

**Repeated elevational clines of early life-history traits and their proximate mechanisms in brown trout**

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**RUNNING HEAD**

Elevational clines in brown trout traits

## ABSTRACT

1. Climate warming imposes a severe threat to freshwater ecosystems, which are dominated by ectotherms such as fish and aquatic insects. To better predict the effects of climate warming on thermally sensitive ecosystems, information on how temperature affects individual traits within populations is fundamental.

2. Patterns of intraspecific variation in thermal reaction norms along geographic thermal gradients provide valuable information. Immediate temperature effects on individual traits can be inferred from the shape of the thermal reaction norm. The way that temperature and associated environmental conditions (to which populations have been exposed over generations) affect individual traits through transgenerational plasticity in, and/or natural selection on, these traits can also be inferred from patterns of trait variation along a geographic thermal gradient.

3. Many studies have documented patterns of intraspecific variation in thermal reaction norms along geographic thermal gradients. However, most previous studies cannot exclude the possibility that the observed geographic patterns are solely explained by random processes, such as isolation by distance, due to the lack of replication of geographic gradients. Here, we show consistent patterns in intraspecific trait variation along geographic thermal gradients using *Salmo trutta* (brown trout), which is an ecologically and economically important fish in alpine streams.

4. We kept trout embryos collected from 52 families from 14 populations along wide and replicated elevational gradients from three Alpine drainages (Danube, Po, and Rhine) in two temperature treatments (3.2 and 6.2 °C). Timing and body size at emergence from the nest, which are key early life-history traits of trout affecting their early growth and survival, were measured.

5. Besides faster embryonic development at warmer temperatures, we found that offspring from low-elevation parents took very slightly more days to reach emergence from fertilisation and were larger than offspring from high-elevation parents. Importantly, this was evident for all three drainages. Further analyses found that the higher number of days until- and larger body length at-

emergence of low-elevation trout were mainly due to the larger eggs laid by low-elevation females, which had larger body size than high-elevation females.

6. Trout female body size, which is positively correlated with egg size, is susceptible to temperatures and associated environmental conditions. Consequently, climate warming may not only immediately accelerate development rate but also shift timing and size at emergence through egg size-mediated maternal effects within a relatively short time scale.

## 1| INTRODUCTION

Climate warming can affect various individual traits by accelerating physiological processes immediately and by indirectly influencing the strength and even nature of ecological interactions (Beacham & Murray, 1990; Rudolf & Roman, 2018; Bonacina *et al.*, 2022; Lindmark, Ohlberger & Gårdmark, 2022), eventually altering whole populations (Yang & Rudolf, 2010; Ohlberger, 2013). In addition to such immediate effects, environmental conditions, including temperature, to which populations have been exposed over generations can also affect various individual traits through transgenerational plasticity in- and/or natural selection on- the traits (Endler, 1986; Angilletta, 2009; Bell & Hellmann, 2019; Leicht & Seppälä, 2019), again eventually influencing whole populations (Pelletier, Garant & Hendry, 2009; Donelan *et al.*, 2020). Therefore, to better predict the effects of climate warming on natural populations, it is necessary to investigate both immediate temperature effects and effects of temperature to which populations have been exposed over generations on individual traits. Such studies are especially urgent for freshwater ecosystems as they are dominated by ectotherms, such as fish and aquatic insects, whose physiological processes are highly dependent on ambient temperatures (Angilletta, 2009).

A practical approach to investigating immediate temperature effects and effects of temperatures and associated environmental conditions to which populations have been exposed over generations on individual traits is to examine intraspecific variation in thermal reaction

norms along the geographic thermal gradient (Conover, Duffy & Hice, 2009). Representative geographic thermal gradients are elevational and latitudinal gradients. It is reasonable to assume that each geographic population has been exposed to geographically specific environmental regimes for generations. This considered, any detected patterns in intraspecific trait variations for a given rearing temperature and shape of reaction norm along geographic thermal gradients are expected to reflect the consequences of transgenerational plasticity and/or natural selection driven by local environmental conditions. Also, the immediate temperature effects on the traits are shown as thermal reaction norms.

Many previous studies have described intraspecific variations in thermal reaction norms along latitudinal, elevational, and other geographic thermal gradients (Laugen, Laurila & Merilä, 2003; Conover *et al.*, 2009; Kojima *et al.*, 2020). However, most previous studies did not test the consistency of the patterns of trait variations along geographic thermal gradients, which is most often associated with the lack of independent replicates of the geographic thermal gradients (but see Kavanagh *et al.*, 2010). Therefore, they cannot exclude the possibility that observed patterns in trait variations along geographic thermal gradients are solely explained by random processes, such as isolation by distance (Endler, 1977; Ibrahim, Nichols & Hewitt, 1996). To obtain robust evidence for inferring the effects of temperatures and associated environmental conditions to which populations have been exposed over generations, an experiment testing the consistency of the patterns of intraspecific trait variations along geographic thermal gradients is necessary.

Here, we report results from a common-garden experiment using *Salmo trutta* (i.e., brown trout), which is a common fish species in European streams, aimed at examining the consistency of the patterns of intraspecific trait variations along elevational gradients. Importantly, their wide elevational distribution in each drainage allowed us to have replicated elevational gradients (e.g., Fig. 1a,b). Specifically, we focused on the timing of- and body size at- the emergence from the redd, i.e., the nest. In salmonid fishes, hatchlings suffer from predation after emergence and

engage in intensive resource competition among conspecifics (Jonsson & Jonsson, 2011). The timing of- and body size at- emergence affect growth and survival during this critical period (Einum & Fleming, 2000b; Skoglund, Einum & Robertsen, 2011). At the same time, similar to most ectotherms, individuals during early life-stages are highly vulnerable to ambient environmental conditions (Miller *et al.*, 1988; Jonsson & Jonsson, 2011). All this considered, optimal early life-history tactics are expected to be associated with elevational gradients along which environmental factors, such as temperature and community composition, vary. Moreover, the embryonic development of most salmonid fishes, including brown trout, is currently facing an exaggerated increase in ambient temperatures, partly due to hydropower plants. The embryonic development occurs in winter (Beacham & Murray, 1990; Jonsson & Jonsson, 2011), where energy requirements are high and associated release of water from reservoirs above hydropower plants results in up to a 5°C increase in downstream temperatures, due to the heat storage in reservoir water (Olden & Naiman, 2010). Given the ecological and economic importance of salmonid fishes (Jonsson & Jonsson, 2011), the information on how an increase in temperature affects the early life-history traits of brown trout could provide insights to help mitigate the effects of climate warming on the fish and the whole alpine river ecosystems.

In the present study, we kept brown trout embryos collected from 52 families from 14 populations along wide and replicated elevational gradients from three Alpine drainages (Fig. 1a,b) in two different rearing temperatures. We examined (1) reaction norms of timing and body size at emergence from the nest, (2) how they varied along elevational gradients, and (3) whether patterns of variation in the early life-history traits are consistent across three drainages. Moreover, we measured maternal body size, egg volume, and several traits at hatching, which are acknowledged as traits affecting timing and body size at emergence (Einum & Fleming, 2000b a; Rollinson & Hutchings, 2010; Thorn & Morbey, 2018). Then, we examined the links between maternal traits and offspring traits to explore the mechanisms underlying variations in timing and

body size at emergence along elevational gradients.

## 2| MATERIALS AND METHODS

### 2.1| Study system

*Salmo trutta* in the Swiss canton of Graubünden provides an excellent opportunity to have replicated geographic thermal gradients. Headwaters of three major Alpine drainages (i.e., Danube, Po, Rhine) are located within the relatively small region (Fig. 1a), which encompasses a part of the Alps. This allowed us to collect trout from a relatively wide elevation range from each drainage. Notably, previous studies demonstrated that distinct genetic characters of populations from the three drainages have persisted despite intensive stocking activities using several million captive-reared trout (Keller, Taverna & Seehausen, 2011). Independent evolutionary histories, therefore, have not been erased completely. Also, a consistent pattern of elevational changes of genetic structure was observed across drainages, suggesting natural population structure may also not have been erased completely (Keller *et al.*, 2011). We selected 14 collection sites to cover a wide elevation range across the three drainages. The elevation ranges of collections sites in Danube, Po, and Rhine drainages were 1134 – 2073 m (number of collection site [ $N$ ] = 2), 343 – 1872 m ( $N$  = 6), and 771 – 1886 m ( $N$  = 6), respectively (Fig. 1a,b, Table S1). Because of the relatively narrow elevation range of the Danube drainage within the area and the small number of accessible streams in the drainage, we were only able to collect trout from two sites in the drainage. Water temperature at each collection site was measured from November 2018 to February 2019 using temperature loggers (Hobo Pendant data loggers, Onset Computer Corporation, USA), with the exception of the 968 m elevation site in the Rhine drainage, where the logger was washed away (site ID 13 in Fig. 1a-c). This confirmed that trout in higher elevation sites have generally experienced colder temperatures (Fig. 1c).

### 2.2| Gamete collection and in vitro fertilisation

From late October to early November 2018, we collected parent fish from each of 14 selected sites. Adult trout were caught by electrofishing and then brought to hatcheries in Graubünden. The trout were kept until they were ripe for spawning. In early November, we conducted in vitro fertilisation at canton hatcheries. Before stripping eggs or sperms, each parental trout was measured for standard length (hereafter, body length). We created two to six full-sib families from each site by pairing randomly selected males and females and obtained 52 families in total (Fig. 1b). The eggs were transferred to an experimental room in Eawag Kastanienbaum (transport time was less than 6 hours). Eggs of each family were photographed (Tough TG-5, Olympus, Tokyo, Japan). The captured images were used to measure the diameter of 10 eggs randomly subsampled from each family. We measured the maximum and minimum diameter of each of 10 subsampled eggs and then calculated the family-mean egg diameter (mm) and volume (mm<sup>3</sup>). All measurements in this study were conducted using Image J (National Institute of Health, USA).

### **2.3| Common-garden experiment**

We used eight vertical incubators (MariSource Inc., WA, USA) as experimental arenas, each supplied by a recirculating supply of 200 L of oxygen-saturated water at an average flow of 0.6 L per second. Thirty plastic mesh tubes (4.2 cm diameter × 6 cm tall, an acrylic panel was attached to the bottom) were placed in each of six trays within an incubator to keep the eggs and hatchlings from each family separately (i.e., 180 mesh tubes per incubator). Thirty litres of water in all incubators was exchanged every two weeks to maintain water quality. A chiller (TK-2000, TECO, Ravenna, Italy) installed in each incubator enabled us to control the water temperature. We established cold (constant 3.2 °C) treatment using four incubators and warm (constant 6.2 °C) treatment using the remaining four incubators. The temperatures of both treatments were within the range of site mean temperatures during the incubation period in the wild (Fig. 1c), and at the same time, within the suitable temperature range for embryonic development (Jonsson & Jonsson, 2011). Within 12 h of fertilisation, 20 randomly selected eggs from each family were

assigned into one of 180 mesh tubes in each of the eight incubators. Thus, each of the 52 families had four replicates in each of the two temperature treatments (i.e., 208 replicates for each temperature treatment in total). The day we assigned eggs into the incubator was defined as day 1 of the experiment (Table S1).

The developing embryos were checked daily. All dead and/or unfertilised eggs were removed to maintain the water quality. We counted the number of newly hatched individuals in each replicate daily. The newly hatched individuals in each replicate were transferred to an empty mesh tube within the same tray every two days to keep eggs and hatchlings from each replicate separately. Before transferring the newly hatched individuals, we scanned the lateral aspect of all hatchlings (CanoScan 9000F Mark II, Canon, Tokyo, Japan). We measured the body length, yolk sac length, and yolk sac height of five hatchlings randomly selected from each of 416 replicates using the scanned images. For the 16 replicates with less than five hatchlings (probably due to the low in vitro fertilisation rate of certain families), we measured all hatchlings. We estimated yolk sac volume using the following equation:  $V = (\pi/6) \times L \times H^2$  (Blaxter & Hempel, 1963), where  $L$  is the yolk sac length and  $H$  is the yolk sac height. For later analysis, we calculated replicate-mean values of all measured traits. Moreover, within 48 hours after hatching, one randomly selected hatchling from each of 416 replicates was measured for oxygen consumption rate ( $\text{O}_2$   $\mu\text{g/h}$ ) (details are described in the Supplementary Information). The oxygen consumption rate of each individual was measured at the temperature corresponding to their incubation temperature (i.e., 3.2 or 6.2 °C).

The hatchlings were kept until emergence (here, it is defined as the timing when hatchlings almost completely absorbed their yolk sac). The yolk sac absorption of hatchlings was identified visually following the approach used in Metcalfe and Thorpe (1992). We checked the size of the remaining yolk sac of each individual once per week. When the yolk sac almost disappeared, the hatchlings were removed from the mesh tube, euthanised using an overdose of MS-222, and then



scanned from the lateral side. We calculated the degree-days from day 1 to day at the yolk absorption of each hatchling by adding up the daily mean temperature during the period. Also, we measured the body length of five hatchlings randomly selected from each replicate using the scanned images. Then, we calculated the mean degree-days from fertilisation to yolk absorption (hereafter, degree-days until emergence) and mean body length at yolk absorption (hereafter, body length at emergence) in each replicate, which were subsequently used in the statistical analyses. We calculated the survival rate from hatching to emergence by dividing the number of individuals at emergence by the number of individuals at hatching. In three out of the 416 replicates, we lost hatchlings when moving them from the incubators to the scanner and back to the incubators. We, therefore, obtained complete data set from the 413 replicates and used these data for the later analyses.

## **2.4| Data analyses**

All statistical analyses described below were conducted using R (version 4.0.4. R Development Core Team, 2019) within R studio interface (version 1.4.1717. R studio team 2021).

### **2.4.1| Variations in thermal reaction norms of degree-days until- and body length at-emergence along elevational gradients**

We performed linear mixed models (LMM) using the lmer function in the lme4 package (Bates *et al.*, 2015) to assess how degree-days until- and body length at- emergence vary depending on temperature treatment, the elevation of collection site, and drainage. The interaction between temperature treatment and elevation of collection site was considered in the models to examine how the thermal reaction norms vary with the elevation of collection site. We also considered the interaction between drainage and elevation of collection site in the models to examine how the patterns of variation in early life-history traits along the elevational gradients differ among the drainages. We considered family ID nested within collection site ID as a random factor to account for non-independence among replicates originating from the same family caught at the same site.

A random slope of tray within incubator was also considered since water temperature in lower trays might have been warmer than in upper tray because of the structural issues of the incubators. Then, the initial models (Table 1) were simplified using the backward stepwise selection with the step function (Satterthwaite approximation) in the lmerTest package to select minimum adequate models. Both the degree-days until emergence and body length at emergence were log-transformed. Prior to the analyses, we examined whether the data satisfied the assumptions of linear models. The normality of data from each site in each temperature treatment was visually assessed by using QQ-plot, and we used Levene's test to examine the homoscedasticity of variance. The data generally satisfied the assumptions of linear models, although there were a few exceptions.

We performed the same LMM analysis and subsequent model selection on the survival rate from hatching to emergence. Since we kept the hatchlings within the suitable temperature range for development (Jonsson & Jonsson, 2011), the survival rate was generally high (mean survival rate was 94.4 %). In the final model, no explanatory variables were included (Table S2). Therefore, we did not consider the survival rate further in this study.

#### **2.4.2| Mechanisms underlying the trait variation along elevational gradients**

To explore general mechanisms underlying the consistent trait variation along elevational gradients detected in the first analyses, we examined the links between the elevation of collection site, maternal body length, egg volume, traits at hatching, and the degree-days until- and body length at- emergence in each temperature treatment by using piecewise structural equation models (piecewiseSEM) (Lefcheck, 2016). While we acknowledged that paternal traits could affect the traits of hatchlings, we did not consider paternal effects. This is because variations in the early development of salmonids are largely attributable to the female parent (Janhunnen, Piironen & Peuhkuri, 2010; Thorn & Morbey, 2018). Also, while we created families by pairing randomly selected females and males within sites, their body lengths were highly correlated with

each other because both decreased with the elevation of collection site. Due to the data structure (i.e., no egg volume data for each replicate), family-mean trait values (log-transformed) were used for the analyses (number of families  $[N] = 52$ ).

Prior to the second analyses, to reduce the complexity of the piecewiseSEMs as recommended by Lefcheck (2016), we screened the following six measured traits potentially explaining the variation in degree-days until- and body length at- emergence to remove those not correlated with the elevation of collection site: (1) maternal body length, (2) family-mean egg volume, and (3-6) family-mean traits at hatching (i.e., degree days from fertilisation to hatching, body length, yolk sac volume, and oxygen consumption rate) (Fig. 2). Since traits at hatching are highly correlated with egg volume (Vøllestad & Lillehammer, 2000; Régnier *et al.*, 2013; van Leeuwen *et al.*, 2017), we used residuals of each of the traits at hatching after regression against the egg volume. The residuals were calculated from linear regressions of log-transformed traits at hatching on log-transformed egg volume. We performed LMM analyses to examine whether the traits vary with elevation. We considered site ID nested within drainage as a random factor. For the traits at hatching, we also considered temperature treatment and interaction between temperature treatment and elevation of collection site as additional explanatory variables. The initial models (Table 2) were again simplified using the step function.

Then, using the elevation of collection site, the traits in which a significant effect of elevation of collection site was detected in the preliminary analyses (i.e., maternal body length and egg volume [see results]), and degree-days until- and body length at- emergence, we constructed piecewiseSEMs for each temperature treatment separately. All variables were standardised (i.e., mean was 0 and SD was 1) prior to the analysis. The piecewiseSEM models were initially composed of the four component models describing all direct and indirect effects of elevation of collection site on the degree-days until- and body length at- emergence (Fig. 3a). In each component model, site ID nested within drainage was considered as a random factor to account

for non-independence among families originating from the same site within the same drainage. To avoid overparameterisation of the models, we removed random factors whose standard deviation was estimated to be zero from the models. The initial models were assessed using Shipley's test of the d-separation. When the d-separation test revealed significant unconsidered paths, the paths were added to the model if there was a plausible biological explanation for it. Then, using the variance inflation factor (VIF), we assessed the level of multicollinearity among explanatory variables in each component model. VIF never exceeded 2, indicating acceptable levels of multicollinearity (Dormann *et al.*, 2013). The models were then simplified following the backwards stepwise removal of the non-significant paths ( $P > 0.05$ ). We assessed the models by using Fisher's  $C$  statistics and AICc (AIC corrected for small sample sizes) value to select among alternative models.

### 3| RESULTS

#### 3.1| Variations in thermal reaction norms of degree-days until- and body length at-emergence along elevational gradients

The temperature treatment, elevation of collection site, and interaction between temperature treatment and elevation of collection site were included in the final models of both degree-days until emergence (i.e., degree-days from fertilisation to yolk absorption) and body length at emergence (Table 1). The fact that the interaction between the elevation of collection site and drainage was not included in the final models (Table 1) suggest that the patterns of trait variations along the elevational gradients detailed below were consistent across the drainages.

The degree-days until emergence in the warm treatment ( $796.5 \pm 30.7$  degree-days [treatment-mean  $\pm$  SD]) was 1.1 times greater than in the cold treatment ( $708.8 \pm 24.1$  degree-days) (estimate  $\pm$  SE =  $7.2 \times 10^{-2} \pm 7.2 \times 10^{-3}$ ,  $t$ -value = 10.0,  $P < 0.0001$ , Table S3) (Fig. 1d). In terms of the number of days until emergence, hatchlings kept in the warm treatment reached the

emergence stage about three months earlier than in the cold treatment. The thermal reaction norm varied with the elevation of collection site. Specifically, the degree of difference in the degree-days between warm and cold treatments increased by about 16 degree-days as the elevation of collection site decreased by 1000 m (estimate  $\pm$  SE =  $-7.9 \times 10^{-6} \pm 1.5 \times 10^{-6}$ ,  $t$ -value = -5.4,  $P < 0.0001$ ) (Fig. 1d). The degree-days until emergence increased with decreasing the elevation of collection site. For instance, in the cold treatment, the degree-days until emergence increased by about 13 degree-days (i.e., about four days) as the elevation of collection site decreased by 1000 m (Fig. 1d) (estimate  $\pm$  SE =  $-8.2 \times 10^{-6} \pm 3.4 \times 10^{-6}$ ,  $t$ -value = -2.4,  $P = 0.04$ ).

Although temperature treatment was included in the final model (Table 1, Fig. 1e), the body length of hatchlings in the warm ( $21.3 \pm 1.3$  mm [treatment-mean  $\pm$  SD]) and cold ( $21.3 \pm 1.3$  mm) treatments were almost the same (estimate  $\pm$  SE =  $3.9 \times 10^{-3} \pm 2.2 \times 10^{-3}$ ,  $t$ -value = 1.8,  $P = 0.08$ , Table S4). The hatchlings originating from low-elevation sites grew slightly larger when reared in the warm treatment, while hatchlings originating from high-elevation sites grew slightly larger when reared in the cold treatment (Fig. 1e) (estimate  $\pm$  SE =  $-2.9 \times 10^{-6} \pm 1.4 \times 10^{-6}$ ,  $t$ -value = -2.1,  $P = 0.04$ ). The body length at emergence increased with decreasing the elevation of collection site (Fig. 1e). For instance, in the cold treatment, the body length of hatchlings increased by 5% per 1000 m reduction in the elevation (estimate  $\pm$  SE =  $-2.3 \times 10^{-5} \pm 6.8 \times 10^{-6}$ ,  $t$ -value = -3.4,  $P = 0.001$ ) (Fig 1e). Drainage was included in the final model (Table 1). Post-hoc comparison using Tukey's test found that hatchlings originating from the Danube ( $21.6 \pm 0.9$  mm [drainage-mean  $\pm$  SD]) were larger than from the Po ( $20.8 \pm 1.5$  mm) ( $t$ -ratio = 2.8,  $P = 0.02$ ). Hatchlings originating from the Rhine ( $21.6 \pm 1.2$  mm) were also larger than from the Po, while the difference was marginally non-significant ( $t$ -ratio = -2.4,  $P = 0.05$ ). No significant difference was found between Rhine and Danube ( $t$ -ratio = 0.9,  $P = 0.6$ ).

### **3.2| Mechanisms underlying the consistent patterns of elevational trait variation**

#### ***Variation in maternal body length, egg volume, and traits at hatching***

Among the six traits of interest (maternal body length, egg volume, and residuals of traits at hatching after regression against egg volume [body length at-, degree-days until-, yolk sac volume at-, and oxygen consumption rate at- hatching]), the elevation of collection site was included in the final models of maternal body length and egg volume (Fig. 2 and Table 2).

Specifically, maternal body length and egg volume increased by 16% (estimate  $\pm$  SE =  $-6.5 \times 10^{-5} \pm 2.4 \times 10^{-5}$ ,  $t$ -value = -2.7,  $P$  = 0.02, Table S5) and 21 % (estimate  $\pm$  SE =  $-8.3 \times 10^{-5} \pm 3.2 \times 10^{-5}$ ,  $t$ -value = -2.6,  $P$  = 0.03, Table S6) per 1000 m reduction in elevation, respectively (Fig. 2a,b).

Regarding the residuals of traits at hatching after regression against egg volume, only temperature treatment was included in the final models (Fig. 2c-f and Tables 2, S7-10).

#### ***Links between the elevation of collection site, maternal body length, egg volume, and degree-days until- and body length at- emergence***

All final piecewise SEM models had adequate fit to the data (Fisher's  $C$  statistic with  $P > 0.05$ , Fig. 3b,c). They revealed that egg size-mediated maternal effects are the key mechanisms underlying the increase in the degree-days until- and body length at- emergence with decreasing the elevation of collection site. Specifically, females originating from low-elevation sites were larger than from high-elevation sites, thereby producing larger eggs. The hatchlings from larger eggs used more degree-days until emergence and grew larger than those from smaller eggs. On the other hand, for a given egg volume, hatchlings from larger females used fewer degree-days until emergence than those from smaller females. The direct negative link between the elevation of collection site and degree-days until emergence was included in both temperature treatments, even after accounting for the effects of maternal body length and egg volume.

## **4| DISCUSSION**

Many studies have documented patterns of intraspecific trait variations along geographic thermal gradients and have provided valuable information for inferring how temperatures and associated

environmental conditions (to which populations have been exposed over generations) affect traits through transgenerational plasticity in- and/or natural selection on- these traits (Conover *et al.*, 2009). However, since most studies did not test the consistency of the pattern (but see Kavanagh *et al.*, 2010), they cannot rule out the possibility that the patterns were solely explained by random processes. Here, by conducting a common-garden experiment using the brown trout from wide and replicated elevational gradients from three Alpine drainages, we showed consistent patterns of variation in key early life-history traits along elevational gradients. Specifically, degree-days until- and body length at- emergence, both affecting early growth and survival of fish (Einum & Fleming, 2000b; Skoglund *et al.*, 2011), increased with decreasing elevation of origin consistently across the three drainages.

We also found variations in thermal reaction norms of early life-history traits along the elevational gradients. Similar to previous studies (Beacham & Murray, 1990; Jonsson & Jonsson, 2011; Réalis-Doyelle *et al.*, 2016), warmer rearing temperatures markedly increased degree-days until emergence. Immediate temperature effects increased slightly with decreasing elevation of origin. Also, an increase in temperature slightly enhanced growth in size of hatchlings originating from lower elevations, but in contrast, it slightly reduced growth of hatchlings originating from higher elevations. These results suggest that the effects of climate warming on trout populations could vary with elevation, given the importance of early life-history traits in determining early growth and survival (Einum & Fleming, 2000b; Jonsson & Jonsson, 2011; Skoglund *et al.*, 2011). We have to mention, however, that the degrees of variation in thermal reaction norms are considered negligibly small, although patterns were statistically significant. The subtle differences in thermal reaction norm in timing and body length at emergence are unlikely to be reflected in early growth and survival, considering within-stream heterogeneity in temperature (Leach & Moore, 2014; Sullivan *et al.*, 2021).

An increase in egg size with decreasing elevation of origin was a key mechanism underlying

the increase in the degree-days until- and body length at- emergence with decreasing elevation of origin. For the degree-days until emergence, the egg size-mediated maternal effects generally followed the relationship between egg size and development rate predicted by processes controlled by metabolic rate (Gillooly, Londoño & Allen, 2008). However, this may not always be the case for salmonids. Although there are studies that have shown deceleration in early development rate with increasing egg size (Einum & Fleming, 2000a; Rollinson & Hutchings, 2010), some studies showed no relationship or even the opposite relationship (Einum & Fleming, 1999; van Leeuwen *et al.*, 2017). The discrepancy of reported patterns among studies suggests diversity in egg-size mediated maternal effects. Interestingly, for a given egg size, hatchlings from larger females developed faster than those from smaller females. Non-egg size-mediated maternal effects suggest that larger females producing larger eggs could somehow compensate for egg size-mediated maternal effects. It has been shown that, for example, hormone and carotenoid content of eggs can affect early development of hatchlings (Burton *et al.*, 2011; Wilkins *et al.*, 2017). For a better understanding of non-egg size-mediated maternal effects, examining how egg chemical composition varies with maternal body length is an interesting next step. Even after accounting for maternal effects, the direct link between the elevation of origin and degree-days until emergence still remained. This suggests the possibility of local adaptation and grandparental effects on embryonic development.

The relationship between offspring size and offspring fitness is fundamental in determining maternal investment per offspring (offspring size) (Smith & Fretwell, 1974; Venable, 1992; Rollinson & Hutchings, 2013). The present study suggests a shift in the relationship, and hence in optimal egg size, along the elevational gradient. In salmonid fishes, hatchlings engage in intensive intraspecific resource competition and suffer from predation during early free-swimming stages (Jonsson & Jonsson, 2011). During the early critical period of salmonid fishes, larger body size and faster development are generally thought to be advantageous for survival



and growth (Einum & Fleming, 2000b; Skoglund *et al.*, 2011). However, it is difficult for hatchlings to increase body size and accelerate their development rate simultaneously because of the constraints imposed by egg size. Therefore, the relationship between egg size and hatchling fitness could be determined by the relative importance of fitness benefits obtained from an increase in body size and fitness losses caused by a deceleration in development rate through an increase in egg size. The increases in degree-days until- and body length at- emergence with decreasing elevation of origin suggest that fitness benefits obtained from an increase in body size could become relatively more important at lower elevations, and thus an optimal egg size at low elevations shifts towards larger size.

The shift in the relative importance of fitness benefits obtained from an increase in body size and fitness losses caused by a deceleration in development rate through an increase in egg size along the elevational gradient seems reasonable. Given the acceleration of development rate at warmer temperatures shown in the present and many previous studies (Beacham & Murray, 1990; Jonsson & Jonsson, 2011; Réalis-Doyelle *et al.*, 2016), hatchlings may develop faster in warm, low elevations. Moreover, in our study system, an increase in body size through an increase in egg size may only slightly decelerate development rate. The slight deceleration of development rate due to an increase in egg size could be compensated easily by, for example, nest environments chosen by parents, considering the marked effects of rearing temperatures on development rate and within-stream temperature variation (Leach & Moore, 2014; Sullivan *et al.*, 2021). Importantly, hatchlings are likely to suffer more intensive competition and predation at lower elevations due to, for example, higher conspecific and predator density (Schager, Peter & Burkhardt-Holm, 2007; Buisson, Blanc & Grenouillet, 2008; Jones *et al.*, 2019). All this considered, fitness benefits obtained from an increase in body size could become relatively more important at lower elevations.

Alternatively, there is a possibility that consistent patterns of variation in early life-history

traits along elevational gradients are just by-products reflecting a shift in female body length at reproduction along elevational gradients. For instance, in most ectotherms, an increase in size at maturation is often caused by a decrease in temperature and an improvement in food conditions (Berrigan & Charnov, 1994). While temperatures increase with decreasing elevations, food conditions can improve as elevation decreases, considering the higher temperature and nutrient availability at low elevations (Ward, 1998). If the latter is relatively more important in determining the female size at reproduction, female size should increase with decreasing elevation. In support of this, Bærum *et al.* (2013) showed that an increase in temperature during the growth period enhances growth of matured brown trout, perhaps by increasing prey availability. The plastic increase in female size at reproduction in response to warmer temperatures could result in an increase in egg size and, eventually, degree-days until- and body length at- emergence. Considering the possible importance of female body size plasticity in shaping variation in early-life history traits, we cannot completely rule out the possibility that consistency in the patterns of variation in early life-history traits across drainages is due to stocking activities that homogenise populations, although populations from the three drainages still possess distinct genetic characteristics (Keller *et al.*, 2011).

Especially in alpine streams, where salmonid fishes are often dominant fish and function as keystone species, hydropower plants arguably plays a key role in recent temperature changes, while also affecting various environmental factors (Halleraker *et al.*, 2007; Olden & Naiman, 2010; Lange *et al.*, 2018). Hydropower operation caused increases of up to 5 °C in downstream temperature during winter but decreases of up to 15 °C in downstream temperature during summer (Olden & Naiman, 2010). The increase in winter temperature, when embryonic development of most salmonids occurs, could significantly accelerate emergence, as the present study and previous studies have shown (Beacham & Murray, 1990; Jonsson & Jonsson, 2011; Réalis-Doyelle *et al.*, 2016). In addition to the immediate temperature effects, given the

importance of egg size in determining timing and body size at emergence, there is a possibility that the decrease in summer temperature and associated changes in environmental conditions could affect early life-history traits by influencing maternal reproductive strategies. Moreover, we note that the actual timing of emergence is determined by spawning date and embryonic development rate, while we focused only on the latter in this study. Therefore, studying how decreased summer temperatures and associated changes in environmental conditions affecting female reproductive traits (such as size and timing at spawning and egg size and quality) is a valuable next step to better understand the effects of hydropower plant-induced temperature changes on the key early life-history traits of this economically and ecologically important fish.

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#### DATA AVAILABILITY STATEMENT

The data associated with this paper are available from the Dryad Digital Repository <https://doi.org/10.5061/dryad.crjdfn37r>

#### CONFLICT OF INTEREST

There is no conflict of interest.

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## FIGURE LEGENDS

**Figure 1.** Overview of collection sites and variations in traits at emergence along the elevational  
gradient. (a) Maps of collection sites. Original maps used to describe collection sites were  
downloaded from [https://d-maps.com/carte.php?num\\_car=24779&lang=en](https://d-maps.com/carte.php?num_car=24779&lang=en), [https://d-maps.com/carte.php?num\\_car=10439&lang=en](https://d-maps.com/carte.php?num_car=10439&lang=en), <https://www.freecountrymaps.com/blank-maps/wgs84/transparent/watermarkless/switzerland.png>,

[maps.com/carte.php?num\\_car=2251&lang=en](https://maps.com/carte.php?num_car=2251&lang=en). (b) Elevation above sea level (m) of each collection site. (c) The relationship between elevation of collection site and water temperature during the incubation period. (d) The relationships between the elevation of collection site and degree-days until emergence in the cold and warm treatments. (e) The relationships between the elevation of collection site and body length at emergence in the cold and warm treatments. In (d) and (e), the circles filled with white and other colours (black, blue, and red) with error bars denote population mean  $\pm$  SD in the warm and cold treatment, respectively. Solid lines in (d) and (e) are predictions from the final models (Table 1), and shaded areas are 95 % CI drawn using the sjPlot package. Note that Y axes are log-scaled in (d) and (e).

**Figure 2.** (a) The relationship between elevation of collection site and maternal body length. (b) The relationship between elevation of collection site and egg volume. In (a) and (b), the filled circles with error bars denote population mean  $\pm$  SD. Solid lines in (a) and (b) are predictions from the final models (Table 2), and shaded areas are 95 % CI drawn using the sjPlot package. Relationships between temperature treatment and residuals of (c) body length at hatching-, (d) degree-days until hatching-, (e) yolk sac volume at hatching-, and (f) oxygen consumption rate at hatching- after regression against egg volume. Error bars denote SD. Note that Y axes are log-scaled in (a) and (b).

**Figure 3.** Initial (a) and final path models of the cold (b) and warm (c) treatments. Unidirectional arrows represent supported relationship (positive [blue] and negative [orange]) between variables. The thickness of lines is scaled to the magnitude of standardized correlation coefficients.

## TABLE LEGENDS

**Table 1.** List of explanatory variables and random factors included in the final models (in bold) after backwards models selection using step function (Satterthwaite approximation) and those included in the initial models explaining traits at emergence. Npar is an abbreviation for the

number of parameters in the model.  $\Delta AIC$  gives the difference in AIC between the final and initial models.

**Table 2.** List of explanatory variables and random factors included in the final models (in bold) after backwards models selection using step function (Satterthwaite approximation) and those included in the initial models explaining maternal traits and traits at hatching. Npar is an abbreviation for the number of parameters in the model.  $\Delta AIC$  gives the difference in AIC between the final and initial models.

FIGURES

Figure 1.

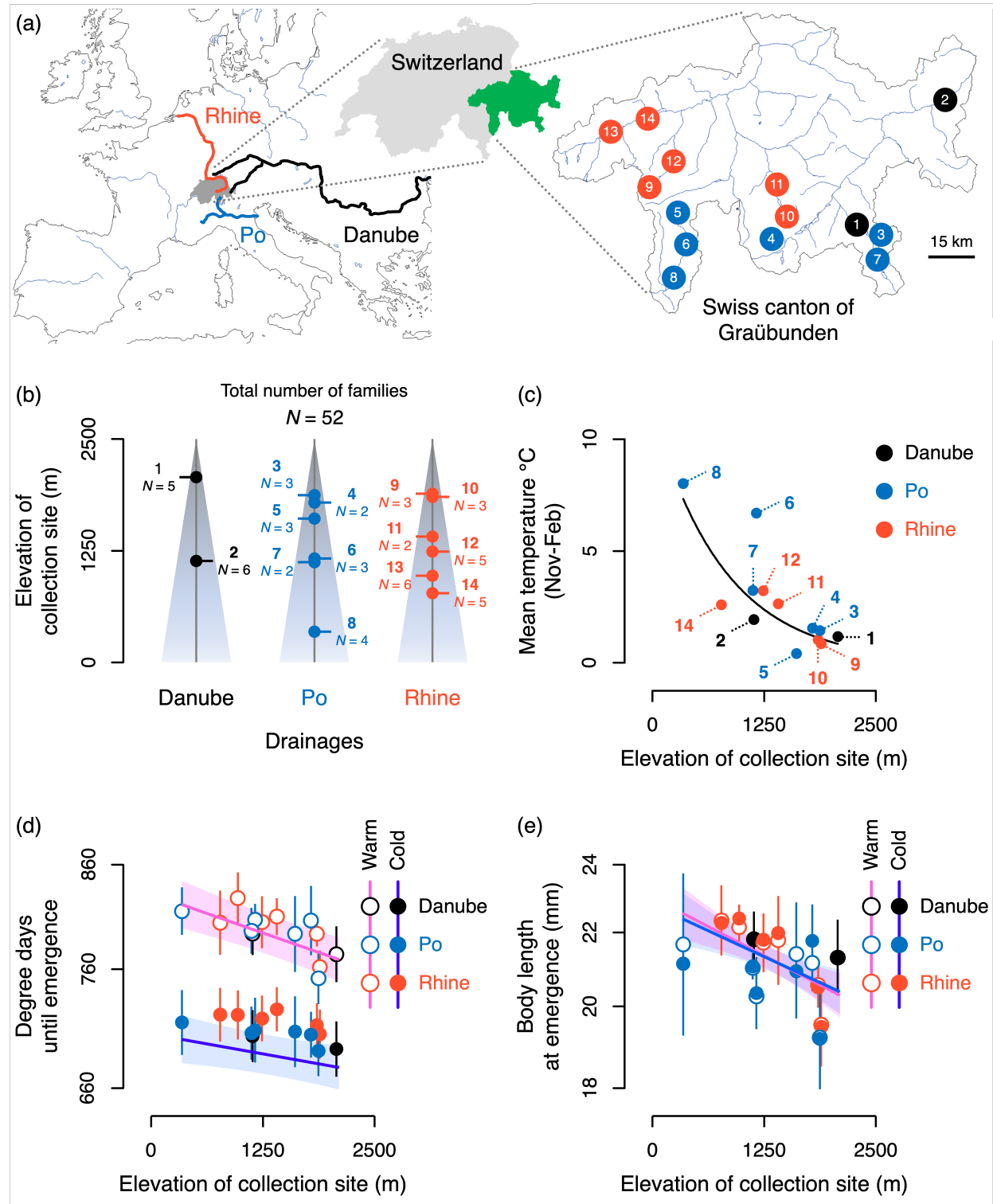


Figure 2.

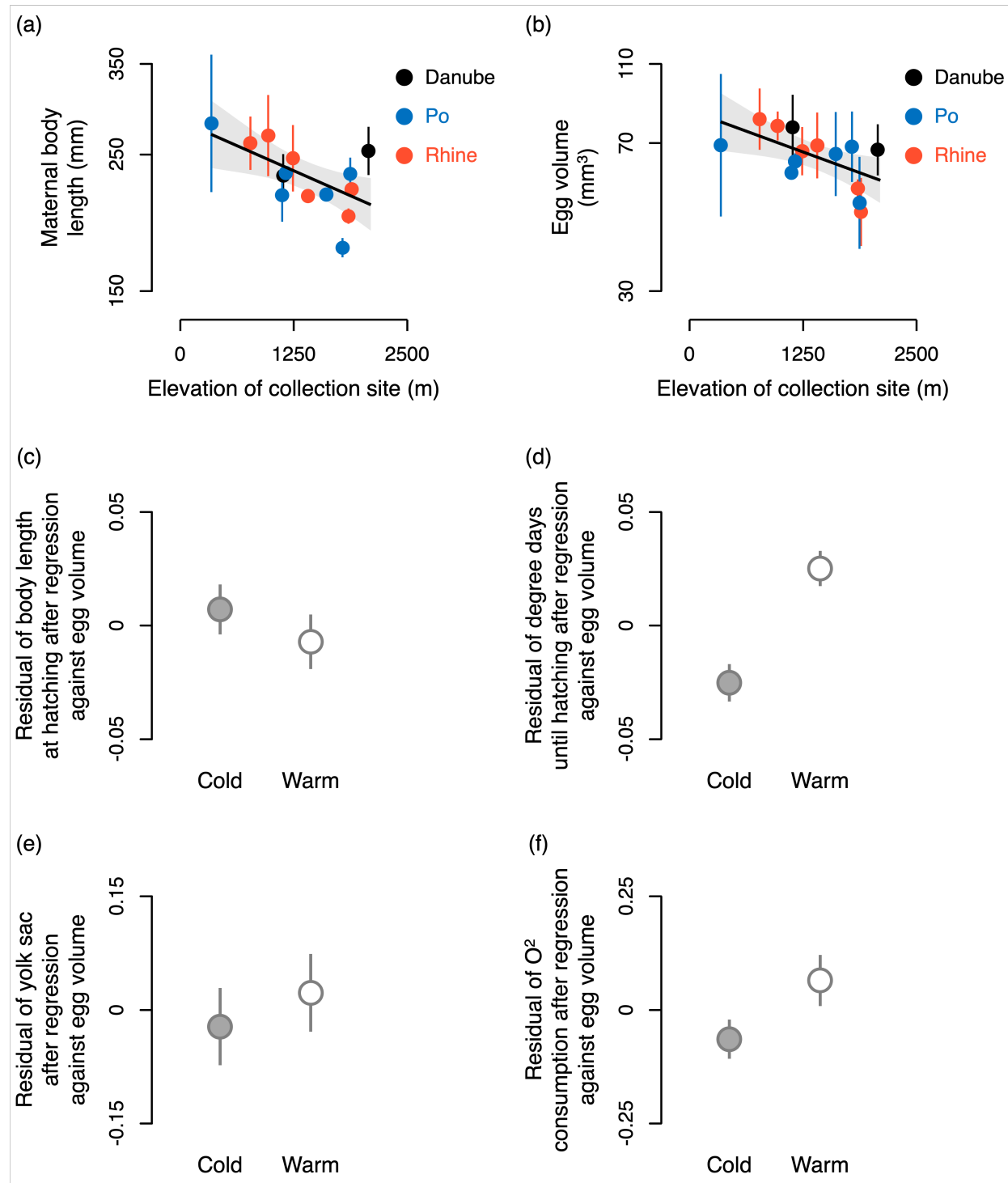
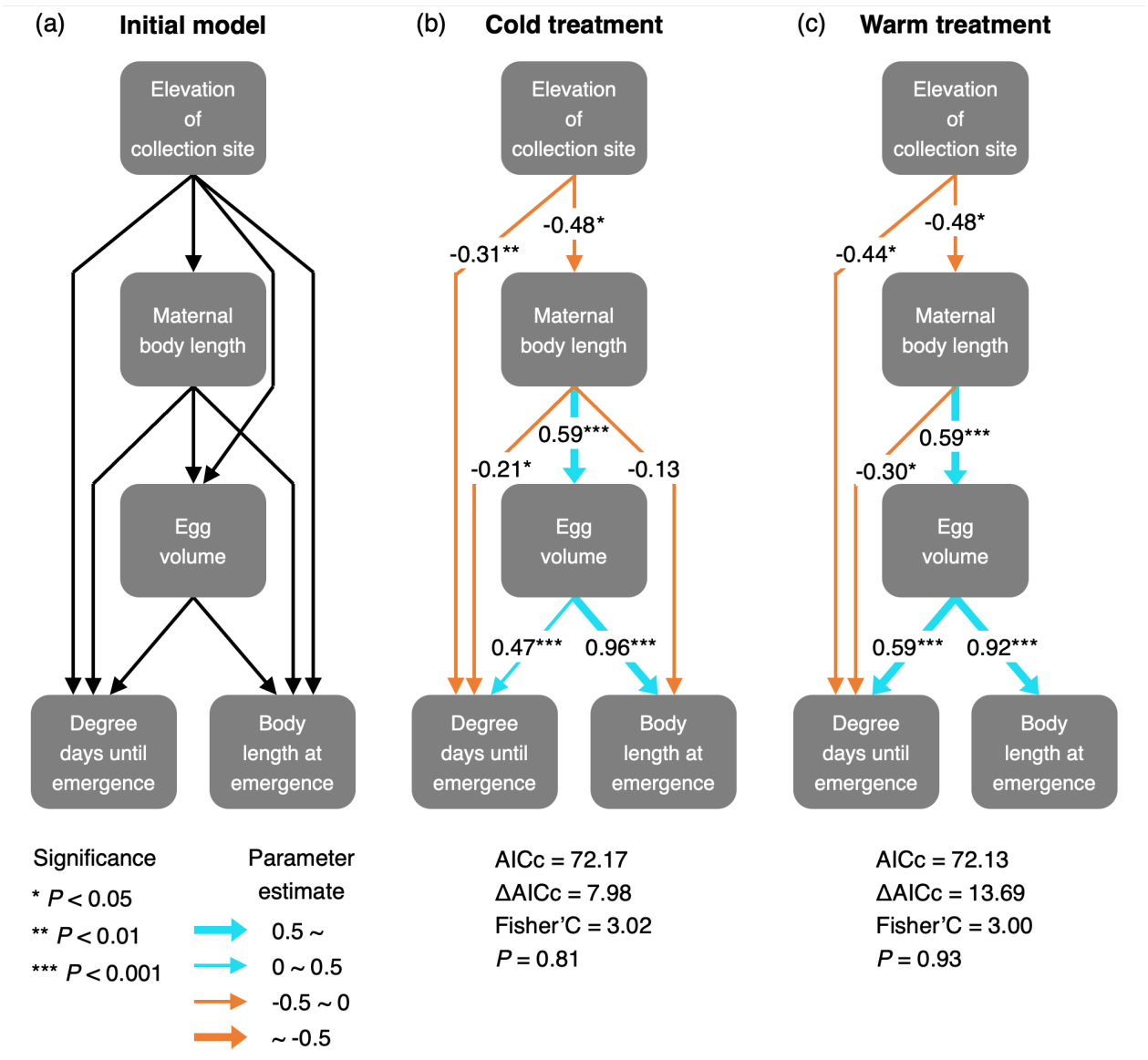


Figure 3.



**Table 1**

	<b>Npar</b>	<b>AIC</b>	<b><math>\Delta AIC</math></b>
<b><i>(1) Degree-days until emergence</i></b>			
<b>Treatment + Elevation of collection site +</b>	10	-2700.7	0
<b>Treatment: Elevation of collection site +</b>			
<b>(1 site/family) + (1+tray incubator)</b>			
Treatment + Elevation of collection site + Drainage +	14	-2697.7	3.0
Treatment: Elevation of collection site +			
Elevation of collection site: Drainage +			
(1 site/family) + (1+tray incubator)			
<b><i>(2) Body length at emergence</i></b>			
<b>Treatment + Elevation of collection site + Drainage +</b>	9	-2679.8	0
<b>Treatment: Elevation of collection site +</b>			
<b>(1  family: site) + (1 incubator)</b>			
Treatment + Elevation of collection site + Drainage +	14	-2675.5	4.3
Treatment: Elevation of collection site +			
Elevation of collection site: Drainage +			
(1 site/family) + (1+tray incubator)			

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**Table 2**

	<b>Npar</b>	<b>AIC</b>	<b><math>\Delta AIC</math></b>
<b>(1) Maternal body length</b>			
<b>Elevation of collection site + (1 site:drainage)</b>	4	-314.2	0
Elevation of collection site + (1 drainage/site)	5	-312.2	2.0
<b>(2) Egg volume</b>			
<b>Elevation of collection site + (1 site:drainage)</b>	4	-213.5	0
Elevation of collection site + (1 drainage/site)	5	-211.5	2.0
<b>(3) Degree-days until hatching</b>			
<b>Treatment + (1 site:drainage)</b>	4	-723.7	0
Treatment + Elevation of collection site +	7	-719.2	4.5
Treatment: Elevation of collection site + (1 drainage/site)			
<b>(4) Body length at hatching</b>			
<b>Treatment + (1 site:drainage)</b>	4	-642.8	0
Treatment + Elevation of collection site +	7	-641.5	1.3
Treatment: Elevation of collection site + (1 drainage/site)			
<b>(5) Yolk sac volume at hatching</b>			
<b>Treatment + (1 site:drainage)</b>	4	-365.7	0
Treatment + Elevation of collection site +	7	-365.3	0.4
Treatment: Elevation of collection site + (1 drainage/site)			
<b>(6) Oxygen consumption rate at hatching</b>			
<b>Treatment</b>	3	-324.1	0
Treatment + Elevation of collection site +	7	-317.6	6.5
Treatment: Elevation of collection site + (1 drainage/site)			