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ORIGINAL ARTICLE

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Chinook salmon emergence phenotypes: Describing the relationships between temperature, emergence timing and condition factor in a reaction norm framework

Abby E. Fuhrman[1] | Donald A. Larsen[1] | E. Ashley Steel[2] | Graham Young[3] | Brian R. Beckman[1]

[1]Environmental and Fisheries Sciences Division, Northwest Fisheries Science Center, National Marine Fisheries Service, NOAA, Seattle, WA, USA

[2]US Forest Service, Pacific Northwest Research Station, Seattle, WA, USA

[3]School of Aquatic and Fishery Sciences, University of Washington, Seattle, WA, USA

Correspondence
Abby E. Fuhrman, Environmental and Fisheries Sciences Division, Northwest Fisheries Science Center, National Marine Fisheries Service, NOAA, Seattle, WA, USA.
Email: Abby.Fuhrman@noaa.gov

Abstract

Water temperature can have a profound influence on development and distribution of aquatic species. Salmon are particularly vulnerable to temperature changes because their reproductive and early development life phases are spent in freshwater river systems where temperature fluctuates widely both daily and seasonally. Flow regulation downstream of dams can also cause temperature regime changes, which in turn may spur local adaptation of early life-history traits. In a common garden laboratory incubation experiment, we exposed spring Chinook salmon (*Oncorhynchus tshawytscha*) embryos to four temperature regimes: warm stable, cold stable, daily variation and below dam. We found that fry from warmer thermal regimes emerged earlier than those from colder regimes both in terms of calendar date and temperature units and that warmer temperatures caused fry to emerge less developed. There was also a significant effect of family on both emergence timing and development level at emergence. By combining measurements of physiological and behavioural traits at emergence and interpreting them within a reaction norm framework, we can better understand which populations might be more vulnerable to altered thermal regimes.

KEYWORDS
emergence timing, phenotypic plasticity, reaction norm, Salmon, temperature

1 | INTRODUCTION

Salmon behaviour and physiology are intertwined with water temperature, especially during the freshwater phase of their lifecycle. Adult spawn timing is influenced by the local thermal environment, and over time, offspring emergence period is selected to correspond with ideal flow, temperature and food availability (Brannon, 1987; Skoglund, Einum, & Robertsen, 2011). Once eggs are deposited in the gravel, the thermal regime experienced during incubation determines development rate (Alderdice & Velsen, 1978). Modifications to freshwater river systems, like dams and climate change, alter water temperature profiles during salmon development and may disrupt selection patterns over time (Angilletta et al., 2008; Crozier et al., 2008). Rapid onset of anthropogenic changes to river and stream thermal regimes underscores the need for better measurements of phenotypic plasticity during salmon development (Burt, Hinch, & Patterson, 2010).

Understanding plasticity of certain developmental traits in response to environmental changes will help in estimating the degree to which these traits might contribute to the adaptive potential of a population. The range of expression for a phenotypic trait across different environments within a single genotype is known as a reaction norm (Schlichting & Pigliucci, 1993; Woltereck, 1913). The foundation for future reaction norm research should be based on the notion that plasticity is most likely heritable, and should also take into account the idea that population differences in reaction norms suggest that adaptation is functioning on a local scale (Hutchings, 2011). Evaluating reaction norms for traits that have major fitness consequences is becoming an important tool for salmon conservation and recovery efforts.

For salmonids, emergence timing and condition at emergence influence early growth and survival and thus have direct impacts on fitness (Einum & Fleming, 2000). Early studies established species-specific development rate and condition at emergence under constant incubation temperatures (Alderdice & Velsen, 1978; Beacham & Murray, 1990; Heming, 1982). Although the rate of development may differ based on thermal regime and species, all developing salmon (alevins) eventually reach a certain morphological threshold where they are physically capable of swimming movements. Swim-up and surfacing behaviour have been correlated with emergence age in rainbow trout (Dill, 1977; Huntingford, 1993). The time at which emergence occurs is related to water temperature, but is also influenced by other environmental factors including but not limited to light, sediment size and dissolved oxygen (Geist et al., 2006; Heard, 1964; Witzel & MacCrimmon, 1981). Furthermore, studies on Chinook salmon development have established that there is a genetic component to emergence timing (Beckman, Gadberry, Parkins, & Larsen, 2008), and that genetics may dictate the magnitude of response to temperature variability (Steel et al., 2012). However, only a few studies have expanded knowledge about the potential for adaptive variation in emergence timing and condition at emergence in changing environments. Hendry, Hensleigh, and Reisenbichler (1998) documented evidence for plasticity of yolk conversion efficiency in Lake Washington sockeye populations that experience unique thermal regimes due to temporal differences in spawn timing. A common garden experiment on sockeye populations from the Fraser River found evidence for inter- and intraspecific phenotypic plasticity in survival rates at different incubation temperatures (Whitney, Hinch, & Patterson, 2013).

Salmon populations that spawn in rivers with thermal regimes altered by dams present a unique opportunity to study reaction norms and improve knowledge about the capacity for populations to adapt. The release of thermally stratified water from dams can delay seasonal cooling typically found in late autumn and early winter. Flow regulation may also reduce daily temperature variation downstream (Rounds, 2010). This interruption in normal temperature pattern typically occurs at a critical time for salmon, while embryos and alevins are immobile in the gravel during incubation. The unseasonably warm temperatures downstream of dams during the late fall can cause salmon to develop at a faster rate and exhibit swim-up emergence behaviour as much as 2 months earlier than normal (Webb & Walling, 1993). Many strategies have been implemented to combat the problem of early emergence, including more informed regulation of water temperature downstream, and transporting adults upstream of impoundments to spawn and recolonise (Keefer et al., 2010).

Additional research is needed to understand how families and populations differ in their developmental response to temperature variation. We conducted a common garden incubation experiment using populations of Chinook salmon (*Oncorhynchus tshawytscha*) from the Willamette River Basin, Oregon and Yakima River Basin, Washington to address the following two questions. Does the condition, size, or emergence timing of fry differ between families and across populations? How does the reaction norm (interaction between genotype and environment) depend on the thermal regime experienced during incubation? Our approach combines measurements of physical and behavioural aspects of emergence to better evaluate variation in emergence phenotypes within and across populations.

2 | MATERIALS AND METHODS

2.1 | Gamete collection and fertilisation

Chinook salmon eggs and milt were collected from four separate hatchery populations: three from Oregon's Willamette River Basin, and one from the Yakima River in Washington (Figure 1). At each location, eggs were stripped from six females (Table S1). Each lot of eggs was placed in a 0.74 L Ziploc (SC Johnson, Racine, WI, USA) bag, which was then filled with oxygen and sealed. A similar procedure was repeated for collection of milt from six males at each facility. Bags with gametes were insulated from direct ice contact with a wet towel and transported to the Northwest Fisheries Science Center (NWFSC) in Seattle, Washington, during the week of September 17, 2012.

Transport and artificial fertilisation occurred on September 18 for Yakima, September 19 for South Santiam and McKenzie and September 20 for Clackamas gametes. One-to-one family crosses were produced at the NWFSC according to standard salmon hatchery spawning protocols (Stickney, 1991). Egg lots were strained to remove excess ovarian fluid and weighed to the nearest 0.1 g. A subsample of unfertilised eggs (n = 10) from each family was weighed and frozen at −20°C for later analysis. Each lot of eggs was divided equally by weight into eight plastic cups. Milt from one male was removed from the transport bag using a sterile 10 ml syringe and distributed equally among the eight egg lots. After combining eggs and milt, fertilisation, water hardening and disinfection were initiated by adding 750 ml of iodine–water mixture (1:200) to each cup. Water was decanted after ten minutes and embryos were placed into 10 cm mesh-lined square bottomless plastic planter cups. This process was repeated to generate six unique families from each population (six 1 × 1 male–female crosses, each split into eight lots). Garden planter cups of embryos were nested inside larger 0.95 L containers and supplied with upwelling water from one of four temperature regimes via siphon tubes at a rate of 2 L × min^{-1} (Figure S1).

2.2 | Experimental design and apparatus

All embryos were incubated in a common pool of re-circulating water from which four thermal treatment regimes were created, each with temperatures falling between 5–10°C. Two treatments maintained relatively constant temperatures throughout the experiment, while the other two treatments had a mixture of daily and seasonal temperature variation in an effort to mimic natural (with daily variation) and below-dam (seasonal shift) environments (Figure 2). More specifically, the daily variation and below-dam treatments were designed with direct reference to temperature differences above and below Cougar Dam on the South Fork of the McKenzie River in Oregon as described by a USGS report on thermal effects of dams in the

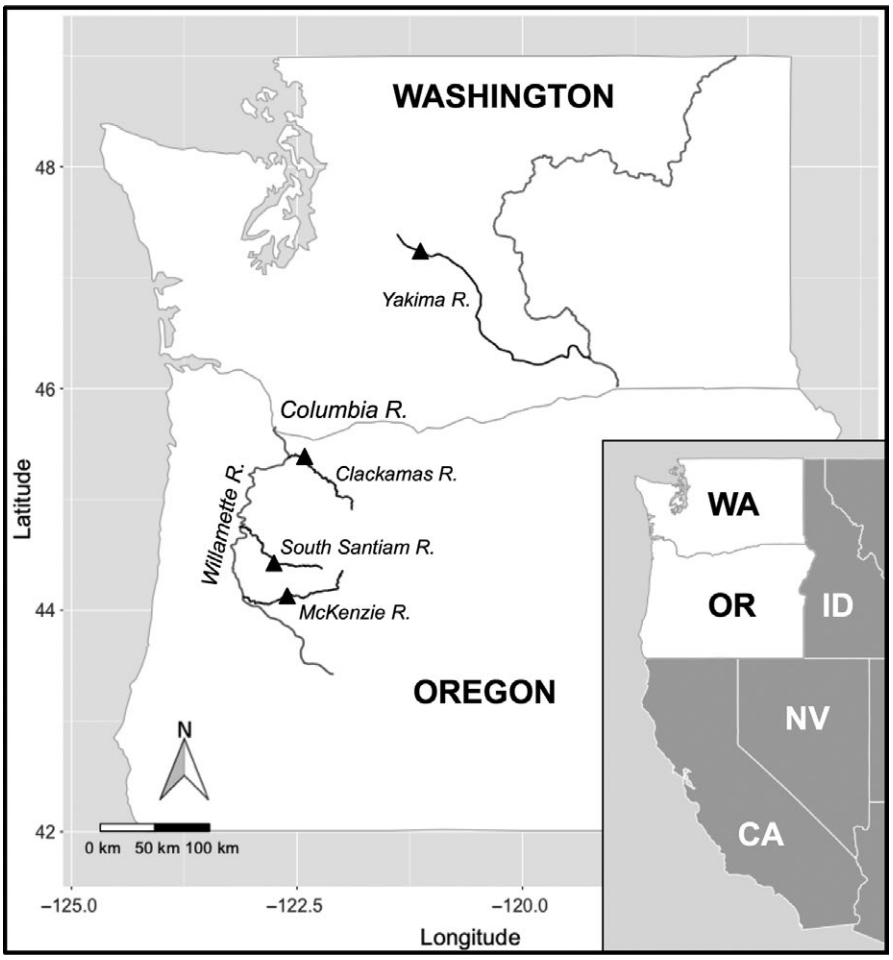


FIGURE 1 Salmon eggs and milt were collected from fish at hatcheries (Δ) on tributaries of the Willamette and Columbia River systems

Willamette River Basin (Rounds, 2010). The cold and warm stable treatments were designed to be a similar overall average temperature as the daily variation and below-dam treatments respectively. De-chlorinated municipal water was chilled to approximately 5°C, circulated to eight 105 L head tanks and aerated with medium pore air diffusers. Water in head tanks was heated with two immersion heaters (Process Technology ELSA1111-P1, Mentor, Ohio) using digital thermostat controllers (Process Technology DRAE15-1, Mentor, Ohio). To achieve daily temperature variation for the natural treatment, power to heaters was controlled by heavy-duty appliance timers (Intermatic HB113, Spring Grove, IL, USA) programmed to turn heaters on during daylight hours. All four treatment regimes were replicated for a total of eight thermal treatments (4 regimes × 2 replicates). Temperature was recorded hourly using temperature data loggers (HOBO, Onset Computer Corp, Bourne, MA, USA) placed in each head tank. An additional temperature data logger was submerged in one incubation chamber per treatment to confirm the similarity of temperature in head tanks and chambers.

2.3 | Incubation

Salmon embryos were incubated in accordance with regulations set forth by the Institutional Animal Care and Use Committee for use of animals in scientific research, under University of Washington protocol 2313-09. They were kept in complete darkness, except when water flow rates were being monitored. During monitoring events, red lights were used as embryos are initially sensitive to natural light. Clear vinyl aquarium tubing (6.4 mm inside diameter) originating from thermal regime treatment head tanks supplied water to each individual egg container via simple gravitational siphons. Equal flow rates from siphon tubes (0.75 L × min^{-1}) were attained by placing all cups at the same elevation. Once eye pigmentation was visible, unfertilised and dead eggs were identified by applying a mechanical shock (pouring embryos from the cup into a bucket from a height of 0.5 m), counted, removed and discarded.

To maximise experimental efficiency, we discarded the two families from each population with the lowest survival across treatments. Embryos (n = 80) from the remaining family groups in each treatment were transferred to individual incubation chambers measuring 10.2 cm in diameter and 35.6 cm tall. Artificial substrate was created with 13-mm diameter plastic bio-filtration balls weighed down by a single layer of 13-mm diameter black glass marbles. Water was supplied to each chamber at 1.5-L min^{-1} using a pump connected to a valve manifold (1.27 cm diameter by 50.8-cm long) fitted with eight 0.64 cm tube adapters. To maximise replication of families across treatments, emergence chambers were divided into half by a plastic 3 mm mesh divider placed lengthwise through the middle of the chamber and secured on each side with silicon aquarium sealant. Two families from the same

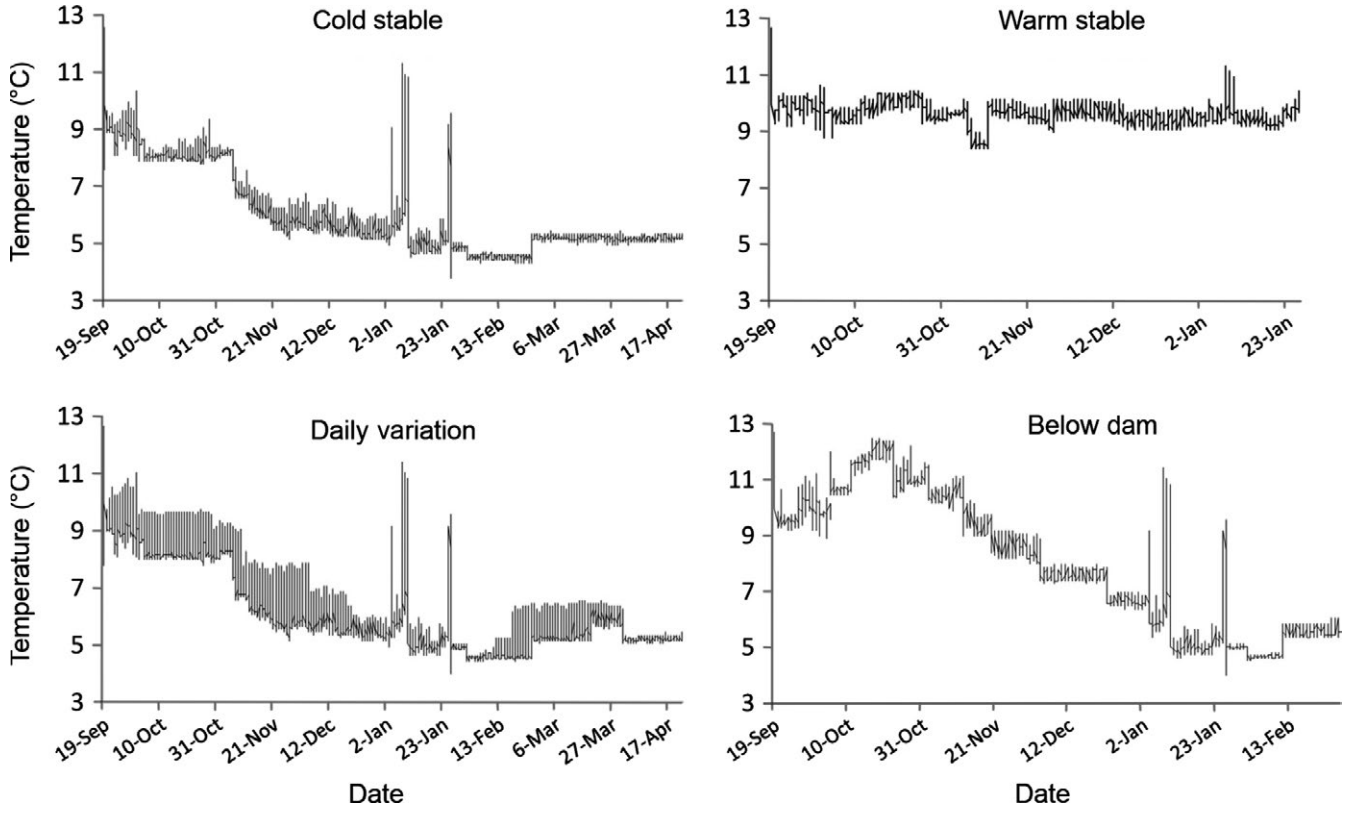


FIGURE 2 Temperature regime treatments. Note the temperature spikes at the beginning and end of January were due to a chilling pump failure

population were loaded into divided emergence chambers, one family per side (Figure S2). To monitor hatch timing, additional embryos (n = 80) from each family/treatment group were placed in the corresponding family's collection cup. When embryos in the collection cups hatched, alevins were removed, counted and euthanised with a lethal dose (300 ppm) of buffered tricaine methanesulphonate (MS-222, Western Chemical, Ferndale, WA) every 24 h. The period of development between hatching and emergence was long enough to permit all alevins in collection cups to be counted and removed before fry in the chambers started to emerge. A subsample of hatched alevins (n = 10) per family/treatment group were weighed to the nearest 1.0 mg and frozen at −20°C for subsequent analysis.

2.4 | Sample collection

After embryos in the incubation chambers hatched, they were allowed to develop undisturbed. Fry that exhibited swim-up behaviour by volitionally exiting the incubation chamber were contained in the collection cup and counted every 24 h. A 9 cm gap between the substrate and outflow spout on the emergence chamber ensured that fry would need to exhibit swim-up behaviour to exit the chamber. Each emerged fry was euthanised with a lethal dose of MS-222, visually inspected for development level and given a score of 0–5 depending on amount of yolk sac remaining (0 = newly hatched, 5 = no visible yolk sac) (Figure 3). Fork length (L ± 1.0 mm)

FIGURE 3 Development level assignment (1–5) corresponding to amount of yolk remaining at emergence. Left panel shows side view and right panel shows ventral view of same fry

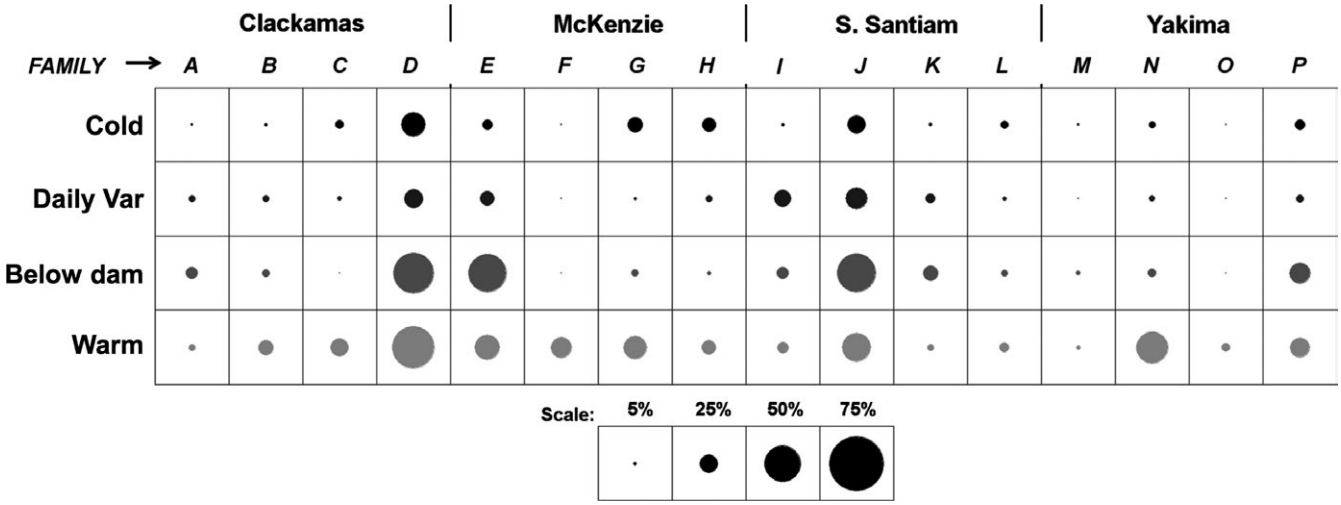


FIGURE 4 Visual representation of the proportion of fish that emerged prematurely (DL 0–3) from each family and temperature treatment (averaged across replicates)

and wet weight (W ± 1.0 mg) were measured from a subsample of emerged fry per family/treatment group at the beginning (n = 13), middle (n = 13) and end (n = 13) of emergence. Another subsample (n = 10) from the 39 fry that were weighed and measured in each family/treatment group were frozen at −20°C for later analysis (n = 10 × 4 × 4 × 8 = 1,280). Individual emergence time was recorded by calendar date and converted to temperature units, or TUs (*TU* =°C × *Days*). Dry weight (DW) was determined for subsamples of emerged fry by freeze drying whole samples for 2 days using a Dura-Top MP freeze dryer (FTS Systems, Stone Ridge, NY) until constant weight was achieved.

2.5 | Statistical Analysis

Fry were divided into one of two groups (premature or buttoned up) based on their condition at emergence. Bams' condition factor (KD) was calculated for the subsample of fry that were weighed and measured, and compared to five development level (DL) assignments (Figure 3). Values of KD ≤ 1.95, indicating complete yolk absorption (Bams, 1970) corresponded to DL 4 and 5; thus, DL 0–3 fry were classified as premature, and 4–5 as buttoned up.

$$\mathrm{KD} = \frac{10 \times \left[\text{weight (mg)}^{1/3}\right]}{\text{fork length (mm)}}$$

We examined the influence of family and temperature treatment on the proportion of fry that emerged prematurely using tests for equality of proportions. We were able to test for a potential interaction between emergence timing and family because families were paired within a single chamber in different combinations across replicates. To rule out an interaction effect, a binomial test (Zar, 2007), with n = 64 and p = .5 was used to contrast paired family differences in emergence timing with differences between the same families from unpaired chambers. The paired family difference minus the unpaired family difference was defined as a success in the binomial test if the value was >zero:

$$\left(\left|F_{1\text{paired}} - F_{2\text{paired}}\right|\right) - \left(\left|F_{1\text{unpaired}} - F_{2\text{unpaired}}\right|\right) > 0$$

Statistical analysis on size at emergence only included those fry that were buttoned up, approximately 84% of all emerged fry (n = 8,150). Egg weight varied among families and is known to affect size at emergence (Beacham & Murray, 1990); therefore, we used multiple regression analysis with mean family egg size as a covariate to test for effects of temperature and population on fry DW, L and KD at emergence. The metrics used to describe emergence timing were calendar days to emergence and TUs at emergence for mature fry, averaged by family. As families were split across treatments and replicates, we analysed main effects of thermal treatment on TUs and calendar days to emergence using a split-plot ANOVA with a completely randomised design on whole plot treatments, at an alpha of 0.05. One-way ANOVA was used to test for overall population effects on KD at the time of emergence (family average).

3 | RESULTS

Visual inspection provided no evidence of differences between thermal replicates. Observed temperatures never differed by more than 0.5°C among replicates and the overall mean temperatures of replicate treatments were within 0.3°C. Two temperature spikes occurred in January due to a cooling pump failure. These temperature spikes were short in duration (lasting less than 10 hr) and were experienced by alevins in all treatments because water was re-circulating and chilled in a common pool.

3.1 | Development level

Development level of fry that were weighed and measured was correlated with KD and there was no overlap between the mean and standard error across the five visually estimated development

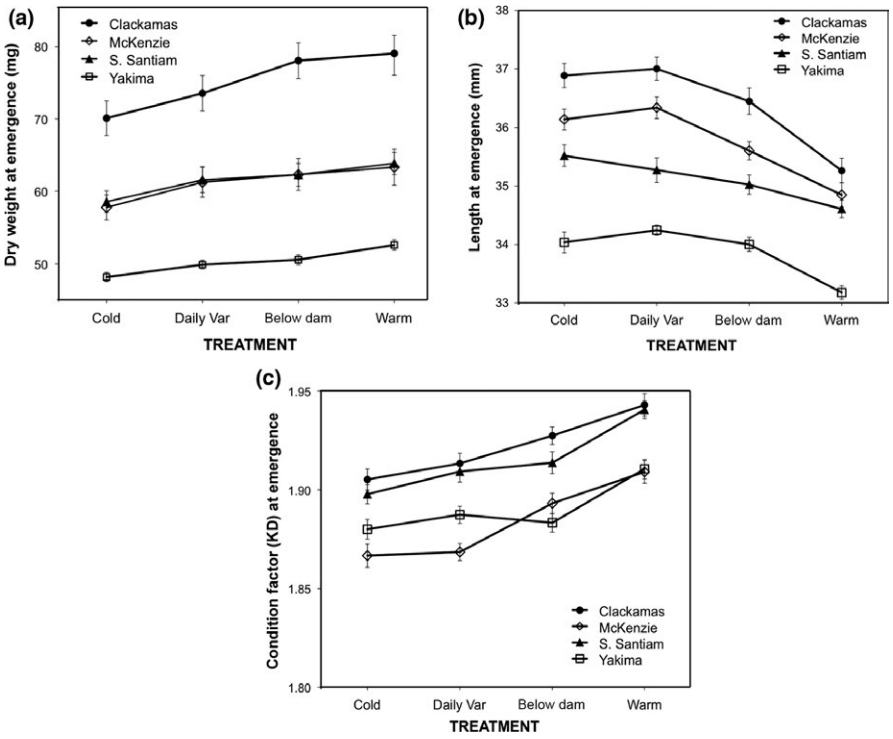


FIGURE 5 Plots showing estimated population means of the response variables (a) dry weight, (b) length and (c) Bams' condition factor (KD) across temperature treatments for fully developed fish (DL 4 and 5) with 95% confidence intervals

levels. Some families showed a tendency to emerge prematurely regardless of temperature treatment (Figure 4). Over 40% of fry that emerged prematurely originated from 3 of the 16 families. Equality of proportions tests for premature emergence indicated significant differences between families in each treatment (p-value < .001) and differences across treatments for all families combined (p-value < .001). Pairwise comparisons indicated that the warm treatment had a significantly higher proportion of prematurely emerging fry than all other treatments. There was no evidence of differences in proportion of prematurely emerging fry between treatments experiencing daily variation and those in cold stable treatments (p-value = .99). Finally, we concluded that pairing of families in chambers did not have a significant effect on emergence timing (binomial test, p-value = .382) or KD at emergence (binomial test, p-value = .708).

3.2 | Allometry of mature fry

Regression models revealed that fry DW and L at emergence were positively correlated with unfertilised egg weight ($r^2_{DW}=0.79$, $r^2_{L}=0.70$). Analysis of covariance found a significant effect of thermal regime treatment on DW (p-value < .001) and L (p-value < .001) at emergence, with fry in the warm constant treatment emerging heavier and shorter (Figure 5a,b). There was no interaction between egg size and treatment for DW (p-value = .461) and L (p-value = .818). Condition factor at emergence was not explained by egg size ($r^2_{KD}=0.5$) but was affected by temperature treatment (p-value = .0012) (Figure 5c). Because egg size was initially different across populations (p-value < .001) and replication was limited, it was not possible to test the interaction of population and treatment on fry size.

3.3 | Emergence timing for mature fry

The behaviour of emergence as measured in calendar days was influenced by temperature treatment (p-value < .001). Fry from colder treatments did not emerge until nearly 2.5 months after those in the warm treatments (Figure 6a). Emergence timing as measured by accumulated TUs was influenced by both treatment (p-value = .0039) and population (p-value = .009) with fry from Clackamas and McKenzie populations emerging from warm treatments at fewer TUs. An interaction was detected between treatment and population (p-value = .003) on emergence timing (TUs), although the difference in response to the below-dam treatment for the Yakima population (Figure 6b) may have been influenced by the temperature spikes in January, to which they did not show a marked response. The results of the split-plot ANOVA using families instead of populations for analysis also indicated significant effects of treatment and family on emergence timing (TUs) (p-values < .001), although no interactions between family and treatment were observed.

3.4 | Emergence phenotype reaction norm

Mean family KD at emergence across all treatments (standardised by TUs) was significantly influenced by population ($F_{3,56}$=3.124, p-value = .033), and a significant interaction effect was also detected ($F_{3,56}$=3.66, p-value = .017). This analysis included fry that emerged

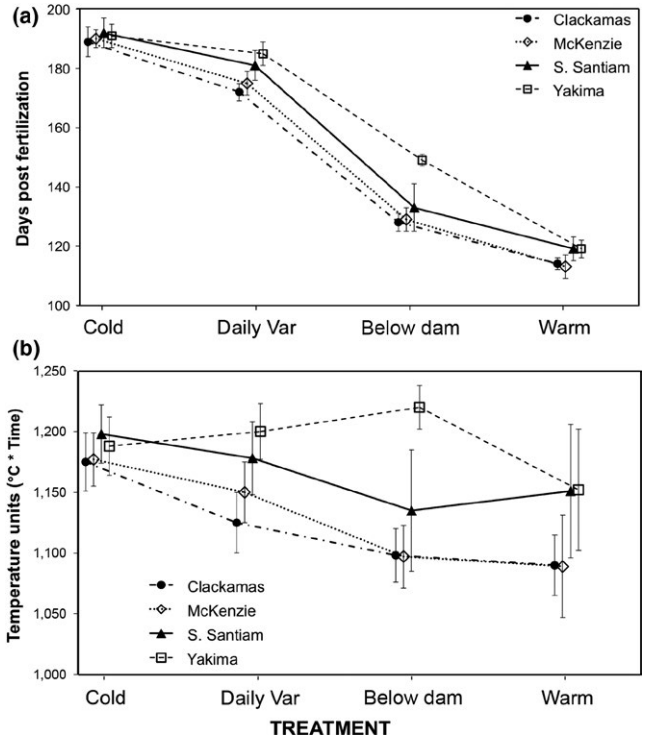


FIGURE 6 Mean emergence time for DL 4 and 5 fry (across families) shown in calendar days (a) as well as in temperature units (b) with 95% confidence intervals

prematurely in terms of development level to capture a more complete representation of family variation in emergence phenotype (Figure 7).

4 | DISCUSSION

Coupled measures of behaviour (emergence timing) and morphology (length and weight at emergence) are needed to describe an overall emergence phenotype for salmon. Observations about emergence phenotype allow us to assess ecologically and evolutionarily meaningful differences within and among populations of Chinook salmon during this key life-history transition. Moreover, given that incubation temperatures directly alter both emergence timing and physical condition at emergence (Skoglund, Einum, Forseth, & Barlaup, 2011), population responses need to be assessed within a reaction norm paradigm (Hutchings, 2011). Therefore, we examined emergence phenotype across a range of temperatures, and explicitly placed emergence phenotype within a framework that explored interactions between genetics and environmental conditions (G × E).

Our results clearly demonstrate differences in emergence phenotype between populations of spring Chinook salmon and even between families within populations. Fry from Santiam and Yakima populations accumulated more TUs before emergence than fry from Clackamas and McKenzie populations. Fry from Clackamas and McKenzie populations also tended to have a higher KD at emergence. Thus, emergence phenotype varied along an axis of early emergence with high KD and later emergence with low KD. Furthermore, the degree of difference in emergence phenotype between populations varied with incubation regime, with greater differences between populations at warmer temperatures. This combined evidence suggests that a G × E interaction shapes emergence phenotype among populations across temperature regimes.

4.1 | Emergence phenotype

The need to sustain or re-build salmon populations that live in thermally altered streams has generated questions about how early development is affected by these changing environments. Specifically, the effects of temperature variation on early development might ultimately influence population sustainability (Angilletta et al., 2008; Steel et al., 2012). This study responds to these questions by integrating measures of behavioural emergence and physical condition within variable thermal regimes that mimic natural environments. We expand on earlier experiments that demonstrated the dependence of emergence traits on both

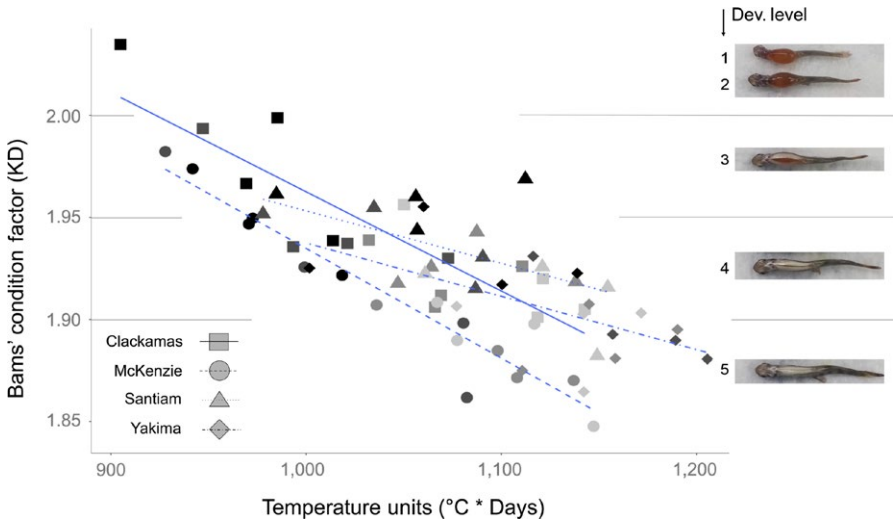


FIGURE 7 Reaction norm trend lines showing significant population effect ($F_{3,56}$ = 3.124, p = .033), and interaction effect ($F_{3,56}$ = 3.66, p = .017) on KD at emergence. Symbol shading indicates thermal regime treatment group: dark to light = warm to cold

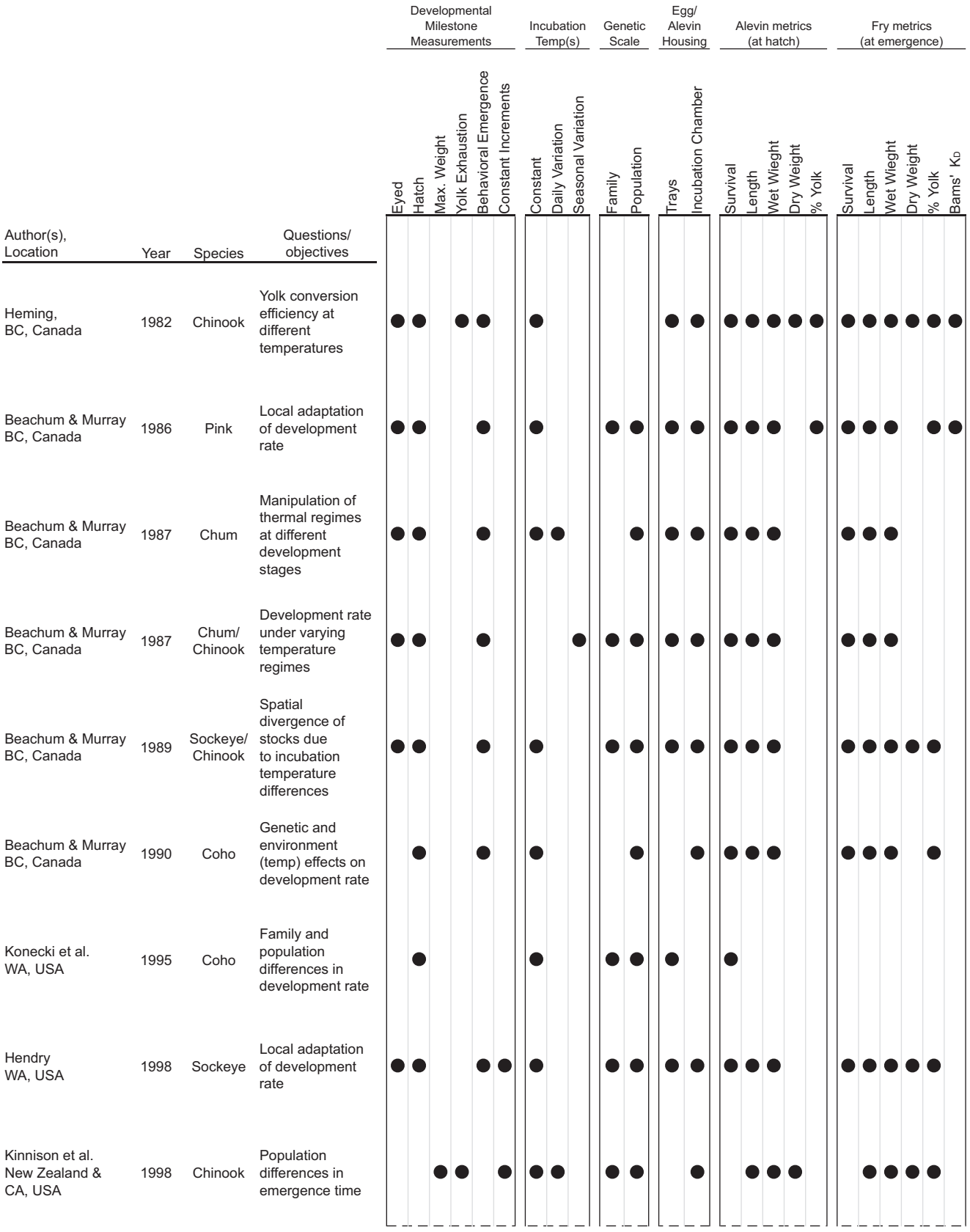
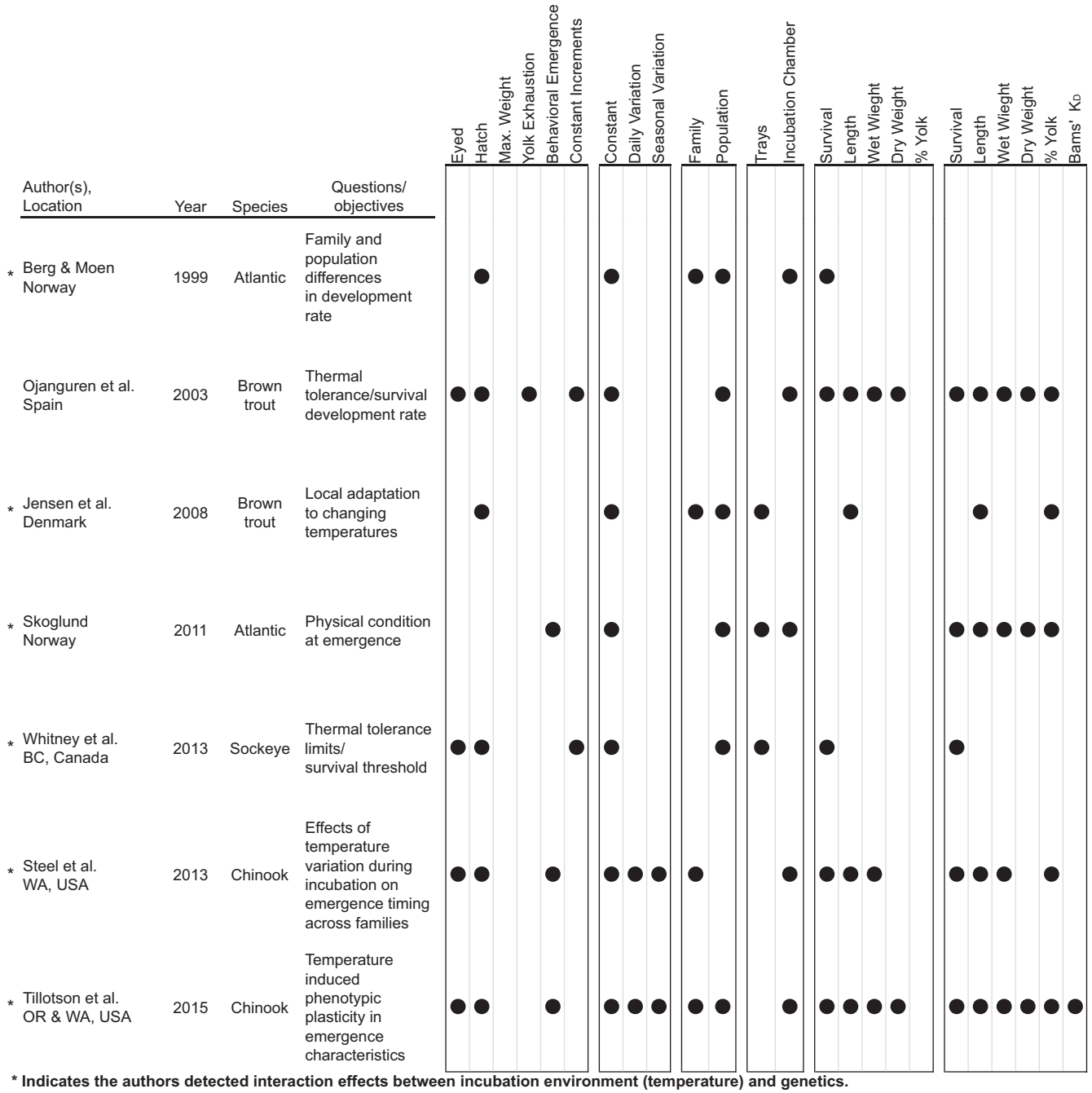


FIGURE 8 Summary review of methods used in previous studies comparing salmon development at various temperatures



	Author(s), Location	Year	Species	Questions/ objectives
*	Berg & Moen Norway	1999	Atlantic	Family and population differences in development rate
	Ojanguren et al. Spain	2003	Brown trout	Thermal tolerance/survival development rate
*	Jensen et al. Denmark	2008	Brown trout	Local adaptation to changing temperatures
*	Skoglund Norway	2011	Atlantic	Physical condition at emergence
*	Whitney et al. BC, Canada	2013	Sockeye	Thermal tolerance limits/ survival threshold
*	Steel et al. WA, USA	2013	Chinook	Effects of temperature variation during incubation on emergence timing across families
*	Tillotson et al. OR & WA, USA	2015	Chinook	Temperature induced phenotypic plasticity in emergence characteristics

*** Indicates the authors detected interaction effects between incubation environment (temperature) and genetics.**

FIGURE 8 (Continued)

temperature and genetics (Figure 8) by assessing emergence, in addition to hatch timing at both the population and family level. The timing of transition from embryo to alevin within the gravel is a key developmental milestone, but likely does not have as much impact on survival as the transition from alevin to free-swimming fry. We also used seasonally variable thermal regimes in addition to constant temperatures for our experiment. The importance of this feature is that the embryo to alevin transition typically occurs during the autumn prior to large winter decreases in temperature. Thus, much of the impact of varying temperature regime occurs during the alevin stage and not during the embryo stage. It is well known that cold temperatures during early development (fertilisation to eyed embryo) may be lethal (Murray & McPhail, 1988). Ecologically pertinent emergence phenotype data will only be produced if environmentally realistic thermal regimes are matched to temporally realistic development stages. Furthermore, our analysis determined that the correlation between hatch timing and emergence timing may depend on temperature regime and population of origin (Figure S3).

4.1.1 | Selection on emergence phenotypes

Synchronicity of emergence timing within populations has been hypothesised to result from selection against the behaviour of emerging "too early" or "too late" through mortality due to either predation or starvation associated with varying seasonal environmental and

ecological conditions (Brannas, 1995; Einum & Fleming, 2000). Implicit within this hypothesis is that optimal emergence timing will vary with differing thermal environments (Brannon, 1987). Our results show that at the time of emergence, morphology is not fixed (Figure 3). Therefore, emergence behaviour could be the product of a trade-off between energy reserves and development stage (Lelong, Bolliet, Bancel, & Gaudin, 2008). Thus, our conceptualisation of selection on the timing of emergence needs to expand to include selection for fry morphology at the time of emergence. Our results show that degree of yolk sac depletion and abdominal body wall fusion at emergence varied between individuals, families and populations and this variance increased at warmer temperatures. Such morphological differences undoubtedly influence a fry's ability to swim and thus elude predators, compete for territories and capture prey. The ultimate significance of these morphological differences awaits experiments explicitly testing for morphological effects on fry swimming performance and/or growth.

4.1.2 | Family variation in emergence phenotypes

Family level variation in emergence traits within populations has also been observed (Burt et al., 2011; Beacham & Murray 1985; Beacham & Murray 1986; Beckman et al., 2008; Steel et al., 2012). In our experiment, the strongest indicator of family level variation was the tendency for individuals from certain families to emerge with a large amount of visible yolk sac still remaining. Premature emergence represents an unexpected phenotype; a large amount of yolk present at the fry stage clearly hinders swimming and predator avoidance (Fresh & Schroder, 1987). Moreover, these fry obviously have neither the need, nor ability to feed. In natural environments, prematurely emerging fry could exit the water column and re-enter benthic gravels; thus, premature emergence might represent a brief interlude during early development rather than a mal-adaptive behaviour. Unlike emergence in natural environments, our experimental apparatus did not permit re-entry into benthic substrates and thus may not represent ecological reality. Nevertheless, the distinct family difference in premature emergence behaviour suggests that the behaviour could be genetically derived and may predispose these animals to brief periods of predation exposure.

4.1.3 | Egg size and the emergence phenotype

A robust literature documents both family and population level differences in egg size among salmonids (Beacham & Murray, 1990, 1993; Fleming & Gross, 1990) as well as the ecological and evolutionary basis for these differences (Einum & Fleming, 2004). Egg size also has a significant effect on the emergence phenotype. The observed positive relationship between egg size and fry weight at emergence was expected because larger eggs are documented to produce heavier fry (Beacham & Murray, 1990). Given the strong effect fry size has on postemergent ecological success in establishing territory (Chapman, 1962), egg size needs to be considered as an essential factor affecting the emergence phenotype. However, the weak relationships between egg weight and KD at emergence and between egg weight and emergence timing suggests that additional factors beyond egg size influence the emergence phenotype.

There was a significant effect of incubation temperature on fry length; fry from warmer incubation regimes were shorter at emergence than fry from the same family incubated at cooler temperatures. This temperature effect on length at emergence could be due to relative increases in metabolic demand at warmer temperatures as opposed to cooler temperatures, leaving less energy to support growth at warmer temperatures. Earlier research has shown that incubation temperature is a critical factor in determining alevin length (Beacham & Murray, 1990). Furthermore, Hendry et al. (1998) and Jensen et al. (2008) both demonstrated that temperature effects on metabolic rate could vary between populations as fry developed most efficiently at temperatures that mirror their local thermal environment. More focused research on metabolic rate, yolk utilisation and growth under different thermal regimes would help clarify the link between metabolic rate and emergence timing reported in studies of brown trout (Régnier, Labonne, Gaudin, & Bolliet, 2012) and Atlantic salmon (Metcalfe, Taylor, & Thorpe, 1995).

4.2 | Variation in emergence timing

Variation in emergence timing, both in terms of TUs and calendar days, for button-up fry was evident within families across thermal regimes. In comparing the natural to below-dam regimes for example, individual family means differed anywhere from 65 TUs earlier to 77 TUs later in the natural thermal regime. These temperature unit discrepancies translated to a difference across families in calendar date ranging from 28 to 54 days later in the natural regime (Figure 6). In this experiment, fertilisation and egg incubation began for all fry during the same week, and we still observed significant variation in TUs to emergence within families. It is important to note that spawning in hatcheries may span up to a month in some cases, and natural spawning periods could extend even further. Considering that early and late spawning adults are somewhat reproductively isolated and that selection may act differently over the course of a season (Hebert, Goddard, Smoker, & Gharrett, 1998), a compelling argument can be made for adaptive variation in emergence timing within populations (Hendry & Day, 2005).

Emergence time of individual fry is informative and relatively easy to quantify, but the overall emergence period of a group might be a more useful metric to estimate responses to smaller scale ecological events, like short spikes in temperature, increases in flow, or changes in food availability. Preliminary observations of our data on emergence period duration by family point to significant differences among populations both within and across temperature treatments (Tillotson, 2015). Research on fry emerging from redds in the wild suggests that the trade-off between first access to territory and exposure to predators favours a more compressed emergence period (Garcia de Leaniz, Fraser, & Huntingford, 2000; Gustafson-Marjanen & Dowse, 1983). Duration of emergence period is very likely under selection in the wild, but it is not a trait that contributes to fitness in a hatchery, because emergence behaviour does not exist in a hatchery environment. The

decoupling of morphological and behavioural aspects of emergence in a hatchery population may relax selection for synchronous emergence.

4.3 | Reaction norms

TUs are often used to standardize the thermal experience of salmon embryos during the course of their development before emergence. However, Steel et al. (2012) suggest that the rate of temperature delivery (variable vs. constant) even at the daily time scale may be an important factor influencing an individual's developmental trajectory. To compare physical attributes of populations across different environments in a reaction norm framework, it seems appropriate to use TUs as a continuous environmental variable, while remembering that the actual calendar dates on which these thermal units were accumulated varies considerably between treatments. In this sense, we can compare population differences in KD at low or high TUs and also connect those TUs to a calendar date for each treatment. This approach will help when contextualising the life-history trade-offs for each emergence phenotype.

Using this method to compare all four populations shows two distinct patterns (Figure 7). First, Clackamas and McKenzie show a definite negative correlation between TUs to emergence and KD. In warmer regimes, they emerged earlier in terms of TUs and had relatively high KD. For Santiam and Yakima populations, TUs to emergence had only a slightly negative effect on KD. Based on this reaction norm, one could predict that warmer temperatures would have a greater effect on the emergence phenotype of Clackamas and McKenzie populations because not only would they emerge early (fewer TUs) with more yolk remaining, but this would occur much earlier in the calendar year (December or January). It seems possible that the differing emergence phenotypes of Clackamas and McKenzie fry at warmer temperatures, as compared to Yakima and Santiam fry, could have fitness and survival consequences. The potential factors that have shaped these differences in emergence phenotype are many, including founder effects, thermal regime during incubation and postemergence growth opportunities. It is also potentially significant that gametes from all populations were sourced from hatchery programmes.

4.4 | Hatchery selection

Selective conditions in hatchery environments are quite different than those experienced by wild populations. First, fry do not generally emerge volitionally in a hatchery; rather, they are directly transferred from rearing trays to larger tanks, troughs or ponds based on a visual assessment of the fry's ability to swim and feed and/or at a fixed number of TUs. Thus, behaviour (emergence from the gravel) and physical condition at emergence are disassociated in a hatchery environment. In addition, at ponding (transfer from incubation tray to raceway or tank) fry are fed to satiation in an environment with no predators, which results in the majority of fish surviving to feed and grow. Finally, hatchery domestication has been shown to affect development rate (Fraser et al., 2010; Smoker, 1986) because of unnatural temperatures (usually warmer) experienced by salmon embryos during incubation. Discriminating between the effects of domestication due to relaxation of selection at the free-swimming fry stage and potential domestication due to altered thermal regimes would require comparative measurements with wild-type fry and could be of great value in understanding the limitations and potential of using hatchery stocks for re-introduction into natural environments.

Within the populations we examined, the Yakima River population has experienced the least amount of potential hatchery-induced domestication. The programme was initiated relatively recently (1997) and only used naturally produced adults as broodstock (Fast et al., 2015). This practice eliminates the potential for successive generations of domestication. The populations from the Willamette River have a longer history of hatchery propagations and have varying and hard to assess degrees of broodstock integration with naturally produced adults. Nevertheless it is interesting to note that the emergence phenotypes of Yakima and Santiam populations are similar. There have been a number of studies documenting differences between hatchery and wild populations due to early rearing environment (Berejikian, Mathews, & Quinn, 1996; Metcalfe, Valdimarsson, & Morgan, 2003) and some studies have suggested that the fitness of domesticated hatchery populations in natural conditions is reduced as compared to wild populations (Berejikian & Ford, 2004; Williamson, Murdoch, Pearsons, Ward, & Ford, 2010). It might be fruitful to examine emergence phenotypes of these populations to assess whether differences in early life history contribute to differences in fitness.

5 | SUMMARY

We found that the amount of phenotypic variation in emergence timing and condition at emergence is population and, in some cases, family specific. Our findings are consistent with results from earlier studies, which suggest that there is an interaction between genotype and thermal regime during early development (Figure 8). These data will help us understand and interpret changes to emergence patterns for populations that inhabit river systems with altered temperature and flow patterns.

Temperature changes will create challenges for salmon populations during the spawning, development and migration phases of their life cycle. Studies of systems with dams have clearly shown that these challenges result in population declines. We estimate that salmon in systems impacted by climate change will display similar negative responses. After examining issues related to the effects of thermal regime patterns on emergence phenotype, it is clear that there are three major focus areas where additional research could improve our understanding and further inform management decisions. These areas are (i) the relationship between metabolism and emergence behaviour, specifically the important factors determining when an alevin is physiologically competent to emerge; (ii) the relative influence of per cent natural origin broodstock in a hatchery programme in determining the amount of variation in emergence timing at the population level; (iii) the influence of emergence phenotype on fry growth, especially in situations with added environmental stress such as food limitation,

predation and/or high flow. Deciphering the complexities of the relationship between temperature, metabolism and growth, and how the relationship might differ between populations may require constant metabolic monitoring of embryos and fry during incubation.

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