Population Origin and Water Temperature Affect Development Timing in Embryonic Sockeye Salmon

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Published online: 11 Sep 2014.


To link to this article: http://dx.doi.org/10.1080/00028487.2014.935481

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Predicting the future impact of changing thermal regimes on life history stages for wild fish requires a better understanding of the relative importance of population origin and offspring size on embryonic development. We assessed hatch timing and offspring size of Sockeye Salmon \( \textit{Oncorhynchus nerka} \) in relation to egg size (variation from full-sibling families), population origin, and temperature (three treatments of 10, 14, and 16°C). Both hatch timing and hatch duration varied by the interacting effects of population origin and thermal treatment, shown in crossing reaction norms. Hatching was faster, yet more variable, at higher temperatures across many groups, so while fish generally hatched faster, developmental asynchrony also increased among families. On average, fish incubated at 16°C were shorter but not lighter at hatch, showing developmental tradeoffs between basal metabolic requirements and growth. Egg size decreased among populations as migratory distance increased, but development rates were not related to egg size. In this case, embryonic development rates were linked to temperature and population-specific cues for hatch timing more than to the maternal influence of egg size.

Within species, genetically distinct populations are likely to differ in parentally mediated developmental qualities, such as offspring size and reproductive strategies related to local adaptation (in fish: Brannon 1987; Jensen et al. 2008; Hutchings 2011; in birds: Charmantier et al. 2008). In fish, offspring survival during early development is a critical indicator of population viability over time (Bradford 1995; Jensen et al. 2008). Temperature is a regulatory factor that affects most biological processes in ectotherms; elevated temperature can increase basal metabolism and speed development rates, affecting embryogenesis, offspring size, growth, and survival (Velsen 1987; Pankhurst and Munday 2011). Salmonids are particularly susceptible to temperature changes during their sessile embryonic stage and mobility-limited posthatch stages (Brannon 1987) when significant shifts in temperature can result in sublethal or lethal effects on developing eggs and larvae (e.g., Velsen 1987; Blaxter 1992). With small tolerance limits around thermal optima (Romhough 1997), adaptation to thermal conditions during embryonic stages is of critical importance (Janhunen et al. 2010).

Within the different species of Pacific salmon \( \textit{Oncorhynchus} \) spp., genetically distinct populations display measurable physiological and behavioral differences linked to their unique life histories and the adaptive strategies employed to maximize
fitness (Taylor 1991; Hodgson and Quinn 2002; Fraser et al. 2011). Adaptive differences to environmental conditions among populations have been observed in the spawning period (Hodgson and Quinn 2002; Eliason et al. 2011), prompting the question of whether this adaptive diversity persists to the embryo and early-development stages. In the Fraser River of British Columbia, migration effort, elevation change, and migration distance vary among coastal-spawning populations, with short migrations of ~20 km and long migrations for interior-spawning populations, some of which travel over 1,200 km to reach their natal spawning streams (Groot and Margolis 1991). Fraser River Sockeye Salmon *Oncorhynchus nerka* provide a good model for examining the interaction of incubation temperature, population origin, and egg size on early development (see Whitney et al. 2013 for thermal tolerance in terms of survival on these same populations).

Offspring size and viability are influenced by the maternal traits of egg size, thermal history, and population adaptation to environmental conditions (Jonsson et al. 1996; Jonsson and Jonsson 2011). Egg size plays an important role in the development of fish, affecting offspring size, morphology, and competitiveness (Bagenal 1969; Brooks et al. 1997). Developmental timing cues control annual movement patterns in highly migratory species, such as salmonid fish, and these cues are mediated by environmental and genetic influences, such as temperature and population origin. Experimentally, incubation temperature is likely to affect thermally adapted populations differentially depending on their historical thermal regime. Previous research suggests that at a common temperature embryos from populations with cooler incubation environments (cool-experienced populations) develop faster than embryos from populations with warmer incubation environments (warm-experienced populations), an adaptive response that may reduce thermally mediated hatch- and emergence asynchrony among groups (e.g., Brannon 1987; Beacham and Murray 1990). Beacham and Murray (1989) observed that under the same incubation treatment, a cool-experienced population produced longer and heavier offspring than a warm-experienced population relative to egg size, a response attributed to differences in yolk conversion efficiency (Beacham and Murray 1987, 1989). The postemergent competitive benefits of large eggs (e.g., larger alevin and fry, improved offspring condition, lower immediate exogenous energy requirements, and improved competitiveness) may be more important than egg number when considering the additive negative consequences of elevated incubation temperature on offspring viability (Kinnison et al. 2001; Braun et al. 2013).

The evolution of developmental phenology affects a species response to climate change and drives adaptation to shifting environmental conditions (Hodgson and Quinn 2002; Bradshaw and Holzapfel 2008; Jensen et al. 2008). Hatch and emergence timing varies among populations to reflect local spawning conditions and thermal patterns (Beacham and Murray 1987, 1989; Brannon 1987; Berg and Moen 1999) and is related to migration timing to the ocean (Beacham 1988). Over time, environmental conditions at hatch and emergence have resulted in a well-timed development sequence to maximize fitness and population-wide survival; a sequence that is likely to be affected by temperature shifts (Tallman 1986; Blaxter 1992; Pankhurst and Munday 2011).

For juvenile fish, accumulated thermal units (ATUs), or degree-days, represent the sum of temperature exposure since fertilization and can be used as a means to predict developmental events in early life history (Burt et al. 2012b). While less commonly evaluated in an experimental context (but see Springate et al. 1984; Beacham and Murray 1988; Murray et al. 1990; Berg and Moen 1999), hatch timing is closely associated with emergence timing (Crisp 1988). Hatch timing has consequences for the survival of developing embryos within gravel redds related to environmental variables, including dewatering, oxygen availability, and gravel ice in spring (Cope and Macdonald 1998). Measuring hatch duration can describe developmental synchrony within a population and habitat adaptation across populations. Hatch synchrony tends to decrease under suboptimal conditions, which has implications for population viability, but little is known about this relationship as this aspect of development timing is rarely explored in salmonids (Kamlar 2002; Burt et al. 2012b).

The objective of this study was to explore the influence of population, incubation temperature, and egg size on developmental timing and offspring size at hatch in Sockeye Salmon. A previous study looked at survival (Whitney et al. 2013), an important metric to estimate fitness but one that provides an incomplete picture; development timing and offspring size are both key parameters for determining early life competitive advantage among populations. In this study, we assessed population-level differences in offspring development rates and alevin size at hatch using three incubation temperature treatments (10, 14, and 16°C). Temperatures were chosen to span thermally optimal, elevated, and climate-change-associated high incubation temperatures for the species during early embryonic development (McCullough et al. 2001). We predicted that (1) within a common temperature, development timing would vary by population, and specifically that embryos from populations that spawn at cooler temperatures would develop faster than populations that spawn at warmer temperatures. Thus, while warmer incubation temperature should result in faster development overall, population differences in hatch timing will emerge, especially at the more “stressful” high temperatures. Additionally, we predicted that (2) alevin size (i.e., length and mass) would be negatively related to time to hatch, as yolk conversion efficiency would be reduced at high temperatures, so that those fish that developed faster in elevated temperatures would hatch as smaller alevin. In addition, we discuss some of the potential ecological consequences of changes in hatch timing and alevin size relevant to the different populations.
METHODS

Offspring collection.—This study used nine populations of Sockeye Salmon from the Fraser ($n = 8$) and Columbia ($n = 1$) rivers. The Fraser River populations are all abundant, geographically distinct populations within the watershed, which has a variety of migration distances and spawning conditions, and they display a range of life history characteristics (See Whitney et al. 2013 for details; Figure 1). The populations vary in thermal history during spawning and incubation, which is related to both spawn timing and the environmental characteristics of the natal streams (i.e., warmwater inlet versus glacial runoff) (Table 1).

Gametes were collected from 12 to 20 pairs of reproductively mature male and female Sockeye Salmon that were collected from the spawning grounds of each population. Adults were sacrificed by cerebral concussion and measured for size parameters (fork length, postorbital hypural length, postorbital fork length, total mass), and eggs and milt were hand expelled and collected in clean, dry plastic containers. All containers were immediately oxygenated and chilled to 0–4°C for immediate transportation to the laboratory facilities at the University of British Columbia. As some populations were geographically isolated and transport to the laboratory took much longer than for others, all groups were fertilized at a standardized 24 h after collection. Previous work has shown that transporting unfertilized gametes results in higher survival than transporting activated, developing eggs (Jensen and Alderice 1983) and that salmonid eggs and sperm will remain viable with a 95–100% fertilization potential for up to 5 d as long as temperature is low and sufficient oxygen levels are maintained within the containers (Jensen and Alderice 1984).

Fertilization protocol and incubation methods.—Gametes were crossed by a randomized mating design in which each male was paired once with each female ($n = 12–20$ families/population) using a dry fertilization method (details in Patterson et al. 2004). Individual family baskets were then randomly distributed in vertical-stack Heath incubators, with single replicates of each family at 10, 14, or 16°C.

Fertilized eggs were monitored and maintained according to the methods used in Whitney et al. (2013). Mortality between fertilization and hatching was significantly higher for all populations at the 16°C treatment (45 ± 27% [mean ± SD], across all groups), versus 15 ± 15% at 14°C, and only 7 ± 16% at 10°C (for details and survival analysis, see Whitney et al. 2013).

Once hatching was observed for any family in a population at a given temperature, newly hatched alevin were recorded daily and hatching rate and duration were monitored. Within 1 d of each population’s 90–95% hatch point, five alevin (when available) from each family were measured for length and weight at hatch. Alevin were euthanized in dilute MS-222 (tricaine methanesulfonate), blotted to remove excess moisture, weighed (±0.01 mg), and measured for length (length from nose to visible end of body tissue [L₃]; ±0.5 mm). In cases of low survivorship within a family, as many alevin as were available were measured (<5) as among family means were used for population values, and those families with no remaining alevin were excluded from the analyses.

Data analysis.—All statistical analyses were based on population means (i.e., averages of family values for the offspring of each mating pair). Daily counts of egg hatching were used to describe ATUs prior to first hatch ($H₀$), 5% hatch ($H₅$), median hatch date ($H₅₀$), and hatching duration ($H_D$; number of days between 5% and 95% hatch per family). These metrics were chosen because they represent early

![FIGURE 1. Map of the study populations in the Fraser River watershed and the upper Columbia River within British Columbia. Embryos and adult samples from each group were transported back to laboratory facilities at the University of British Columbia in Vancouver, British Columbia, for fertilization, incubation, and husbandry throughout early development. Map courtesy of Whitney et al. 2013.](image-url)
TABLE 1. Environmental characteristics and ecology for eight populations of Sockeye Salmon from the Fraser River watershed and one population from the Columbia River watershed (Okanagan River). Peak spawning date and mean, maximum, and minimum spawning temperatures for Fraser River populations are reported from a 10-d period encompassing the estimated mean peak spawning date during the years 1990–2010. Peak river entry dates in parentheses reflect recent shifts in run timing to earlier river entry.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Gates Creek</th>
<th>Scotch Creek</th>
<th>Chilko River</th>
<th>Horsefly River</th>
<th>Stellako River</th>
<th>Okanagan River</th>
<th>Adams River</th>
<th>Weaver Creek</th>
<th>Harrison River</th>
</tr>
</thead>
<tbody>
<tr>
<td>Run-timing group</td>
<td>Early summer</td>
<td>Early summer</td>
<td>Summer</td>
<td>Summer</td>
<td>Summer</td>
<td>Columbia River</td>
<td>Late</td>
<td>Late</td>
<td>Late</td>
</tr>
<tr>
<td>Peak river entry</td>
<td>Jul 31</td>
<td>Jul 31</td>
<td>Aug 11</td>
<td>Aug 11</td>
<td>Aug 11</td>
<td>Jun 15</td>
<td>Sep 27</td>
<td>Sep 27</td>
<td>Sep 27</td>
</tr>
<tr>
<td>Historical peak spawn date</td>
<td>Sep 3</td>
<td>Sep 10</td>
<td>Sep 28</td>
<td>Sep 10</td>
<td>Sep 28</td>
<td>Oct 17</td>
<td>Oct 11</td>
<td>Oct 19</td>
<td>Nov 12</td>
</tr>
<tr>
<td>Dry egg mass (mg; mean ± SD)</td>
<td>42.7 ± 2.6</td>
<td>35.1 ± 3.0</td>
<td>41.6 ± 2.4</td>
<td>35.9 ± 3.6</td>
<td>34.5 ± 3.2</td>
<td>41.3 ± 6.1</td>
<td>52.2 ± 4.4</td>
<td>72.8 ± 6.8</td>
<td></td>
</tr>
<tr>
<td>Mean ± SD spawning temperature (°C)</td>
<td>9.59 ± 0.76</td>
<td>10.84 ± 2.75</td>
<td>9.95 ± 1.39</td>
<td>12.72 ± 1.92</td>
<td>10.95 ± 2.33</td>
<td>12.9</td>
<td>11.81 ± 1.96</td>
<td>10.43 ± 1.41</td>
<td>8.50 ± 1.13</td>
</tr>
<tr>
<td>Maximum ± SD spawning temperature (°C)</td>
<td>10.22 ± 0.90</td>
<td>11.92 ± 2.79</td>
<td>10.83 ± 1.16</td>
<td>14.06 ± 1.98</td>
<td>11.97 ± 2.44</td>
<td>No data</td>
<td>13.07 ± 2.01</td>
<td>11.57 ± 1.48</td>
<td>9.09 ± 1.29</td>
</tr>
<tr>
<td>Minimum ± SD spawning temperature (°C)</td>
<td>8.90 ± 0.70</td>
<td>9.46 ± 3.02</td>
<td>8.45 ± 2.75</td>
<td>11.21 ± 1.91</td>
<td>9.81 ± 2.27</td>
<td>No data</td>
<td>9.95 ± 2.66</td>
<td>9.42 ± 1.52</td>
<td>7.73 ± 1.40</td>
</tr>
</tbody>
</table>

*aSource for the Fraser River populations: Pacific Salmon Foundation–Fisheries and Oceans Canada. Sources for the Okanagan River population: Hodgson and Quinn (2002); Hyatt et al. (2003); S. Folks, Okanagan Nation Alliance, personal communication.*
development \((H_0, H_3)\), average population incubation requirements \(H_{50}\), and the incubation requirements for the majority of offspring within a population to successfully hatch (a measure of developmental synchrony, \(H_D\)). Dry egg mass, alevin mass \(M_H\), and alevin length \(L_H\) at hatch were also analyzed.

Alevin size response was assessed using a linear mixed model with population and temperature treatment as the fixed effects and family as the random effect. Temperature and population differences were tested using mixed-effect three-way ANOVAs (Type III sum of squares), using population, temperature, and family to determine the statistical significance of the fixed effects of population and temperature, and subsequent Tukey’s honestly significant difference (HSD) post hoc multiple comparisons tests for alevin size and hatch timing analysis. All analyses were conducted using R (R Foundation: www.r-project.org).

Population variation was assessed using a full model with the following form:

\[
y_{jk} = \mu + T_j \times F + P + F(P_k) + E_k + \varepsilon_{jk},
\]

where \(y\) is the response variable \((H_0, H_5, H_{50}, H_D, M_H, \text{ or } L_H)\), \(T\) represents the temperature treatment \((j)\), \(P\) is the population, \(E\) represents egg mass, and \(F\) is the individual family identity (full-sib families; \(k\)) nested within populations. For all analyses, temperature and population were considered fixed effects, varying by the random effect of family. Egg size was found to be nonsignificant and was subsequently removed. The effect of Heath stacks within the incubation design was evaluated but was also found to be nonsignificant and was thus also removed. Differences among fixed effects (temperature treatments and population) were tested using Tukey’s HSD tests.

Additionally, Pearson’s correlations were calculated to assess the relationship between egg mass, alevin size, and hatch timing. Regression analyses were used to compare egg size with migration distance and offspring size \((M_H, L_H)\) with adult female fork length. The significance level for all tests was set at \(\alpha = 0.05\).

### RESULTS

#### Hatch Timing

Temperature, population, and the interaction of temperature \(\times\) population, but not egg mass, significantly affected all hatching characteristics (Table 2). All populations developed faster as incubation temperature increased, but the magnitude of this difference varied by population (Tukey’s HSD among incubation temperatures: development at both \(14^\circ\)C and \(16^\circ\)C was faster than at \(10^\circ\)C, at \(16^\circ\)C it was faster than at \(14^\circ\)C; \(P < 0.0001\)). Alevin from the cool-spawning Chilko River had the longest incubation time to reach hatch at \(10^\circ\)C \((P < 0.01)\), longer than the Adams, Horsefly, and Scotch populations, which spawn at warmer temperatures \((P < 0.01)\), and longer than cool-spawning Harrison and Weaver populations at \(14^\circ\)C \((P < 0.05)\). At \(16^\circ\)C, Okanagan alevin incubated the longest to hatch \(44 \pm 2.03\) d [mean \(\pm\) SD]) among all populations \((P < 0.0001)\), while Harrison fish hatched the fastest \(38.1 \pm 1.8\) d; not significant (Table 3). Considering ATUs rather than days, all populations required more ATUs to reach hatching at higher temperatures, except for Harrison River embryos, which hatched with lower ATUs at \(16^\circ\)C than at \(10^\circ\)C \((P = 0.0506; \text{Figure 2})\). In general, the among-population differences reflected the same pattern as for the metric of days to 5% hatch, whereby Chilko alevin required the longest incubation to hatch at both \(10^\circ\)C and \(14^\circ\)C, but Okanagan populations were the slowest to hatch at \(16^\circ\)C (Table 3).

Development time to median hatch \((H_{50})\) was affected by incubation temperature (ANOVA: \(F_{2, 199} = 52.1, P < 0.0001\)) in the same way, with hatching occurring earlier with higher temperatures (Figure 3). Both population and the interaction of population \(\times\) temperature also significantly affected time to median hatch \((P < 0.0001; \text{Table 4})\). Alevin from the Chilko population required significantly more ATUs \((672 \pm 24.85\) ATUs [mean \(\pm\) SD]) to reach median hatch at \(10^\circ\)C than those from all other populations \((P < 0.001)\), while those from the Adams population required less \((661.5 \pm 37.7\) ATUs) than most other cool-experienced groups (Chilko, Gates, Harrison, Okanagan; \(P < 0.001\)) but the same as other warm-experienced populations \((P > 0.10)\) (Table 3). At \(14^\circ\)C, most populations required more ATUs to reach 50% hatch \((661.5 \pm 37.7\) ATUs [mean \(\pm\) SD of all populations]; \(P < 0.001)\) than

### Table 2

Summary statistics for mixed-model ANOVAs with family as a random effect and temperature and population as fixed effects. Egg mass was significantly different among populations \((P < 0.01)\). The results are presented for six early life history traits: time to first hatch \((H_0)\); in both days and ATUs), hatch duration \((H_0; \text{days}, 5–95\% \text{ hatch})\), time to 50% hatch \((H_{50}; \text{days})\), alevin length, and alevin mass. The F-values are shown with degrees of freedom in parentheses; asterisks represent significance \(* = P < 0.01, ** = P < 0.001, \text{n.s.} = \text{not significant})

<table>
<thead>
<tr>
<th>Effect</th>
<th>(H_0); days</th>
<th>(H_0); ATUs</th>
<th>(H_D)</th>
<th>(H_{50})</th>
<th>Alevin length</th>
<th>Alevin mass</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature</td>
<td>639.16 (2)**</td>
<td>5.10 (2)**</td>
<td>37.84 (2)**</td>
<td>52.10 (2)**</td>
<td>13.49 (2)**</td>
<td>1.53 (2) n.s.</td>
</tr>
<tr>
<td>Population</td>
<td>17.29 (8)**</td>
<td>8.99 (8)**</td>
<td>15.86 (8)**</td>
<td>7.49 (8)**</td>
<td>12.95 (8)**</td>
<td>56.17 (8)**</td>
</tr>
<tr>
<td>Temperature (\times) population</td>
<td>6.57(16)**</td>
<td>5.05 (16)**</td>
<td>8.58 (16)**</td>
<td>3.39 (16)**</td>
<td>6.25 (16)**</td>
<td>1.11 (16) n.s.</td>
</tr>
</tbody>
</table>
### TABLE 3. Summary data of hatch timing characteristics across populations and incubation temperatures. The collection date is shown as is the number of families in each population to survive to hatch by incubation temperature. Abbreviations are as follows: $H_0$ represents the first hatch within a population, $H_5$ is the 5% hatch within a population, $H_{95}$ is the hatch duration between 5% and 95% hatch, $H_{50}$ is the median hatch point, and CR indicates Columbia River. Populations are ranked in order of collection date.

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Gates Creek</th>
<th>Scotch Creek</th>
<th>Chilko River</th>
<th>Horsefly River</th>
<th>Stellako River</th>
<th>Okanagan River (CR)</th>
<th>Adams River</th>
<th>Weaver Creek</th>
<th>Harrison River</th>
</tr>
</thead>
<tbody>
<tr>
<td>Families/population</td>
<td>18; 18; 15</td>
<td>20; 20; 20</td>
<td>10; 11; 7</td>
<td>14; 14; 14</td>
<td>15; 15; 15</td>
<td>20; 20; 20</td>
<td>20; 20; 20</td>
<td>20; 19; 19</td>
<td>16; 16; 9</td>
</tr>
<tr>
<td>$H_0$ days</td>
<td></td>
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<td></td>
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<td></td>
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</tr>
<tr>
<td>10°C</td>
<td>56.7 ± 2.7</td>
<td>59.9 ± 2.15</td>
<td>63.5 ± 1.51</td>
<td>59.0 ± 2.94</td>
<td>59.7 ± 1.4</td>
<td>62.6 ± 1.8</td>
<td>59.6 ± 1.5</td>
<td>59.9 ± 1.35</td>
<td>61.4 ± 1.15</td>
</tr>
<tr>
<td>14°C</td>
<td>44.9 ± 2.1</td>
<td>43.7 ± 1.13</td>
<td>47.8 ± 1.84</td>
<td>42.1 ± 2.13</td>
<td>42.2 ± 1.7</td>
<td>45.2 ± 1.7</td>
<td>43.5 ± 0.9</td>
<td>44.1 ± 1.4</td>
<td>44 ± 1.2</td>
</tr>
<tr>
<td>16°C</td>
<td>39.3 ± 2.8</td>
<td>37.7 ± 2.6</td>
<td>41.7 ± 1.8</td>
<td>36.8 ± 1.7</td>
<td>37.8 ± 2.5</td>
<td>41.7 ± 3.5</td>
<td>38.9 ± 0.9</td>
<td>38.9 ± 1.85</td>
<td>36.7 ± 2.6</td>
</tr>
<tr>
<td>$H_{50}$ days</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>10°C</td>
<td>58.8 ± 3.1</td>
<td>60.3 ± 1.4</td>
<td>65.5 ± 2.95</td>
<td>60.6 ± 2.1</td>
<td>60.2 ± 1.08</td>
<td>62.6 ± 1.2</td>
<td>60.1 ± 1.1</td>
<td>60.3 ± 1.3</td>
<td>62.8 ± 1.0</td>
</tr>
<tr>
<td>14°C</td>
<td>45.4 ± 1.85</td>
<td>44.2 ± 1.05</td>
<td>47.5 ± 1.2</td>
<td>44.2 ± 1.25</td>
<td>45.1 ± 5.34</td>
<td>45.9 ± 1.65</td>
<td>44.3 ± 1.25</td>
<td>44.8 ± 1.08</td>
<td>44.8 ± 1.2</td>
</tr>
<tr>
<td>16°C</td>
<td>40.5 ± 2.3</td>
<td>39.9 ± 1.25</td>
<td>42.9 ± 1.95</td>
<td>39.5 ± 1.0</td>
<td>39.9 ± 1.8</td>
<td>44.2 ± 2.0</td>
<td>40.1 ± 1.3</td>
<td>40.1 ± 1.6</td>
<td>38.1 ± 1.8</td>
</tr>
<tr>
<td>$H_{95}$ days</td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>10°C</td>
<td>9.2 ± 2.8</td>
<td>4.2 ± 1.5</td>
<td>5.2 ± 2.6</td>
<td>3.6 ± 1.0</td>
<td>2.9 ± 1.0</td>
<td>7.6 ± 3.5</td>
<td>2.8 ± 0.9</td>
<td>5.3 ± 2.7</td>
<td>5.8 ± 3.3</td>
</tr>
<tr>
<td>14°C</td>
<td>6.0 ± 2.6</td>
<td>5.35 ± 2.4</td>
<td>6.1 ± 1.6</td>
<td>4.1 ± 1.3</td>
<td>8.9 ± 4.8</td>
<td>8.0 ± 2.6</td>
<td>8.3 ± 2.9</td>
<td>6.4 ± 1.8</td>
<td>6.1 ± 1.9</td>
</tr>
<tr>
<td>16°C</td>
<td>8.1 ± 1.9</td>
<td>7.0 ± 1.8</td>
<td>7.1 ± 1.7</td>
<td>6.4 ± 1.7</td>
<td>7.8 ± 2.4</td>
<td>6.6 ± 1.9</td>
<td>8.2 ± 1.6</td>
<td>6.6 ± 1.6</td>
<td>6.9 ± 1.7</td>
</tr>
<tr>
<td>$H_{50}$; days</td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>10°C</td>
<td>63.3 ± 1.6</td>
<td>61.9 ± 1.2</td>
<td>67.2 ± 2.5</td>
<td>62.1 ± 1.65</td>
<td>60.9 ± 0.9</td>
<td>54.3 ± 2.6</td>
<td>60.6 ± 1.9</td>
<td>61.75 ± 1.2</td>
<td>64.4 ± 1.5</td>
</tr>
<tr>
<td>14°C</td>
<td>47.1 ± 2.2</td>
<td>45.75 ± 1.48</td>
<td>50.4 ± 3.0</td>
<td>45.2 ± 1.3</td>
<td>46.5 ± 2.6</td>
<td>49.5 ± 2.8</td>
<td>47.0 ± 2.9</td>
<td>47.8 ± 2.1</td>
<td>46.5 ± 2.0</td>
</tr>
<tr>
<td>16°C</td>
<td>43.7 ± 2.2</td>
<td>42.4 ± 1.7</td>
<td>45.6 ± 2.6</td>
<td>41.9 ± 1.5</td>
<td>42.4 ± 2.0</td>
<td>46.4 ± 2.5</td>
<td>43.6 ± 2.4</td>
<td>43.0 ± 1.9</td>
<td>41.2 ± 1.3</td>
</tr>
<tr>
<td>$H_{50}$; ATUs</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10°C</td>
<td>633.3 ± 16</td>
<td>619 ± 11</td>
<td>672 ± 25</td>
<td>621 ± 16</td>
<td>608.6 ± 9</td>
<td>642.5 ± 25</td>
<td>605.5 ± 19</td>
<td>617.5 ± 12</td>
<td>644 ± 15</td>
</tr>
<tr>
<td>14°C</td>
<td>658.7 ± 31</td>
<td>640.5 ± 21</td>
<td>705 ± 42</td>
<td>633 ± 18</td>
<td>650.5 ± 36</td>
<td>692 ± 39</td>
<td>658.7 ± 41</td>
<td>670 ± 29</td>
<td>651 ± 28</td>
</tr>
<tr>
<td>16°C</td>
<td>698.6 ± 35</td>
<td>678.4 ± 28</td>
<td>729 ± 41</td>
<td>669.7 ± 24</td>
<td>678.4 ± 31.8</td>
<td>741.6 ± 41</td>
<td>696.8 ± 38</td>
<td>688 ± 31</td>
<td>659 ± 21</td>
</tr>
</tbody>
</table>
The effect of egg mass was included in the original model for each hatch-timing characteristic but was found to be non-significant and was thus removed from the final analyses. Days to median hatch were not correlated with egg weight at either 14°C (\(P = 0.907\)) or 16°C (\(P = 0.178\)) but were slightly related at 10°C (Pearson’s correlations: \(r = 0.168, n = 152, P = 0.0369\)). Egg mass was not correlated with either days (\(r = -0.039, P = 0.41\)) or ATUs to first hatch (\(r = -0.07, P = 0.1735\)) across temperatures. At 10°C, egg size was only moderately related to \(H_0\), in that larger eggs took longer to hatch (\(r = 0.187, P = 0.02\)), while at 16°C the inverse was true (\(r = -0.26, P = 0.0017\)), so that larger eggs hatched faster.

### Offspring Size

The interaction of temperature \(\times\) population was significantly related to differences in alevin length (ANOVA: \(F_{16, 400} = 7.92, P < 0.0001\)) but not mass (ANOVA: \(F_{16, 399} = 0.76, P = 0.7282\)). Across all populations, alevin were shorter at hatch when incubated at higher temperatures than when incubated at 10°C (\(P < 0.0001\)), while alevin mass did not vary across incubation temperatures (Figure 5; Table 4). Egg mass explained a great deal of the variation in alevin mass at hatch, irrespective of thermal treatment (\(R^2 = 0.59, P < 0.0001\)), but not in alevin length (\(R^2 = 0.05, P < 0.0001\)), for which incubation temperature and population played more of a role.

### DISCUSSION

Exposure of Sockeye Salmon to high temperatures (14°C and 16°C compared with 10°C) during incubation caused substantial differences both within and among populations in hatch timing, hatch duration, and offspring size. Egg mass varied among populations, but this trait only affected alevin mass at hatch, not developmental timing or offspring size. Considering the fitness implications of hatch timing and offspring size, these results suggest that Sockeye Salmon development and offspring competitiveness may be affected by temperature regime and that this effect will vary by population but that egg mass is not a main driver of among-population differences in hatching characteristics.

### Hatch Timing: Populations Matter More than Egg Size

Hatch and emergence timing have ecological implications for salmonids within natural aquatic communities, whereby development timing cues relate to life stage survival and subsequent life history transitions (Crozier et al. 2008). In general, incubation studies suggest that development rates reflect adaptation to local conditions (Taylor 1991; Hendry et al. 1998; Jensen et al. 2008) and changes to those thermal
regimes are likely to have both biological and ecological effects on species and ecosystems (Steel et al. 2012).

Incubation requirements did vary by population, but there was no relationship between the development rate of embryos and the average spawning temperature for each of the populations. Similarly, development time to median hatch was not related to spawn date or parental migration experience (Figure 3 versus Table 1). In theory, developmental synchrony should be evident from early development to later life stages, from fry emergence to smolt migration to ocean systems, and from adult movement at sea (Godin 1982; Tallman 1986; Becham and Murray 1987, 1988; Brannon 1987). While most research on development timing has focused on emergence rather than hatch as discussed here, alevin hatch timing has been linked to emergence timing (Godin 1982; Crisp 1988). However, in this study it is not clear why some populations hatch considerably earlier than others under the same thermal experience (e.g., fish from the Adams River hatched 6 d earlier

FIGURE 3. The influence of incubation temperature among populations on time to median hatching from fertilization (d.p.f. = days postfertilization) at three incubation temperatures: (a) 10°C, (b) 14°C, and (c) 16°C. Responses for all treatments are ranked by the order of populations in the 10°C treatment. The black lines show the median response for a population, the boxes contain the data between the 25th and 75th quartiles, whiskers show the 10th and 90th percentiles, and open circles show extreme data; OK = Okanagan.
TABLE 4. Measurements (mean ± SD) for alevin mass ($M_H$) and length ($L_H$) at hatch across populations under the influence of three incubation temperatures (10, 14, and 16°C). All populations are from the Fraser River except for the Okanagan River population, which is from the Columbia River.

<table>
<thead>
<tr>
<th>Metric</th>
<th>Temperature (°C)</th>
<th>Gates Creek</th>
<th>Scotch Creek</th>
<th>Chilko River</th>
<th>Horsefly River</th>
<th>Stellako River</th>
<th>Okanagan River</th>
<th>Adams River</th>
<th>Weaver Creek</th>
<th>Harrison River</th>
</tr>
</thead>
<tbody>
<tr>
<td>$M_H$ (mg)</td>
<td>10</td>
<td>115.3 ± 12.5</td>
<td>91.6 ± 7.9</td>
<td>110.6 ± 7.7</td>
<td>96.4 ± 16.0</td>
<td>91.1 ± 8.1</td>
<td>99.5 ± 11.7</td>
<td>105.4 ± 75.0</td>
<td>128.8 ± 12.9</td>
<td>176.1 ± 16.3</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>104.6 ± 7.9</td>
<td>97.0 ± 64.9</td>
<td>111.1 ± 8.9</td>
<td>93.6 ± 9.2</td>
<td>91.6 ± 16.4</td>
<td>99.4 ± 15.2</td>
<td>99.1 ± 10.8</td>
<td>123.4 ± 13.8</td>
<td>170.7 ± 17.2</td>
</tr>
<tr>
<td></td>
<td>16</td>
<td>107.8 ± 17.9</td>
<td>90.2 ± 11.6</td>
<td>109.0 ± 8.8</td>
<td>94.9 ± 10.5</td>
<td>90.9 ± 7.9</td>
<td>98.8 ± 17.4</td>
<td>97.9 ± 8.9</td>
<td>127.4 ± 13.0</td>
<td>166.5 ± 15.5</td>
</tr>
<tr>
<td>$L_H$ (mm)</td>
<td>10</td>
<td>19.9 ± 1.6</td>
<td>19.4 ± 0.9</td>
<td>21.9 ± 1.0</td>
<td>19.7 ± 1.4</td>
<td>19.0 ± 0.7</td>
<td>20.3 ± 0.8</td>
<td>18.9 ± 0.7</td>
<td>20.2 ± 0.8</td>
<td>21.1 ± 1.1</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>16.9 ± 1.4</td>
<td>17.9 ± 1.0</td>
<td>20.1 ± 0.9</td>
<td>18.6 ± 0.9</td>
<td>19.5 ± 1.3</td>
<td>18.4 ± 1.1</td>
<td>19.2 ± 1.3</td>
<td>18.4 ± 0.8</td>
<td>19.4 ± 1.4</td>
</tr>
<tr>
<td></td>
<td>16</td>
<td>17.4 ± 1.1</td>
<td>17.7 ± 1.1</td>
<td>19.7 ± 1.1</td>
<td>18.3 ± 1.1</td>
<td>17.6 ± 1.3</td>
<td>17.9 ± 1.0</td>
<td>17.8 ± 0.9</td>
<td>18.6 ± 1.0</td>
<td>19.1 ± 0.8</td>
</tr>
</tbody>
</table>
than those from the Chilko River, both at 10°C). Hatching earlier would allow alevin to avoid intergravel ice conditions sooner (Cope and Macdonald 1998) and avoid dewatering events and localized low-oxygen environments, thus increasing competitiveness for those fish. However, there is likely an increase energetic activity cost associated with hatching early into colder winter thermal regimes, especially as alevin, a very vulnerable life stage (Blaxter 1992). Future work should seek to better understand such trade-offs and their ecological relevance to the populations described herein.

Consistent with earlier work on Sockeye Salmon, egg size differed significantly among populations and this variation in size occurred along a gradient of migration distance from river entry, for which longer migrations were associated with smaller eggs (Crossin et al. 2004). While previous work has shown that egg size can be negatively (Beacham and Murray 1985) or positively (Heath et al. 1999) related to offspring survival, it is often unrelated to embryo viability (Hutchings 1991; Nadeau et al. 2009; Burt et al. 2012a). In our study, elevated temperature increased development rates, resulting in earlier hatching than for those embryos incubated at cool temperatures, but egg size did not significantly affect developmental timing characteristics among populations.

While incubation duration is often inversely related to egg size in Pacific salmon (so that smaller eggs hatch faster) (Bagenal 1969), we show that among the populations in our study incubation duration depends more on population origin and water temperature than on egg size, a pattern observed previously for fry emergence (Brannon 1987). Indeed, the population that hatched the fastest at high temperatures (Harrison River) had the largest eggs (Tables 1, 3), although the significant mortality observed for this population (Whitney et al. 2013) may have resulted in a selection bias towards faster-growing families. Moreover, the effect of egg size was not significantly related to time to first hatch, 5% hatch, or 50% (median) hatch. As there was some evidence for faster

![Figure 4](image-url)  
**FIGURE 4.** Hatch duration reaction norms by population under incubation temperatures of 10, 14, and 16°C. Overall hatch synchrony across populations increased with temperature, while hatch synchrony within populations decreased. Hatch duration was measured between 5% and 95% hatch for each family and is shown as averages per population; OK = Okanagan.

![Figure 5](image-url)  
**FIGURE 5.** The effect of temperature on (left panel) alevin length and (right panel) alevin mass across all populations under three incubation temperatures. The black lines show median values, the boxes contain all data within the 25th and 75th quartiles, whiskers show the 10th and 90th percentiles, and open circles show extreme data. Only length varied significantly among temperatures ($P < 0.0001$).
hatching of larger eggs at high temperatures, future research is needed to examine the effects of warming thermal regimes with climate change as an interaction with egg size. Environmental temperature regimes are a selective force driving development rates, and research on Rainbow Trout *O. mykiss* suggests that parallel adaptation for rapid early development may be linked to similar thermal regimes among geographically distinct populations (Miller et al. 2012). In that study, populations adapted to cool temperatures in early life had very rapid development rates, whereas in our study only some of the cold-spawning populations, such as the Harrison River Sockeye Salmon, exhibited rapid embryonic development to hatch. In particular, further work on Harrison River Sockeye Salmon is needed as this population is likely affected by warm lake inflow, which may mediate the usual late-season temperature, suggesting that this late-spawning population is perhaps not as cold adapted as would otherwise be expected.

**Thermal Requirements: Compensation and Hatch Duration**

Thermally elevated basal metabolic rates require a greater proportion of endogenous energy reserves, leaving less for growth and development (Beacham and Murray 1985; Kamler 2008; Jonsson and Jonsson 2011). Blaxter (1992) also suggested that larval length and weight decrease with increased incubation temperature due to the disproportionate increase in development rate versus growth rate so that cell differentiation occurs before sufficient tissue develops to allow for normal weight or length. In poikilotherms, development rates are directly related to temperature (Brett 1971) but exposure to supraoptimal temperature can result in a compensatory response in incubation rate, in which the amount of ATUs required to reach a certain development stage, such as hatching, increases at temperatures beyond a certain threshold (Brannon 1987). Elevated development rates can result in premature hatching if these rates increase proportionately to rising temperature. This disassociation between metabolic and growth rates is generally seen as temperature exceeds an upper thermal limit (Rombough 1994). This relationship between metabolic and growth rates is thought to vary by population and to be linked with historical emergence date (Konecki et al. 1995). Evidence of a thermally driven compensatory response at hatch (Brannon 1987) was observed in our study, in which most populations required more ATUs to reach hatching at the 14°C and 16°C treatments than at the cold treatment (10°C; Chilko). That this was especially evident at both the moderate (14°C) and high (16°C) incubation temperatures suggests that parallels between hatch timing and hatching success should be investigated further.

Hatch duration varied by temperature treatment, with higher temperatures being related to protracted hatching (decreased hatch synchrony) within a population. This has rarely been investigated but was also observed in a separate study on one of our Sockeye Salmon populations (Weaver Creek; Burt et al. 2012b). Kamler (2005) also noted increased hatching asynchrony at elevated incubation temperature. Synchrony in hatch and thus emergence timing should allow populations to “swamp” predators (Godin 1982). Asynchrony in development timing within populations may also be an adaptive response to resource competition, especially in years of high abundance as a means of lowering competition for scarce resources (Godin 1982). While the direct fitness implications of hatch timing are thus less than clear, the association between hatch and emergence timing is strong enough to link the consequences of environmental effects on changing timing sequences for population-specific adaptations.

**Offspring Size and Growth**

Across all populations, alevin hatched from the high-temperature treatment were shorter and lighter than those from the same population raised under the cool-temperature treatment (10°C). This pattern has been shown previously (but mostly for emergent fish) in Sockeye Salmon (Hendry et al. 1998) and other salmonids (Pink Salmon *O. gorbuscha*, Murray and Beacham 1986; Brown Trout *Salmo trutta*, Ojanguren and Brana 2003; Arctic Char *Salvelinus alpinus*, Janhunen et al. 2010). Alevin length, but not mass, was significantly affected by temperature treatment, consistent with the suggestion that under higher temperature alevin utilize their yolk sac less for growth and more for routine basal metabolism (Beacham and Murray 1989; Rombough 1994; Kamler 2008). As shown here, if temperature is high enough during incubation to affect the efficiency of yolk conversion to body tissue, alevin length may be affected more than alevin mass (Beacham and Murray 1985). Indeed, this reduction in growth efficiency was shown in subsequent work to persist through to the emergent stage in these same populations (Whitney 2012), confirming that temperature did not merely result in premature hatching but did affect offspring growth.

**Conclusions**

Suboptimal temperature during incubation is likely to have deleterious consequences for developing embryos beyond a reduction in survival. This research suggests that temperature patterns and population differentiation may affect offspring competitiveness and development timing more than egg size, especially when comparing widely disparate temperatures and geographically isolated populations. The implications of variation in hatch timing are wide ranging, and differences among populations may directly affect survival and thus population viability. Further investigation into how populations vary in early life history traits, and how these genetic differences interact with environmental conditions such as temperature, will be necessary in order to allow us to predict the
evolutionary response of Sockeye Salmon to climate change and peak temperature events. This work highlights the importance of population variation in thermal adaptation and is a reminder of the relevance of maintaining genetic diversity for species conservation.

ACKNOWLEDGMENTS

All research was conducted with the approval of the Animal Ethics Committee of the University of British Columbia, according to the principles of the Canadian Council on Animal Care (#A08-0388). C.W.K. was supported by a Natural Sciences and Engineering Research Council of Canada, Canada Graduate Scholarship and a VanDusen Graduate Fellowship from the University of British Columbia Faculty of Forestry, with project funding deriving from Natural Sciences and Engineering Research Council of Canada Strategic and Discovery Grants to S.G.H. and support from Fisheries and Oceans Canada’s Environmental Watch group. The authors thank members of the Okanagan Nation Alliance, in particular R. Bussanich and S. Folks, for field support and assistance. Many thanks to all the members of the Pacific Salmon Ecology and Conservation Laboratory, especially A. Lotto, J. Burt, E. Vogt, A. Haas, K. Robinson, N. Sopinka, and E. Martins.

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