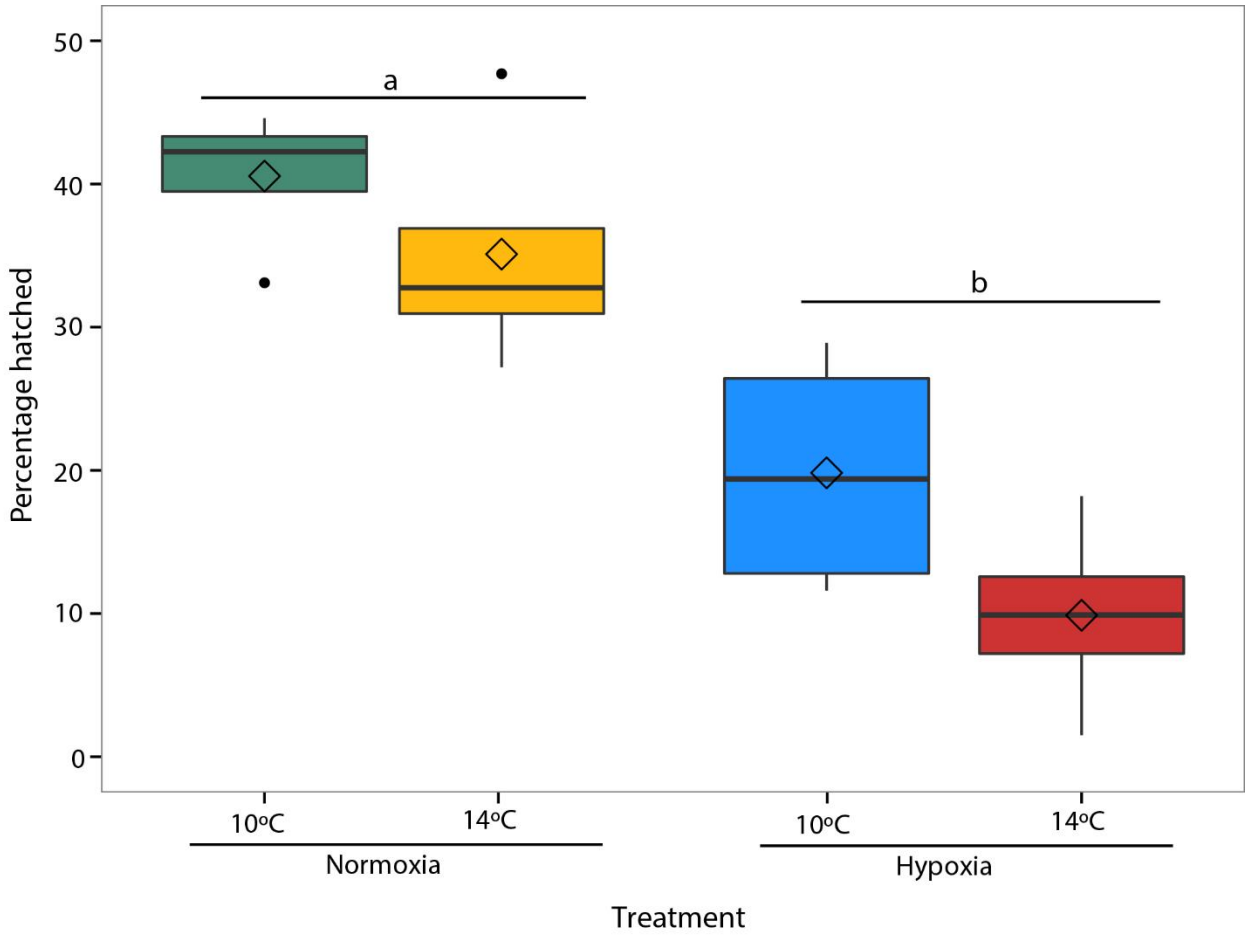
50
40
30
20
10
0
Percentage hatched
a
b
10°C
14°C
10°C
14°C
Normoxia
Hypoxia
Treatment

Figure 1

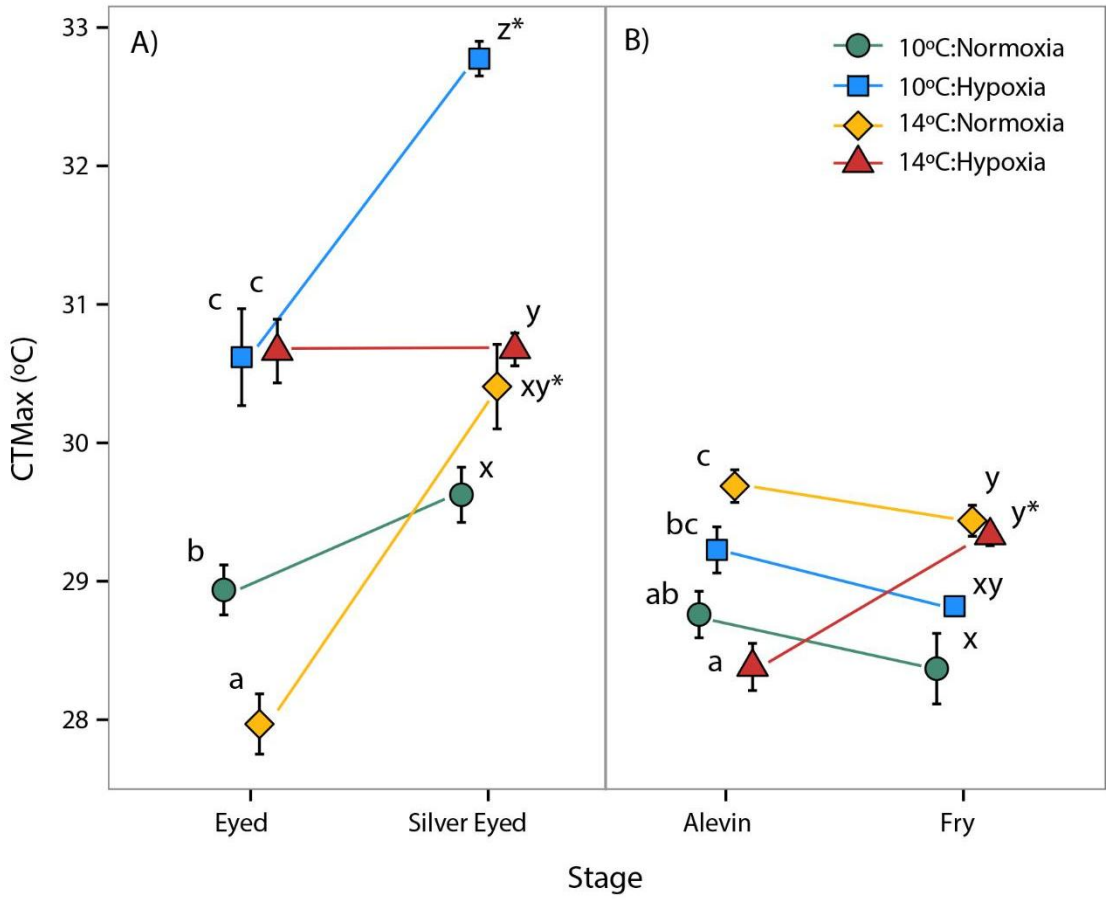
A)
B)
10°C:Normoxia
10°C:Hypoxia
14°C:Normoxia
14°C:Hypoxia
CTMax (°C)
33
32
31
30
29
28
z*
c
c
y
xy*
x
b
a
c
y
y*
bc
xy
ab
x
a
Eyed
Silver Eyed
Alevin
Fry
Stage

Figure 2

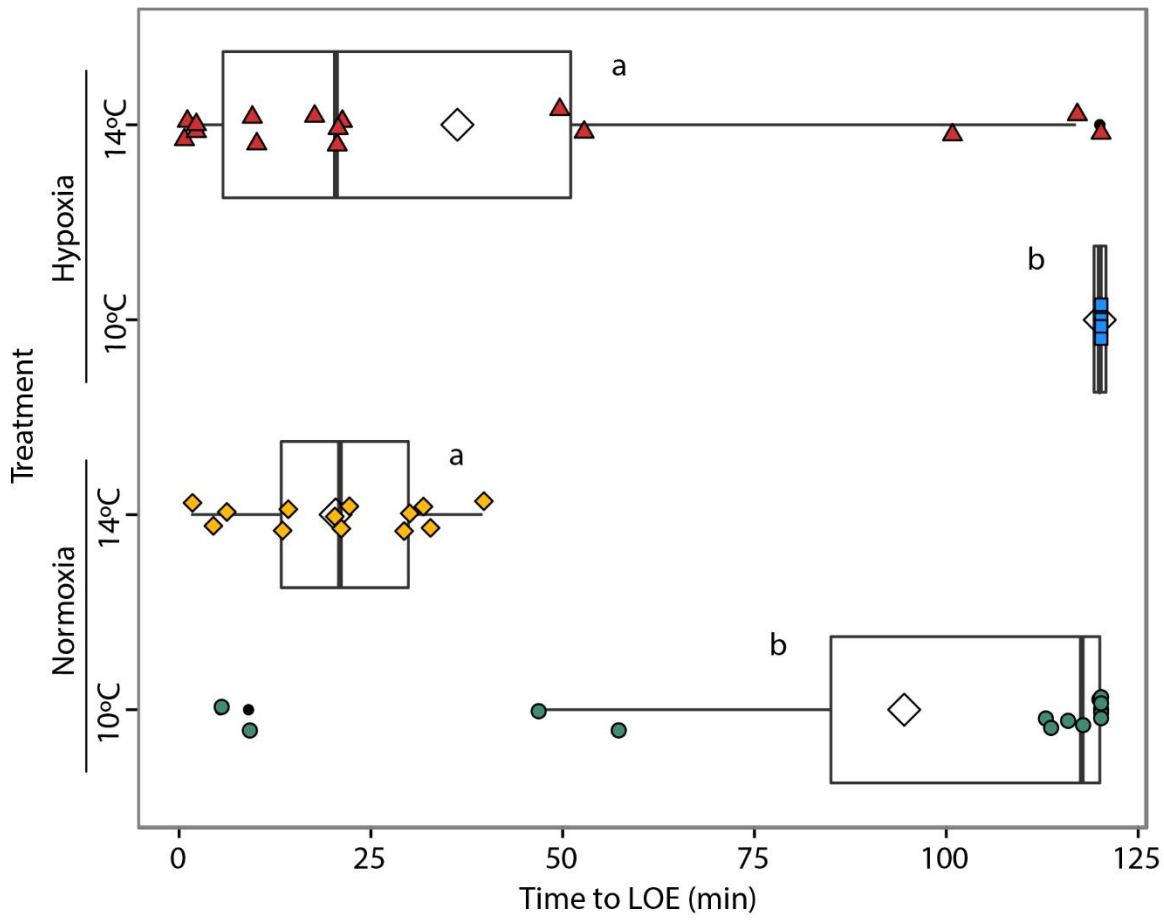
Treatment
Hypoxia
Normoxia
14ºC
10ºC
14ºC
10ºC
a
b
a
b
0
25
50
75
100
125
Time to LOE (min)

Figure 3.

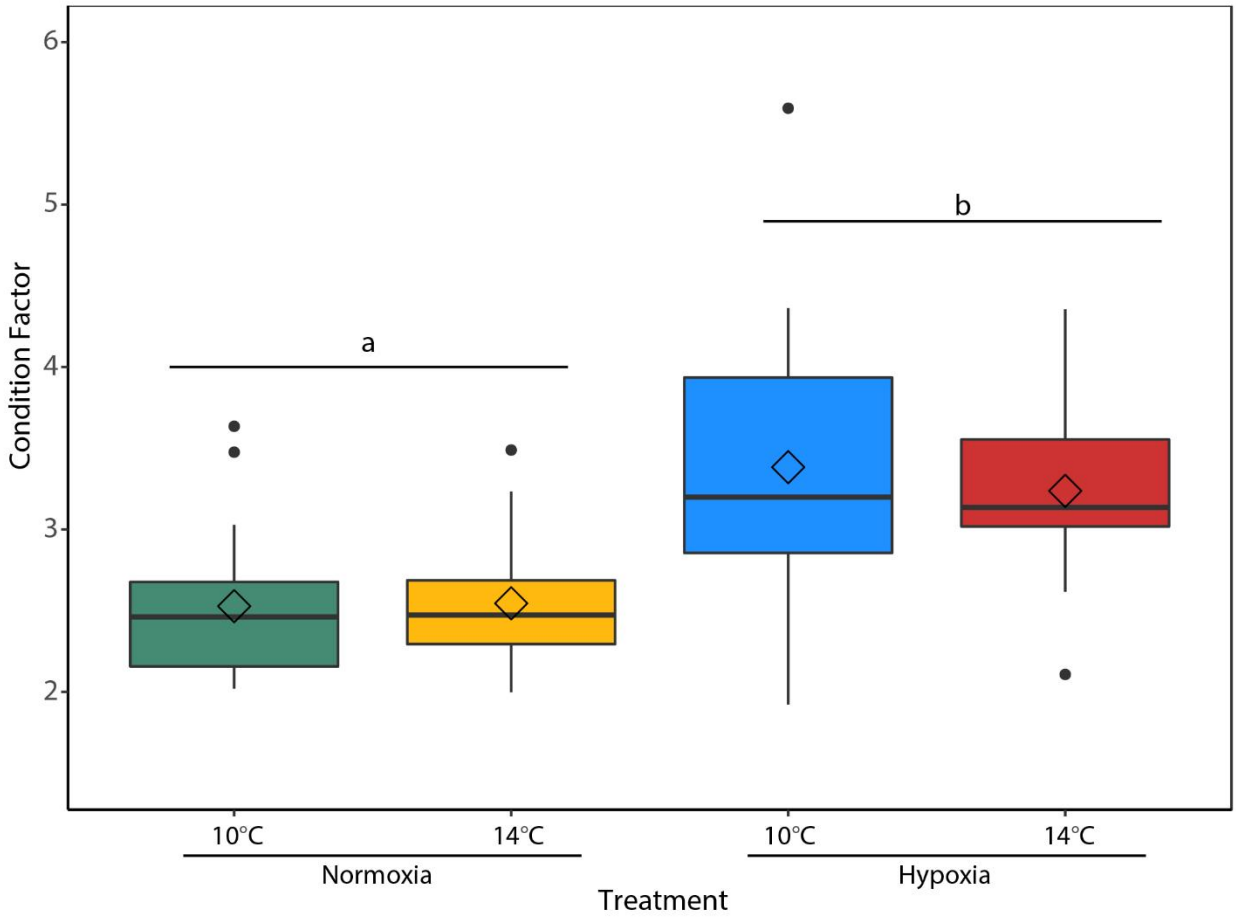
Condition Factor
6
5
4
3
2
a
b
10°C
14°C
10°C
14°C
Normoxia
Hypoxia
Treatment

Figure 4.

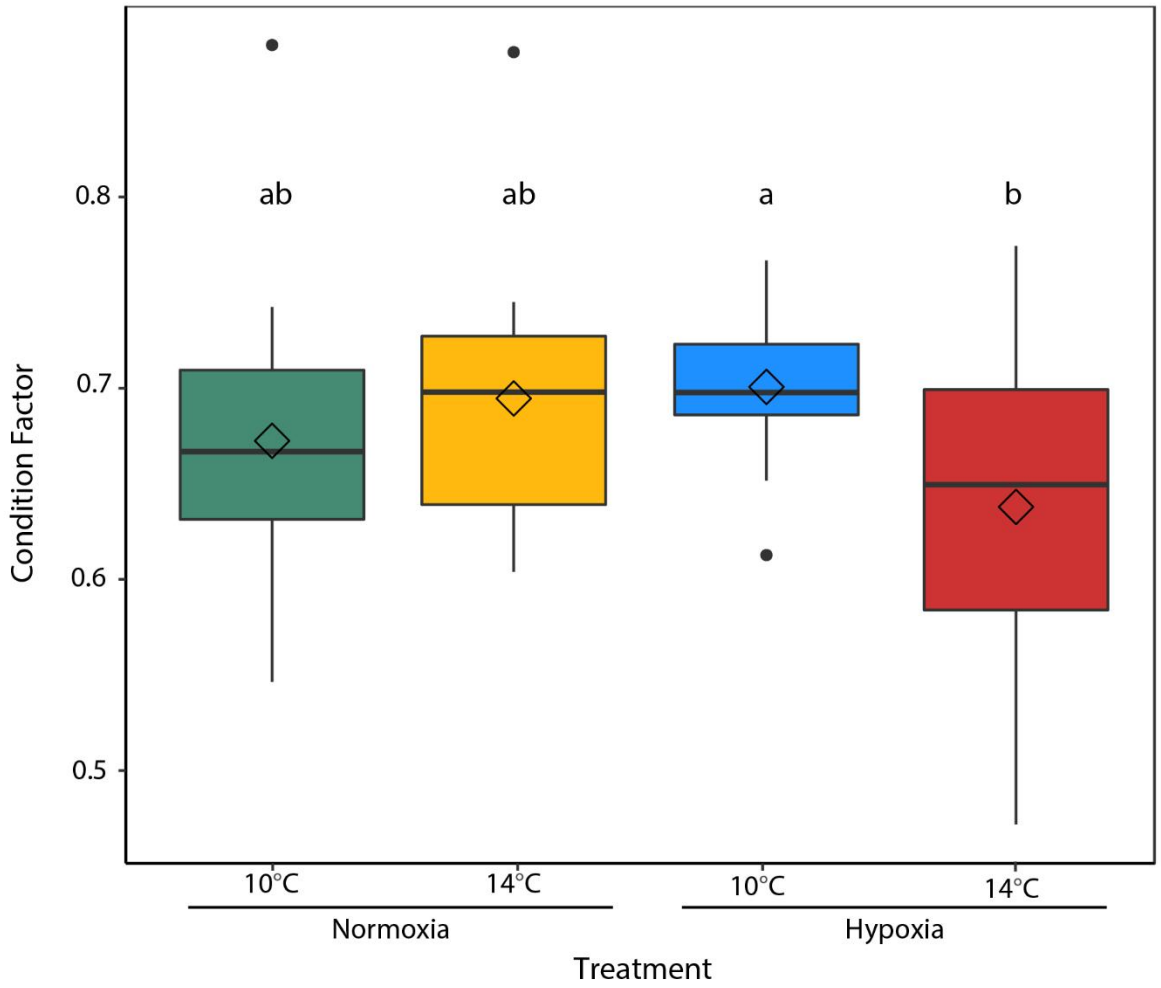
ab
ab
a
b
0.8
0.7
0.6
0.5
Condition Factor
10°C
14°C
10°C
14°C
Normoxia
Hypoxia
Treatment

Figure 5.