Temperature-induced plasticity of emergence phenotypes in Chinook salmon (*Oncorhynchus tshawytscha*)

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Abstract

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Dams supply services that cultivate and sustain civilization such as flood control, navigation, and electricity. Due to thermal stratification in reservoirs the water released by dams is often a different temperature from environments further upstream on both daily and seasonal time scales. The impacts of flow regulation on temperature are important because of the influence that temperature has on development and distribution of species. Salmon are particularly vulnerable to habitat and temperature changes because their reproductive and early development life phases are spent in freshwater systems. Localized temperature regime changes caused by dams may spur local adaptation. In a common garden laboratory incubation experiment, I measured emergence timing of fry from four Spring Chinook populations originating from Northwest river systems affected by hydropower dams. Four family groups from each of four different populations were created by artificially spawning adult salmon (1 to 1 crosses) during the same week of September, 2012. Each family of eggs was exposed to four different thermal regimes from fertilization to emergence to test the following hypotheses: 1) Altered temperature regimes can cause disparity in development timing between families and across populations. 2) Reaction norms for development timing under different temperature regimes are unique depending on family and population origin. 3) The condition of fry at emergence is regulated by an interaction between genotype and thermal regime. 4) The delivery method of temperature (constant vs. variable) affects emergence phenotypes. We found that fry from warmer thermal regimes emerged earlier both in terms of calendar date and temperature units, and that warmer treatments caused fry to emerge less developed. There was also a significant effect of family on both emergence timing, and development level at emergence. The influence of daily temperature pattern was population specific, with most families from the Willamette Basin showing delayed emergence when incubated in a cold stable thermal regime. By combining measurements of physiological and behavioral traits at emergence, our research provides a better baseline for interpreting results in the context of hatchery, natural, and human-altered environments.

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CHAPTER 1

Reacting to the new norm: variation in Chinook salmon emergence phenotypes as a response to altered thermal regimes

INTRODUCTION

Salmon behavior 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, selection shapes offspring emergence to correspond with ideal flow, temperature, and food availability (Brannon 1987, Skoglund et al. 2011b). Once eggs are deposited in the gravel, the thermal regime experienced during incubation determines development rate and time to emergence (Alderdice and Velsen 1978). During the freshwater life stages of salmon, water temperature can fluctuate widely on daily and seasonal time scales. 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 et al. 2010).

Understanding plasticity of certain developmental traits in response to environmental changes will help us estimate the degree to which these traits might contribute to the adaptation 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 (Woltereck 1909, Schlichting and Pigliucci 1993). The foundation for future reaction norm research should be based on the notion that plasticity is most likely heritable, and also take into account the idea that population differences in reaction norms suggest 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.

Emergence timing and condition at emergence are both traits that influence early growth and survival, and thus have direct impacts on fitness (Einum and Fleming 2000). Early studies established species specific development rate and condition at emergence under constant incubation temperatures (Alderdice and Velsen 1978, Heming 1982, Beacham and Murray 1990). However, only a few studies have expanded on knowledge about the potential for adaptive variation in emergence timing and condition at emergence in changing environments. Hendry et al. (1998) documented evidence for plasticity of yolk conversion efficiency (ultimately a determinate of fry size) 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 Frasier River found evidence for inter- and intra-specific phenotypic plasticity in survival rates at different incubation temperatures (Whitney et al. 2013). Studies on Chinook salmon emergence timing have recognized that there is a genetic component to emergence timing (Beckman et al. 2008). Genetics may also dictate the magnitude of response to temperature variability (Steel et al. 2012).

Salmon populations that spawn in rivers with thermal regimes altered by dams present a good opportunity to study reaction norms and improve on our 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 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 emerge from the gravel up to two months earlier than normal (Webb and Walling 1993). Many different 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 recolonize (Keefer et al. 2010).

In order to optimize management strategies, more 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 from the Willamette River Basin, Oregon and Yakima River Basin, Washington to answer two questions: *i*) does the condition, size, or emergence timing of fry differ between families and across populations?; *ii*) how does the reaction norm (interaction between genotype and environment) change depending on the thermal regime experienced during incubation? Our approach combines measurements of physical and behavioral aspects of emergence in order to better evaluate variation in emergence phenotypes within and across populations.

METHODS

Gamete Collection and Fertilization

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 (Fig. 1). At each location, eggs were stripped from six females and each lot of eggs was placed in a 0.74l Ziploc (SC Johnson, Racine, WI) bag, which was then filled with compressed 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 fertilization 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 0.1g. A subsample of unfertilized 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, fertilization, 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 fertilized eggs were placed into 10-cm mesh-lined square bottomless plastic planter cups (Fig. 2a). This process was repeated to generate six unique families from each population (6 1x1 male-female crosses, split into 8 egg lots each). Planter cups of eggs were nested inside larger 32-oz containers and supplied with upwelling water from one of four temperature regimes (Fig. 2b).

Experimental design and apparatus

All eggs were incubated in a common pool of re-circulating water from which four thermal treatment regimes were created with final temperatures falling between 5-10°C. Two treatments were at relatively constant temperature throughout the experiment, while the other two treatments had a mixture of daily and seasonal temperature variation in an effort to simulate natural and below-dam environments (Fig. 3). De-chlorinated municipal water was chilled with heat exchangers 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) to the desired temperature using digital thermostat control (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) programmed to turn heaters on during daylight hours. All four treatment regimes were replicated for a total of eight thermal regimes (4 x 2). Temperature was recorded and stored hourly using temperature data loggers (HOBO, Onset Computer Corp, Bourne, MA) placed in each head tank, as well as in one incubation chamber per treatment to confirm that the temperature in head tanks and chambers was similar.

Egg incubation

Eggs were incubated in complete darkness, except when water flow rates were being monitored, in which case red lights were used as eggs 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 (Fig. 2b). Equal flow rates from siphon tubes (0.75-L/min) were attained by placing all cups at the same elevation. Once eye pigmentation was visible, unfertilized and dead eggs were identified by applying a mechanical shock (pouring eggs from the cup into a bucket from a height of 0.5-m), counted, removed and discarded.

Survival rate was assessed for families in each treatment (Table 1). We discarded the two families from each population with the lowest survival across treatments. Eggs (*n*=80) from the remaining family groups in each treatment were transferred to individual incubation chambers (Fig. 4). Artificial substrate was provided by using 13-mm diameter plastic bio-filtration balls which were weighted down with a single layer of 13-mm diameter black glass marbles. Water was supplied to each chamber at 1.5-L/min 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 maximize replication of families across treatments, emergence chambers were divided in half by a plastic 3 mm mesh divider secured lengthwise down the middle with silicon aquarium sealant. Two families from the same population were loaded into divided emergence chambers, one family per side. To monitor hatch timing, additional eggs (*n*=80) from each family/treatment group were placed in the corresponding family's collection cup which experienced the same temperature regime as the emergence chambers. When eggs in the collection cups hatched, alevins were removed, counted, and euthanized with a lethal dose of tricain methansulfonate (MS-222, Western Chemical, Ferndale, WA) every 24 h. The period of development between hatching and emergence was long enough that all alevins in collection cups were 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.

Sample collection

Eggs were allowed to hatch and the alevins were allowed to develop undisturbed inside the incubation chambers. Fish that volitionally exited the incubation chamber (emerged) were contained in the collection cup and counted every 24h. A 9 cm gap between the substrate and outflow spout ensured that fish would need to exhibit swim-up behavior to exit the chamber. Each fish that emerged was euthanized with a lethal dose of MS-222. Emerged fish were 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) (Fig. 5). Length (±1.0-mm) and weight (±1.0-mg) were measured from a subsample of emerged fish family/ treatment group at the beginning (*n*=13), middle (*n*=13), and end (*n*=13) of emergence. Another subsample (n=10) from the 39 fish that were weighed and measured in each family/treatment group were frozen at -20°C for later analysis (*n*= 10 x 4 x 4 x 8 = 1280). Individual emergence time was recorded by calendar date and converted to temperature units, or TUs ($TU = {}^{\circ}\mathrm{C} \times days$) Dry weight was determined for subsamples of emerged fish 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.

Statistical Analysis

Fish were divided into one of two groups (premature or buttoned-up) based on their condition at emergence. Bams' condition factor (KD) was computed for the subsample of fish that were weighed and measured, and compared to our development level (DL) assignments. KD values ≤ 1.95, indicating complete yolk absorption (Bams, 1970) corresponded to DL 4 and 5 (Fig 6), thus DL 0-3 fish were classified as premature, and 4-5 as buttoned-up.

We examined the influence of family and temperature treatment on the proportion of fish that emerged premature using tests for equality of proportions. We were able to test for potential emergence timing interaction between families paired in single chambers because families were paired in different combinations across replicates. To rule out any interaction effect, a binomial test (Zar 2007), with *n*=64 and p=0.5 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 greater than zero:

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

Statistical analysis on size at emergence only included those fry which were buttoned-up, approximately 84% of all emerged fry (n=8150). Egg weight varied among families and is known to affect size at emergence (Beacham and 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 dry weight, length and condition at emergence. The metrics used to describe emergence timing were calendar days to emergence, and TUs at emergence for mature fry, averaged by family. Since families were split across treatments and replicates, we analyzed main effects of thermal treatment on TUs and calendar days to emergence using a split-plot ANOVA with a completely randomized design on whole plot treatments, using an alpha of 0.05.

RESULTS

Thermal treatment replication

Two sample t-tests for overall mean temperature indicated no significant differences between treatment replicates (all P-values > 0.1). Overall variance was also similar between each set of replicates (Bartlett's test for homogeneity of variances, all P-values >0.05). Two temperature spikes occurred in January due to a cooling pump failure. These temperature spikes were short in duration (lasting less than 10 hours) and were experienced by alevins in all treatments because water was re-circulating and chilled in a common pool.

Development level

Development level of fish that were weighed and measured was positively correlated with Bams' condition factor (KD), r^2=0.56 (Fig.6). This correlation was important because not all fry were measured, but all were given a development level designation at emergence. Using this correlation, we were able to categorize fry as either premature (DL 0-3) or buttoned-up (DL 4 and 5). Some families showed a tendency to emerge premature regardless of temperature treatment (Fig. 7). Over 40% of fish that emerged premature originated from 3 of the 16 families. Equality of proportions tests for premature emergence indicated significant differences between families in each treatment (P<0.001) and differences across treatments for all families combined (P<0.001). Pairwise comparisons indicated that the warm treatment had significantly higher proportion of premature emerging fry than all other treatments, but that daily variation and stable treatments were exactly equal (P=0.99). Finally, we concluded that pairing of families in chambers did not have a significant effect on emergence timing (binomial test, P=0.382) or condition at emergence (P=0.708).

Allometrics for mature fry

Regression models revealed that fry dry weight (DW) and length (L) at emergence were positively correlated with unfertilized egg weight ($r_{DW}^2 = 0.79, r_L^2 = 0.70$). Analysis of covariance indicated a significant effect of thermal regime treatment on DW (P<0.001) and L (P<0.001) at emergence, with fry in the warm constant treatment emerging heavier and shorter. There was no interaction between egg size and treatment for DW (P=0.461) and L (P=0.818). Condition factor at emergence was not related to egg size ($r_{KD}^2 = 0.05$) but was affected by temperature treatment (P=0.0012) (Fig. 8C). Because egg size was initially different across populations (*P*<0.001) and replication was limited, it was not possible to test for interaction effects on fry size between populations within treatments.

Emergence timing for mature fry

Emergence as measured in calendar days was influenced by temperature treatment (P<0.001). Fry from colder treatments did not emerge until nearly 2.5 months after those in the warm treatments (Fig. 9A). Treatment (P=0.0039) and population (P=0.009) both influenced TUs to emergence as fry from Clackamas and McKenzie populations emerged from warm treatments with fewer TUs. An interaction was detected between treatment and population (P=0.003), although the difference in response to treatment 3 for the Yakima population (Fig. 9) may have been influenced by the temperature spikes in January. The results of split-plot ANOVA using families instead of populations for analysis also indicated significant effects of treatment and family on emergence timing (P<0.001), although no interactions between family and treatment were observed.

DISCUSSION

By integrating measurements of emergence behavior (timing) and condition factor at emergence, our results improve our understanding of Chinook salmon developmental reaction norms. These research findings suggest that there is a strong genetic influence on condition at emergence, and that warmer temperatures increase the propensity for certain families to emerge prematurely both in terms of physical development and time. For fry that appear fully developed at emergence, there was a measurable difference in condition factor across different thermal treatments. Eggs incubated in warmer temperatures developed faster, and fry emerged with fewer accumulated temperature units on average (in some cases up to 100 TUs difference). It is also clear that emergence timing and condition at emergence can be variable among families within populations, and that this variance seems to increase with warmer temperatures, even within ranges that may be experienced naturally.

Thermal treatment effects on physiology

Warmer incubation temperatures caused fry to emerge shorter as measured by fork length, a result that was consistent with earlier research (Beacham and Murray 1990). This pattern may be due to decreased yolk to tissue conversion efficiencies at higher temperatures (Heming 1982) since more yolk is used for metabolism rather than tissue growth. However, the relationship between weight at emergence and temperature in this and in past studies is less clear. Discrepancies concerning weight at emergence may be due in part to the number of different methods that have been employed to evaluate development. Researchers have measured responses to temperature using dry weight and wet weight, in addition to recording weight at various developmental benchmarks. However, there is one stage that many researchers use to define a fry as fully developed - the moment when the yolk is observed to be almost completely absorbed. Fry at this stage are commonly referred to as buttoned-up (Beer and Anderson 2001). We recorded both wet and dry weight for a subsample of emergent fry, but relied on the analysis of dry weight of those fry that were buttoned-up to interpret thermal regime effects on size at emergence. Buttoned-up fry from the warm constant and below dam treatments emerged slightly heavier. This outcome could suggest that metabolic demand is lower at warmer temperatures, but this is not likely. Another explanation for heavier fry from warmer treatments could be that they simply emerged slightly earlier, before all yolk was exhausted. Thus, while measurements of weight may help to interpret how differences in development relate to temperature, these measurements must be considered in addition to emergence timing in order to fully assess emergence phenotypes.

There is some evidence for other species that suggests isolated changes in water temperature may provide a behavioral cue for fry to emerge. Emergence responses to temperature have been documented for sea turtle hatchlings, where a decrease in sand temperature prompts sea turtles to emerge at night in order to avoid predation (Glen et al. 2006). Such a mechanism would help explain why, in our study, fry from colder treatments were longer in fork length but weighed less than fry from warmer treatments. Cold treatment fry appear to have used a greater amount of yolk for growth, but may have depleted yolk reserves before an increase in temperature prompted emergence. This scenario seems likely because significantly more fry emerged at DL four or below in the warm constant (40%) vs. the cold constant treatment (22%). Our findings on emergence condition as it relates to thermal treatment were consistent with earlier studies. Heming (1982) observed that Chinook salmon fry incubated at warmer temperatures (10 and 12°C) emerged precociously and were smaller at hatching and emergence than those incubated at 6 and 8°C. Research on adaptive divergence of sockeye stocks in Lake Washington (Hendry et al. 1998) also found that the proportion of yolk remaining at emergence was greater overall for salmon incubated in the highest temperature treatment (12.5°C).

Condition at emergence, in addition to emergence timing, may have important implications for fry as they begin exogenous feeding (Skoglund et al. 2011a). Fully developed fry that emerge slightly earlier may have an easier time establishing feeding territories, and could potentially out-compete smaller fry for food (Mason and Chapman 1965). Fry that emerge before they exhaust their yolk reserves have a longer time frame in which to find food before they must resorb body tissue to maintain physiological processes (Bams 1967). However, fry emerging with more yolk reserves make an easier target for predators (Fresh and Schroder 1987). All these outcomes illustrate a complex series of tradeoffs that promote a wide range of phenotypic expression in newly emerged fry.

Variation in emergence phenotypes is known to exist, but is not well studied at the family or population scale. Our decision to include only button-up fry for the analysis of emergence timing and condition was based on previous pilot study results that indicated the percentage of fry emerging prematurely within family groups was highly variable across thermal regimes (Steel et al. 2012). These results were confirmed in the current investigation when we found that certain families had up to twice as many fry emerge prematurely in the below dam vs. the natural thermal regime. In both studies, premature emergence was not reversible as it may be in the wild where fry could risk predation and high flows for an opportunity to preview environmental conditions. By excluding premature fry from our reaction norm analyses, we were able to reduce the amount of variation across family groups in order to better detect phenotypic differences among populations and across thermal treatments. However, it should be noted that the strong differences in premature emergence which we found to exist within a population (Fig. 7) might present an opportunity for phenotypic divergence and local adaptation to shifting temperatures (Fraser et al. 2011).

Hatchery influence on populations

In order to better interpret the results of this experiment, we must take into account that hatchery rearing conditions impose very different selective pressures than those affecting alevins and fry in the wild. In general, most salmon hatcheries incubate eggs and fry in Heath trays

(MariSource, Fife, WA) without any support structure, which initiates a righting response at the expense of energy for growth and produces smaller fry (Bams 1969). When hatchery fry are deemed ready to feed, they are put into a tank or raceway in a group based on spawning date and developmental state. Since food availability is not a factor, more fully developed fry that can swim and eat might maintain an early growth advantage. Long-term selective pressures for hatchery fish are also different than those in the wild because hatchery salmon are usually bred according to conservation and/or harvest goals (Mobrand et al. 2005). Integrated programs use a certain percentage of natural origin adults in their broodstock in order to reduce genetic divergence between wild and hatchery populations, and thus reduce potential for domestication. Alternatively, segregated programs seek to keep gene flow between wild fish and established hatchery populations to a minimum, thus increasing the potential for the two populations to diverge and become genetically distinct. Over time, it seems likely that selection might favor different emergence phenotypes in wild vs. hatchery environments, but more research is necessary to understand how these differences influence early growth and life history.

The eggs used in our study were collected from salmon at hatcheries with diverse backgrounds. Yakima River spring Chinook salmon eggs were sourced from a relatively new integrated hatchery line with only one generation in culture (described in Knudsen et al. 2006). The Clackamas hatchery maintains a segregated line, operating since the mid-1980s that was developed from other Willamette River Basin hatchery Chinook salmon with historically earlier run timing. Both South Santiam and McKenzie hatcheries operate integrated programs. These two hatchery operations increased production after dams were built during the late 1960’s, but are known to have a low instance of incorporating transfers from other hatcheries so they may still maintain an elevated level of local adaptation (Johnson and Friesen 2010). Although differences in hatchery operations exist, a recent study suggests neutral genetic differentiation is relatively low between each hatchery stock and its respective local wild population in the Willamette River Basin (Johnson and Friesen 2014).

Emergence timing and genetics

We found strong evidence for population level variation in emergence timing. Fry from Santiam and Yakima populations took longer to emerge than those from Clackamas and McKenzie. Some of this variation may be due to the relative degree of domestication selection experienced by each population. If emergence timing is optimized for populations according to local environmental conditions (Brannon 1987), and this phenotypic plasticity is heritable (Whitney et al. 2013), then it is possible that hatcheries seeking to produce fast growing fish could artificially select for earlier emergence timing. It would follow that fry from segregated hatchery programs like Clackamas might emerge earlier compared to those from integrated programs, or programs with very few generations in culture like the Yakima. The lack of selection for emergence behavior in hatchery culture might also explain why all three Willamette populations responded to a brief temperature spike in the below dam thermal treatment, while fry from the Yakima population appeared less sensitive.

Variation in emergence timing for button-up fry was also evident within families across thermal regimes, both in terms of TUs and calendar days. 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 in calendar date ranging from 28 to 54 days later in the natural regime, depending on the family. In this experiment, fertilization 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 et al. 1998), a compelling argument can be made for adaptive variation in emergence timing within populations (Hendry and Day 2005).

In our study, fry incubated in a simulated below dam environment emerged earlier, shorter, and less developed than fry from the same family incubated in a natural type thermal regime. As research on salmon in the Willamette River Basin increases, it is likely we will find that hydropower operations and the resulting mitigation efforts may already be exerting artificial selection pressure on populations (Angilletta et al. 2008). Recovery efforts for Chinook salmon in the Willamette River Basin are largely aimed at introducing adults to spawning and rearing habitat previously blocked by dams and then providing downstream passage for migrating juveniles that have hatched and emerged upstream of these dams. The focus and effort spent on increasing juvenile survival should encourage us to improve our understanding of the degree of local adaptation in developmental traits like emergence, which has a heavy influence on survival, growth, and migration timing of juveniles.

Collecting data on both the behavioral and physical attributes of fry at emergence provides a more complete assessment of their phenotype allowing us to make conclusions in an ecologically relevant context. Implications of local adaptation highlight the need to look beyond thermal tolerance thresholds and focus instead on the overall versatility of populations in their response to novel environmental conditions, especially in the context of climate change (Whitney et al.

2013) and the more immediate environmental changes brought about by dams. Family variation will be important for managers to consider when formulating strategies for rehabilitating or recovering salmon populations affected by hydropower operations. In the Willamette River Basin, adults are transported upstream of dams to spawn in a more natural environment. Certain populations that retain a high level of family variation may be better equipped to adapt to variable conditions upstream of dams.

CHAPTER 2

Variability matters: daily temperature variation affects development and emergence timing of Chinook salmon

INTRODUCTION

A combination of factors influence the temperature fluctuation in a stream reach over the course of a day. Some of these factors include: water source, riparian vegetation, stream depth and discharge rate, air temperature, season, and latitude. In some cases, human activity can alter the complex relationships between these factors and change the range or pattern of daily temperature variation (Olden and Naiman 2010). Dams, for example, tend to reduce the amount of daily temperature variation by releasing water from a stratified reservoir, and/or change the natural flow regime of a river (Webb and Walling 1993). Clear cutting trees near a river can increase both the maximum daily temperature and the range of temperature fluctuation during a day by exposing the water to more sunlight (Brown and Krygier 1970). More recently, the onset of climate change has been linked to changes in the amounts of snow and rainfall per year, which are important factors in regulating stream temperature during the spring and summer (Isaak et al. 2012). These alterations can have important life history consequences for freshwater aquatic species that rely on temperature signals to properly time reproduction, development, and migration (Cassie 2006).

Salmonids for example, are affected both physiologically and behaviorally by temperature anomalies, especially during their time in freshwater. Sockeye salmon adults have been shown to either advance or postpone migration upstream when experiencing higher than average temperatures (Hodgson and Quinn 2002). An increase in temperature can be a signal that initiates smolt migration to the ocean, as shown in Atlantic salmon (Jonsson and Ruud-Hansen 1985). Temperature also determines development rate of salmon eggs and alevins, and ultimately affects the number of days it takes for fry to swim up or emerge from the gravel (Bams 1969). The changes in temperature that happen during incubation are especially important since emergence time sets the baseline trajectory for all the major life history events that follow (Beckman et al. 2007).

Early development is a critical life history period for salmon. Salmon develop all the necessary physical features (fins, jaw, and pigment) during their time in the gravel in order to be successful at swimming and feeding when they emerge. Properly timed development and emergence will contribute to the fry's early growth rate and overall fitness. Survival and recruitment for salmon are heavily dependent on fitness during early life stages (Fuiman and Higgs 1997) thus, much attention has been given to research concerning the effects of water temperature on hatch and emergence timing (Alderdice and Velsen 1978, Beacham and Murray 1990).

As salmon develop from egg to fry, they experience direct effects of temperature. When eggs hatch, sac-fry or alevin are not immediately able to feed so they rely on their yolk sac as their only source of energy. This energy can either be used for tissue growth, or towards other physiological processes like respiration and movement (Bams 1969). When temperature increases, certain processes use more energy, so less of the yolk sac is available for tissue growth. However, some empirical evidence suggests that energy partitioning and the relationship between growth rate and metabolism in larval fish appears to operate differently during embryonic development (Wieser 1989). Research investigating the relationship between development rate and incubation temperature for salmon embryos has thus far focused on the average temperature experience over time (accumulated thermal units or TUs; 1TU = 1°C * 1 day), and tests thermal minimum and maximum thresholds. It is also common practice to use a model to predict hatch and emergence times (Alderdice and Velsen 1978, Beer and Anderson

1997, 2001). Most of the studies examining the relationship between development and temperature utilize different temperature treatments that remain constant throughout the experiment, or rely on an average daily temperature to predict emergence outcomes. Much less is known about how the delivery rate (constant or variable) of TUs might influence metabolism, growth, and emergence timing of salmon fry.

In this chapter, we investigate the importance of daily temperature variation during salmon incubation. We designed a common garden experiment with two thermal regimes that maintained the same average temperature, but differed in their delivery rate of temperature units on a daily scale. We incubated four families from each of four populations in both thermal regimes and measured emergence timing and condition at emergence in order to answer the following questions: *i)* Does delivery rate of temperature units (constant vs. daily variation) affect the timing (or length) of the emergence period? *ii)* How does TU delivery rate affect condition and weight at emergence? *iii)* Are emergence timing and emergence period responses to temperature treatments influenced by family or population origin?

METHODS

See Chapter 1 Methods for experimental design and methods.

Statistical Analysis

For this chapter, we only examined data from two of the four thermal regime treatments: stable and daily variation. Analysis on size at emergence only was restricted to those fry which were buttoned-up, approximately 89% of all emerged fish from the two treatments (*n*=4366). Two-sample t-tests were used to reject the hypothesis that the two replicates of each treatment had different means. Bartlett's test of homogeneity was used to detect any differences in variance between the replicate tanks, and between the two treatments. We used linear regression to determine if there were any differences between treatments in the accumulation rate of TUs. Since families were split across treatments and replicates, we analyzed main effects of thermal treatment on TUs and calendar days to emergence using a split-plot ANOVA with a completely randomized design on whole plot treatments. To analyze trends for emergence period by family we calculated the range of TUs between 20 and 80 percent emergence across treatments, and then subtracted the total number of TUs that were overlapping between treatments (Fig. 13), a number which we refer to as "the overlap score". We repeated the same process to assess within-population variance for each individual treatment. A lower overlap score in this case would indicate synchronicity of emergence period (across treatments, or families). We tested homogeneity of variances in overlap score between populations using Bartlett's test. An ANOVA was used to test for differences in mean overlap score between populations. To determine whether there was a linear relationship between the timing and the length of the emergence period, we used linear regression to test for correlation between TUs to 20 percent emergence and total length of the emergence period for each family. We also analyzed the emergence rate for each population by fitting a logistic regression curve to the proportion of fry emerged (families combined) per TU. We added treatment to the model to test for within-population effect of thermal regime on emergence rate. Treatment, family (egg size) and TU effects on fry dry weight were examined using a linear model and an ANOVA (type III sum of squares). Statistical significance was considered at $\alpha=0.05$. All analyses were conducted using the R programming language (www.r-project.org).

RESULTS

Thermal regimes

There were no significant differences in mean temperature between treatment replicates (all *P*-values > 0.1). Overall variance was also similar between each set of replicates (all *P*-values >0.05). There were significant differences in variance between stable and natural regimes (K^2 = 29.61, P<0.001) as expected. TU accumulation over time from fertilization to hatching was similar between thermal treatments (P = 0.661, Fig. 10). TU accumulation rate from hatching to emergence did differ slightly between treatments, but only by the intercept term (slopes were not significantly different, P = 0.214).

Emergence timing

There were significant thermal treatment effects on emergence timing as estimated by mean TUs to emergence for all fry DL 4 and 5 (P=0.008). Family also affected emergence timing (P=0.0497), but there were no differences detected between populations [P=0.29, (Fig. 11a)]. At most, mean differences between treatments within families were 70 TUs which would translate to approximately 2 weeks at spring temperatures between 4 and 6°C (Fig. 11b).

Emergence period

Emergence rate (% fry emerged per TU) in the Clackamas population was significantly affected by thermal treatment (P=0.032), with fry from the daily variation treatment displaying increasing emergence at fewer TUs relative to those in the stable treatment (Fig. 12A). The effect of treatment was not found to be significant for any other populations (McKenzie (P=0.272), Santiam (P=0.23) or Yakima (P=0.847), Fig. 12B-D). Summary statistics for overlap scores

($\boldsymbol{\mu}$=65.7, $\boldsymbol{\sigma}$ =43.19) indicated that there was a large amount of family variation in emergence period (Fig. 13). Families from Yakima population showed lower overlap scores, and less variance ($\boldsymbol{\mu}$ =22.5, $\boldsymbol{\sigma}$ =8.4, Fig. 14) across treatments. However, there were no significant population differences detected in overlap score variance (Barttlet's test: K^2 = 6.002, P=0.1115), or in mean overlap score (ANOVA: P=0.136). Yakima fry also had the lowest overlap score within treatments (by family) for both the stable and variable thermal regimes. We did find that the length of the family emergence period was related to the number of TUs accumulated at 20% cumulative emergence (R^2=0.703) and that this relationship between timing and length of emergence period was significantly different among treatments (P=0.0187). The metric we used to designate the beginning of the emergence period was 20% cumulative emergence because it approximated the point where the number of fish emerging per TU began to increase more rapidly for all populations. The overall trend was negative for both treatments – emergence was more synchronous among individuals as the initiation of emergence was delayed (Fig.14).

Weight and development level at emergence

Premature emergence, as defined in Chapter 1, was relatively low in most families with only about 10% of fry from all populations/treatments combined emerging at development level 3 or lower (see Ch. 1 and Fig. 5 for explanation of fry development levels). There was no significant difference in premature emergence between treatments (Stable= 11.08%, Daily Variation=11.07%). The overall effect of treatment on dry weight was only marginally significant (P=0.0763). Most individual families showed significant differences in weight between treatments (Fig. 15). Both TU at emergence and egg size had a significant effect on fry dry weight at emergence (P-values <0.001) (Fig. 16). There was also a significant interaction between TU and egg size (P<0.001) on dry weight at emergence (Fig. 17).

DISCUSSION

In this common garden experiment, daily temperature variation experienced by alevins during incubation affected mean emergence timing of most families. There was also a population level response in emergence timing, with 8 out of 10 families from the Willamette River basin showing a significant difference between treatments, and Yakima families showing no difference between treatments. With these results, we were able to confirm speculations from a previous investigation (Steel et al. 2012) that suggested genetics interact with temperature variation to determine the timing and physical characteristics of emergent fry (Steel et al. 2012). This conclusion has important biological and ecological implications for salmon populations that rear in freshwater environments with altered thermal regimes. In our study, fry from the stable regime emerged later (more TUs) than fry from a thermal regime with daily variation. Delayed emergence could also delay time to first feeding, and negatively impact juvenile growth rates, potentially reducing survival of salmon smolts migrating to the ocean. Photoperiod at emergence has also been shown to influence both age at smolting, and age of maturation (Beckman et al. 2007).

Thermal Regimes

Our experiment was designed to approximate temperature pattern changes caused by human activity. Researchers have established that controlled releases of water from hydropower systems can shift the natural seasonality of temperature (Rounds 2010) in addition to reducing the amount of daily temperature variation on a short-term scale (Steel and Lange 2007). Some dams install temperature control systems to adjust for seasonal differences, but the importance of maintaining adequate daily temperature variation in terms of how it relates to emergence timing has not yet been quantified. As we have shown in this experiment, fry from certain populations are more responsive to small increases in temperature towards the end of development. It follows then that small-scale changes in daily temperature patterns should also be factored into management decisions seeking to normalize emergence timing upstream and downstream of impoundments. In addition, these small, local scale changes in temperature patterns are the ones that salmon are more likely to encounter as climate change alters the amount and type of precipitation during the winter and spring. Differences in daily variation may only alter the calendar date of emergence by few weeks at most (Fig. 11). While this is only a fraction of the change in emergence date that a seasonal shift in temperature may cause, daily temperature variation should still be considered as an additional factor impacting salmonid phenotype at emergence.

Emergence Timing

Small scale daily temperature variation resulted in emergence timing differences between treatments. A population-specific response to temperature variation was not detected by ANOVA, although fry from almost all Willamette River Basin families (10/12) emerged earlier (fewer TUs) from the treatment with daily temperature fluctuation (Fig. 11 A). There was a significant amount of family variation in emergence timing, with timing differences between treatments ranging anywhere from 0 to 14 days (Fig. 11 B). However, every family (except B) that exhibited emergence timing differences between treatments showed approximately the same magnitude of response to temperature variation relative to other families from the same population. Therefore, although there were no overall emergence timing differences between populations, it seems likely that the magnitude of response to temperature variation is mediated by genotype, at both the family and population level. One explanation could be that changes in temperature provide a signal for emergence, and each population has a different sensitivity level to temperature changes.

Evidence from research in other aquatic animals, fish, and insects suggest emergence timing is often prompted by thermal cues. Sea turtle hatchlings emerge in response to a decrease in sand temperature, a change that signals the transition from day to night (Glen et al. 2006). On beaches in Newfoundland, emergence of larval capelin is correlated with strong onshore winds that move warm surface water towards the beach, reducing presence of predators and increasing food availability (Frank and Leggett 1982). Mayfly metamorphosis and emergence has been linked to an increase in stream temperature, a signal that indicates low flow and the exposure of large rocks on which to lay eggs (Harper and Peckarsky 2006). These results reinforce the fact that we must consider the entire thermal experience during incubation - both the average temperature along with short term daily and weekly variability - in order to better understand plasticity in emergence.

Emergence responses and sensitivity to changes in temperature may also be dependent on the overall development stage. The idea that salmon eggs reach developmental thresholds before hatching is well documented (Bams 1969, Balon 1984). Most commonly, the eyed stage of development is understood as a point in time where eggs are less sensitive to light and movement (Jensen and Alderdice 1989). We hypothesize that as alevins develop into fry they also reach a developmental threshold beyond hatching - a point at which increases in temperature have the potential to elicit an emergence response by fry. If thermal cues induce an emergence response in salmon, our data indicates that this response is likely to happen closer to the exhaustion of the yolk sac. In Chapter one, we discussed the unanticipated January temperature spikes that produced similar spikes in emergence, particularly for fry incubated in the warmer thermal treatments. The same temperature increases did not provoke a response from less developed alevins in the treatments that had a colder average temperature. A similar phenomenon was observed when temperatures started to increase in early spring, although only fry from Willamette populations exhibited an emergence response. At this point, it is difficult to tell whether Yakima fry did not respond because they had not yet reached their developmental threshold or whether they were just less sensitive to small temperature increases. Regardless, further investigation of developmental thresholds in alevins may help to define the relative importance of genetics, physiology, and behavior in determining responses to environmental change (Balon 1984).

Emergence period

Time to emergence is informative and easy to quantify, but the overall emergence period may be a more ecologically relevant metric to compare genetic variation across treatments. Mean emergence time represents a more general evaluation that could be used to determine phenotypic plasticity of a population at the seasonal scale. The 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. We found there was a large amount of family variation in emergence period (Fig. 13). However, we considered population scale trends to be more important because synchronization of emergence across families has important implications for survival. Fry that emerge first will have first access to food and territory, but may be more vulnerable to predators. Fry that emerge late will be less vulnerable to predators, but lack competitive advantage for food and territory. This risk-reward tradeoff was studied in Atlantic salmon (Brannas, 1995) and is the suggested mechanism for evolution of synchronous emergence. Yakima fry were more synchronous in emergence period both within and across treatments. This could certainly be tied to the fact that emergence period was more compressed for a family that took more TUs to reach 20% cumulative emergence (Fig. 14), and

Yakima fry reached this benchmark a little later than Willamette populations. Length 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 as a behavior does not exist in a hatchery environment. The decoupling of morphological and behavioral aspects of emergence in a hatchery population may be the reason fry from Willamette showed more variation in emergence period than Yakima, which has only 4 generations in culture. Research on the actual length of emergence period in fish is sparse, although a study on emergence trends in sea trout found that duration of their emergence period is influenced by spawning date in addition to water temperature (Elliott and Hurley 1998).

Weight at emergence and metabolism

In Chapter one, we discussed how fry dry weight at emergence is dependent on several factors, including egg size, time to emergence, and incubation temperature. Upon further inspection, we found that the presence of daily temperature variation may also influence dry weight. Fry emerging from the treatment with daily variation were slightly heavier than fry from the same family emerging from the stable temperature treatment. There was a negative relationship between TU at emergence and dry weight. This observation suggests that because fry emerged with more TUs in the stable treatment they weighed less since more of their yolk reserves were depleted in the pre-emergence development period. However, there was a significant effect of treatment on dry weight in fry from some families in the Yakima population even though they showed similar TUs to emergence (Fig. 15). The difficulty of interpreting this model is rooted in the fact that we designated egg size as a continuous variable even though we only used data for mean unfertilized egg size. If we used family in the model instead of egg size, we find that there is a significant effect of treatment ($P<0.001$), but still an interaction between TU and family (Fig.

17). Deciphering the complexities of the relationship between temperature, metabolism, and growth, and how the relationship might differ between families requires constant and detailed metabolic monitoring of fry during incubation. A better understanding of this process would be well worth the effort. We do know that the weight of a fry at emergence is important in determining early growth and life history trajectories (Metcalfe 1998), even without a complete understanding of how genetics influence and regulate metabolic responses to temperature.

Population specific responses

Emergence timing response to temperature variation appears to be population specific. Fry from the Willamette families were clearly more sensitive to temperature variation as they approached the end of development. The strength of this sensitivity may be related to the history of hatchery influence and domestication selection. Since the Willamette populations have spent more time in culture, they may have adapted to a more stable thermal regime during incubation. This adaptation could cause Willamette fry to be more responsive to temperature spikes. Yakima fry, having spent only 3 to 4 generations in culture, may still recognize natural temperature variation and have the ability to buffer physiological and behavioral responses to small increases in temperature. For Willamette fry, their acute response to temperature variation may be disadvantageous in the wild, especially if there are earlier flood events and more precipitation in the form of rain due to climate change.

Family variation in emergence phenotypes

Steel et al. (2012) provided a baseline for research examining the effects of temperature variability during salmon incubation. Results concerning the presence of family variation in emergence response were of particular interest in the planning of our experiment. Interestingly, the one population our study had in common with that of Steel et al. (2012) showed less family variation in emergence condition and timing than fry from the Willamette River Basin populations. However, there were several key differences in our study that may have contributed to these results. Our questions were focused around exploring population specific responses, so we used only four families from each population instead of eight. Also, because we replicated temperature regimes across families, much of our data was averaged across replicates possibly masking some of the variance within families. Finally, we were fortunate to be able to incubate eggs from fertilization to emergence under their designated regime, whereas fry from Steel et al. (2012) experiment all experienced the same regime up until eyed-stage. The differences between these study results have generated more questions about how the thermal experience between fertilization and eyed-stage might influence emergence, beyond just survival.

Conclusions and future research directions

We found that some families responded differently to constant vs. variable thermal regimes, just as Steel et al. (2012) suggested. It would be beneficial to extend this research to look at early growth rates, because the rate at which fry grow during their first year can determine when and if they smolt, and ultimately regulates their path to maturity (Beckman et al. 2007). It would also be interesting to do more detailed testing on metabolic mechanisms during development, specifically investigating the scope of efficiency at varying vs. constant temperatures. Finally, studying fry development in systems before and after dam removal could reveal whether certain populations have adapted to more constant daily temperatures by spawning below dams. Estimating evolutionary and ecological implications of early development in salmon as it relates to temperature variation, will undoubtedly be important for mitigating the impacts of hydropower operations. In addition, the results presented here could be helpful in forecasting emergence response to temperature abnormalities brought about by climate change.

IMPLICATIONS

Our research investigated the developmental interaction between genotype and environment, by exposing different families and populations of salmon embryos to four thermal regime treatments. This data will help us understand and interpret real time changes to emergence patterns for populations that inhabit river systems with unnatural temperature and flow patterns. These temperature changes will create challenges for salmon populations during the spawning, development, and migration phases of their life cycle. 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 because temperature is very important in steering life history trajectories for salmon. Our findings suggest that there is an interaction between genotype and thermal regime during early development, and that the amount of phenotypic variation in emergence timing and condition at emergence is population specific. What follows is a series of potential management issues, questions, and suggestions based on findings from our research, as well as some recommendations for the direction of future research.

Introduction of populations above dams, or after dam removal to recolonize natural habitat

Question: What attributes of a population might make it a suitable choice to recolonize the system?

We found a fair amount of variation in emergence phenotypes (both timing and physical condition) across families within each population, especially considering that we only collected data from four families. The relative amount of family variation in emergence phenotypes will be an important attribute to consider. Populations showing more phenotypic variability might have the ability to adjust or eventually adapt to a wider range of environments. This variation will certainly be affected by the amount of domestication selection imposed by hatcheries, as well as the thermal regimes that populations experience before they are transported to a new environment. We recommend that programs seeking to reintroduce populations above dams use wild broodstock if possible. If using hatchery adults is necessary, we suggest that there should be some estimation of the amount of family variation in emergence timing within the population before transplantation. The group being transplanted should maintain a high level of variability in emergence phenotypes to give them the best chance at survival in a novel environment.

Population history of hatchery propagation

Question: How might this influence a population's ability to cope with or respond to thermal regime changes?

We noticed a difference between Willamette and Yakima populations in their emergence response to daily temperature variation patterns, and acute spikes in temperature. We speculate that this is due to the number of generations each population has been in culture (Yakima = fewer). Another other major difference we might expect to see would be the response of integrated vs. segregated hatchery programs. We suspect that integrated programs, which utilize a certain percentage of natural origin adults in their broodstock, will produce fry that are closer to wild-type fry. These integrated hatchery fry may still retain the ability to buffer abnormal responses to temperature spikes. Regardless of the hatchery program goals, we suggest that reduced selection on emergence phenotypes in a hatchery environment will almost certainly influence the magnitude and direction of the reaction norm response.

Tradeoffs between developmental metabolism and emergence behavior as influenced by thermal regime

Question: How would selection on emergence timing act in natural vs. hatchery systems?

In our experiment, there was no option for fry to burrow back into gravel as there would be in a natural system. This, along with the small sample sizes we collected for dry weights made it difficult to interpret metabolic differences between families and populations. The peaks in emergence that happened in the Willamette populations as a result of the temperature spikes suggest that fry reach a developmental threshold at some point during the later stages of yolk absorption where they rely on temperature cues to signal emergence. Selection in a hatchery system does not exist for this behavior, whereas selection in a natural environment could be heavily regulated by alteration of thermal regimes. If temperature patterns in river systems with salmon continue to change rapidly over shorter time scales, we predict that wild populations with low levels of phenotypic variation will struggle to persist.

Future research directions

After examining issues related to the effects of thermal regime patterns on salmonid emergence, 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: 1) the relationship between metabolism and emergence behavior; specifically, what are the important factors determining when an alevin is physiologically competent to emerge? 2) The relative importance of percent natural origin broodstock in a hatchery program as it relates to determining the amount of variation in emergence timing at the population level. 3) The influence of different emergence phenotypes on early growth, especially in situations with added environmental stress such as food limitation, predation, and/or high flow.

FIGURES

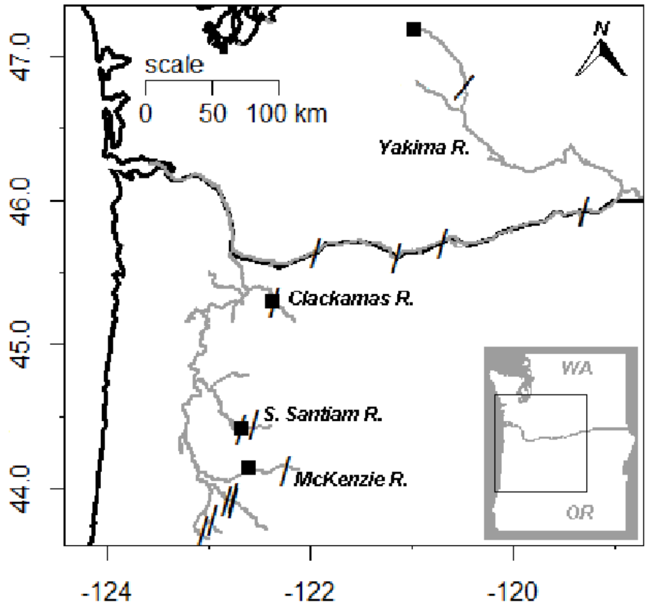


Fig. 1 Eggs were collected from hatcheries on tributaries of the Willamette and Yakima River systems. Hatchery locations where gametes were collected are indicated by squares (■) and major dams are represented by diagonal lines

Fig. 2 After fertilization, eggs were placed in mesh lined plastic planter cups, nested within larger cups and supplied with oxygenated upwelling water from the appropriate temperature treatment via siphon tubes at a rate of 2 liters/min.

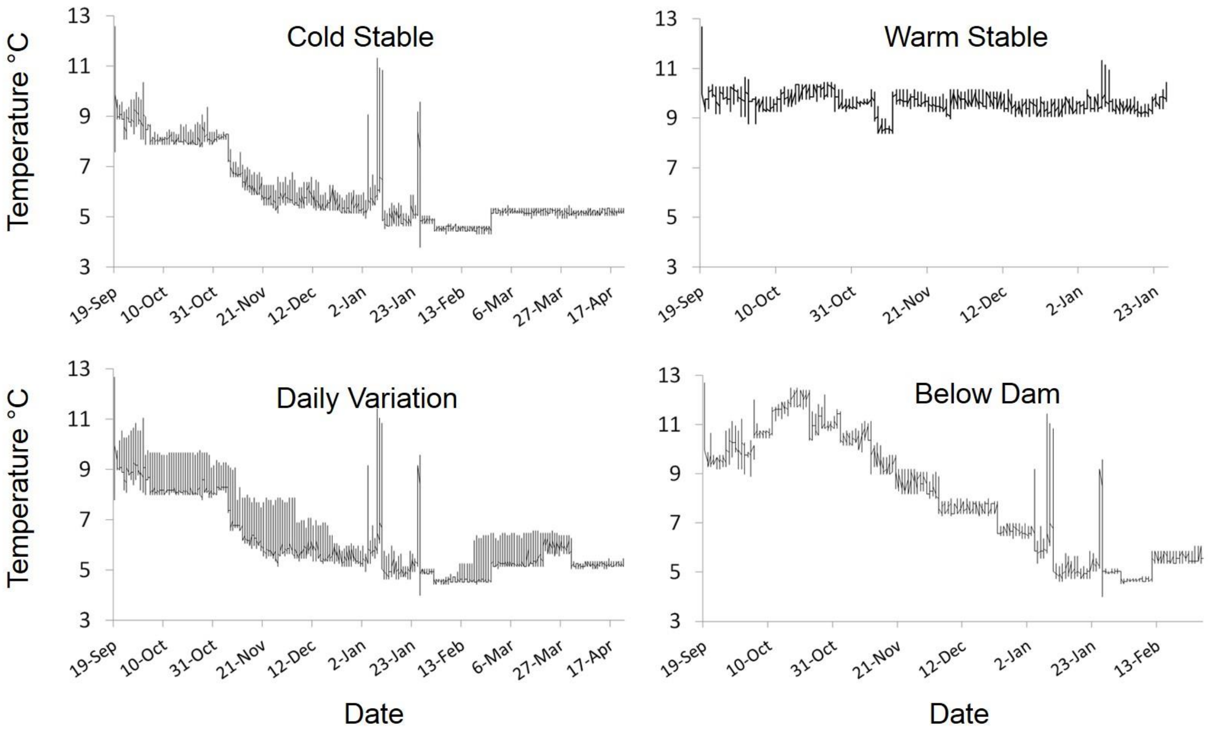


Fig. 3 Temperature regime treatments. Note the temperature spikes at the beginning and end of January were due to a chilling pump failure but are not believed to have affected the overall outcome of the experiment as they were short in duration.

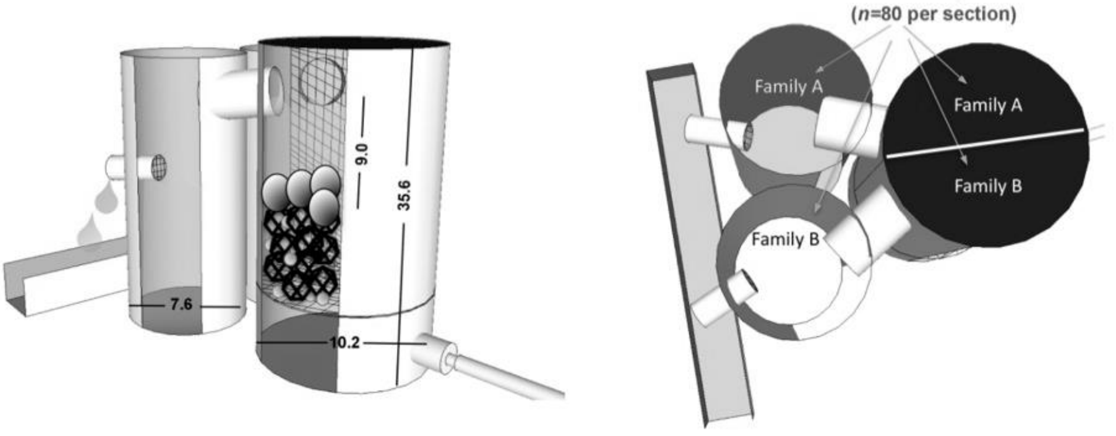


Fig. 4 Cut-away side and top view of egg placement within incubation chamber and catch cups. All dimensions in centimeters.

Fig. 5 Development levels corresponding to amount of yolk remaining at emergence

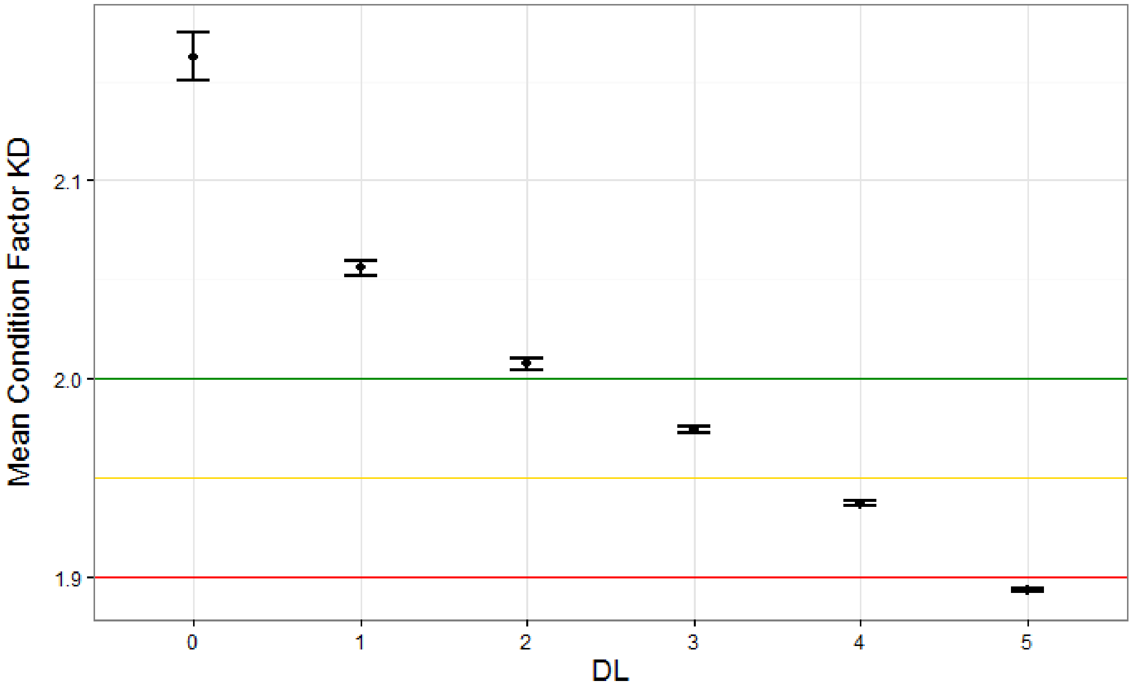


Fig. 6 Correlation of Bams' condition factor to visual estimate of development level (0-5), r^2=0.56

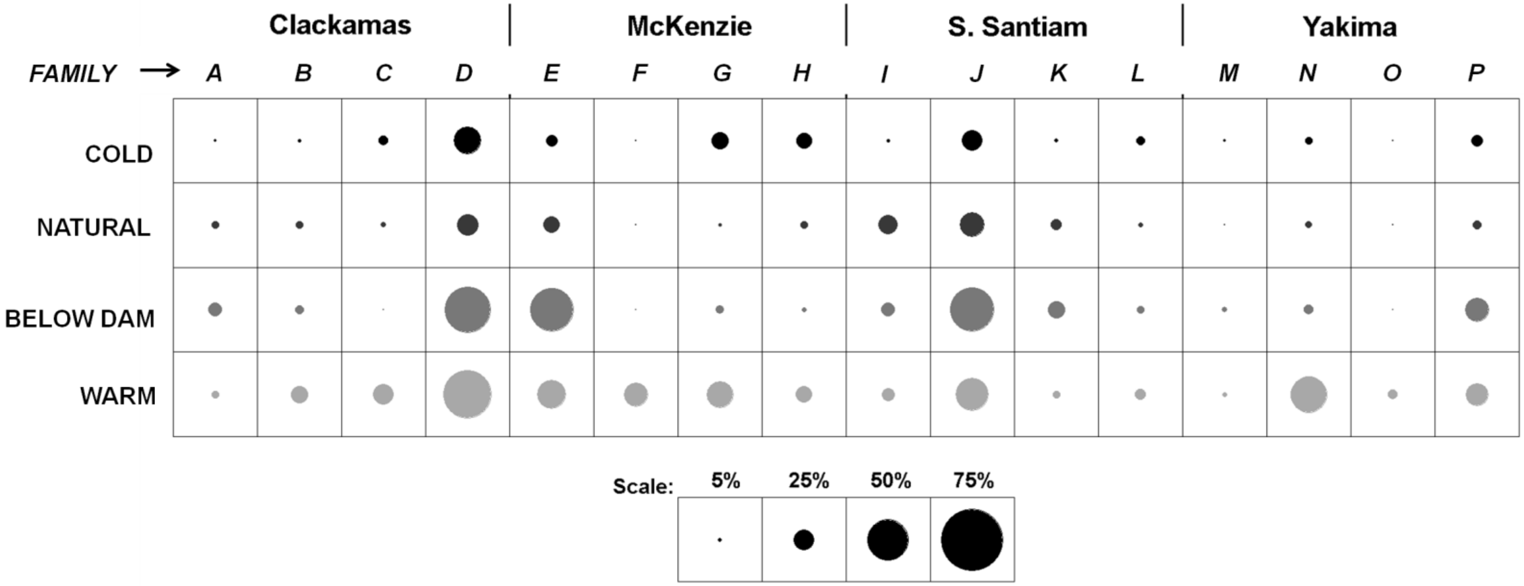


Fig. 7: Visual representation of the proportion of fish that emerged prematurely (DL 0-3) from each family and temperature treatment (averaged across replicates). Each column represents one family, and rows represent the four thermal regime treatments.

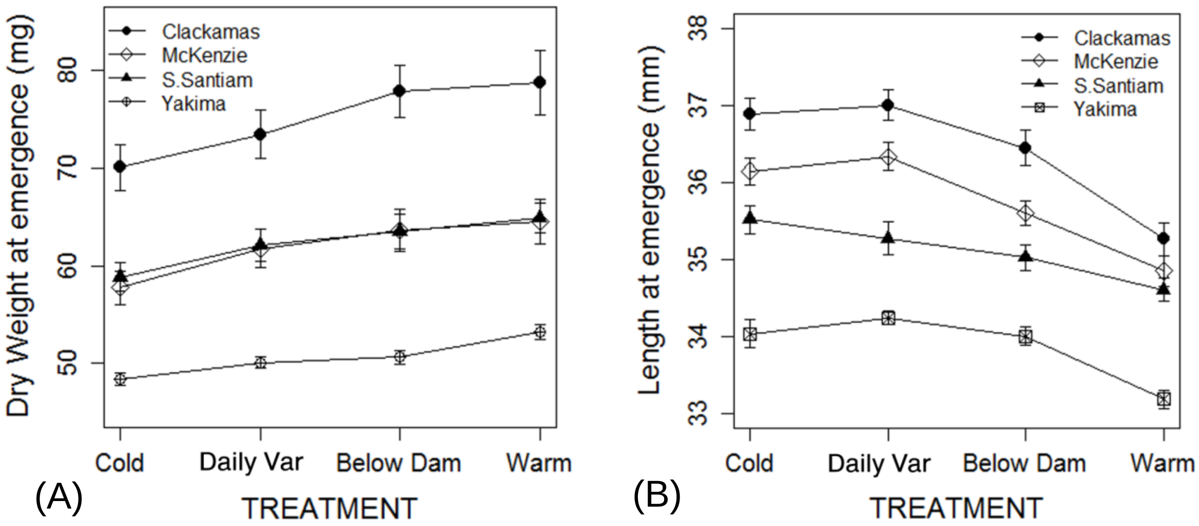
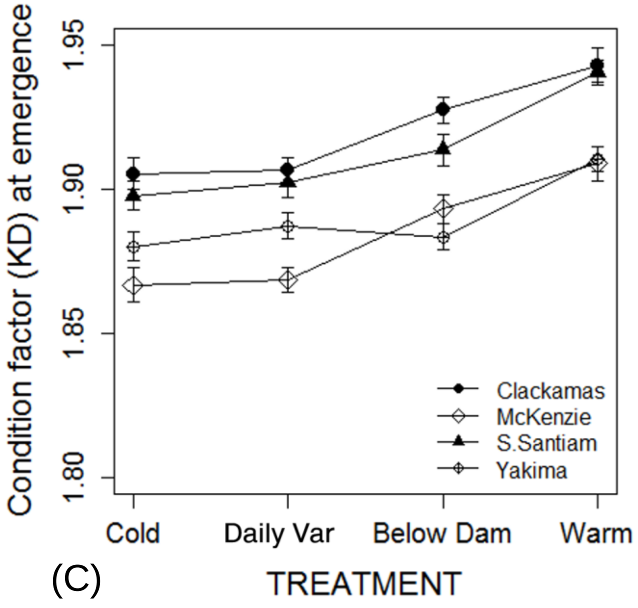


Fig. 8 Reaction norm plots showing estimated population means of the response variables dry weight (A), length (B) and Bam's condition factor (C) across temperature treatments with 95% confidence intervals for fully developed fish (DL 4 and 5)



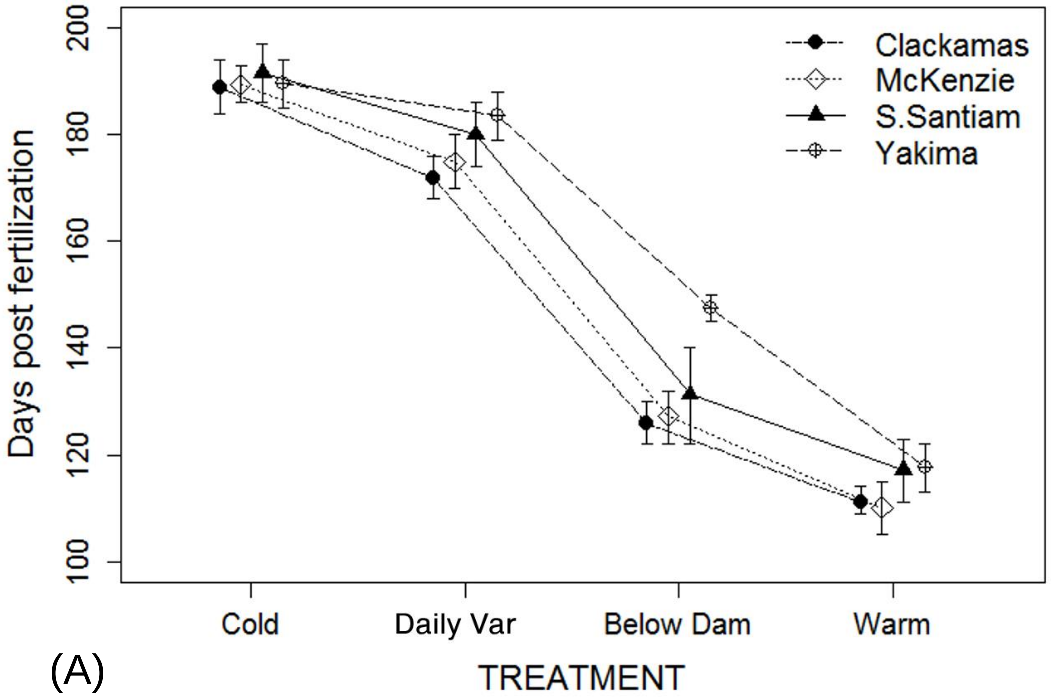
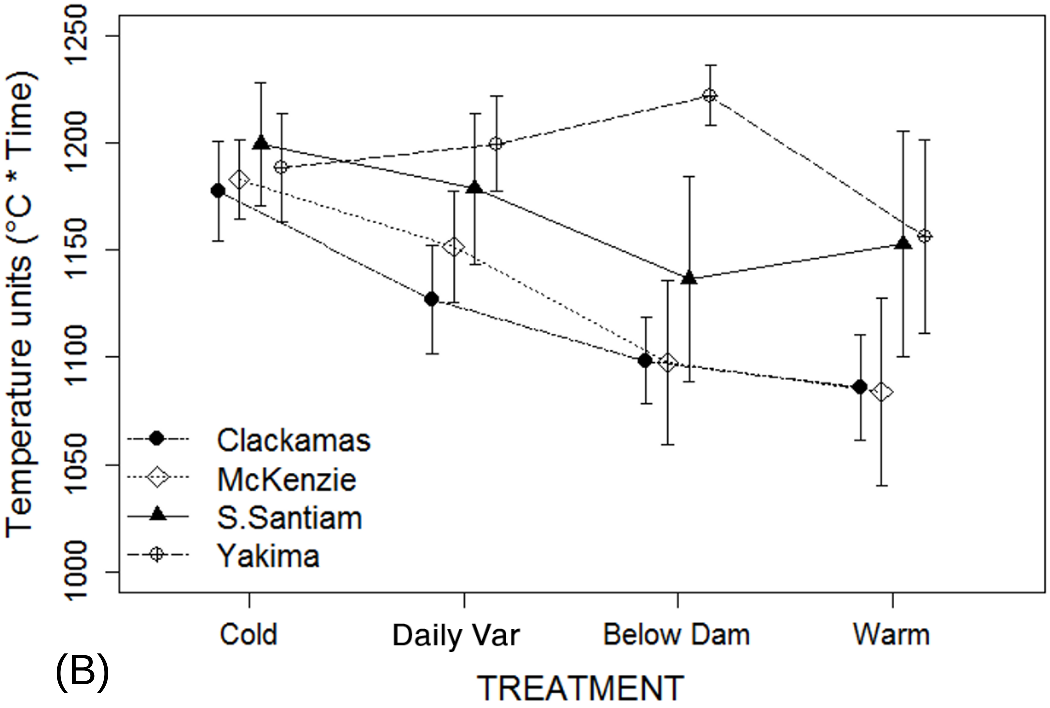


Fig. 9 Mean emergence time for DL 4 and 5 fry (across families) shown in calendar days (A) as well as in temperature units (B).



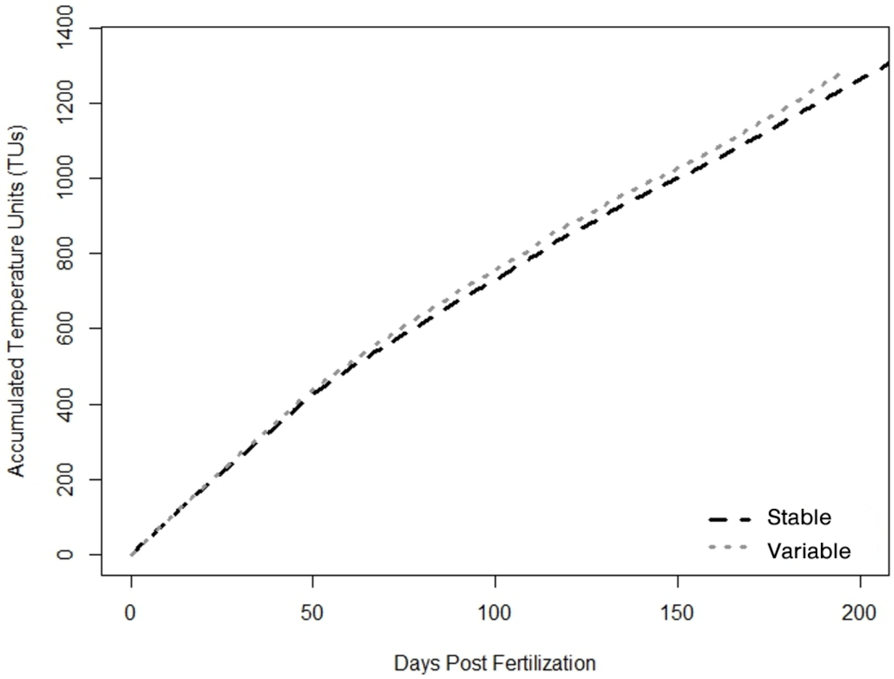


Fig. 10: Accumulation rate of temperature units post fertilization for both treatments

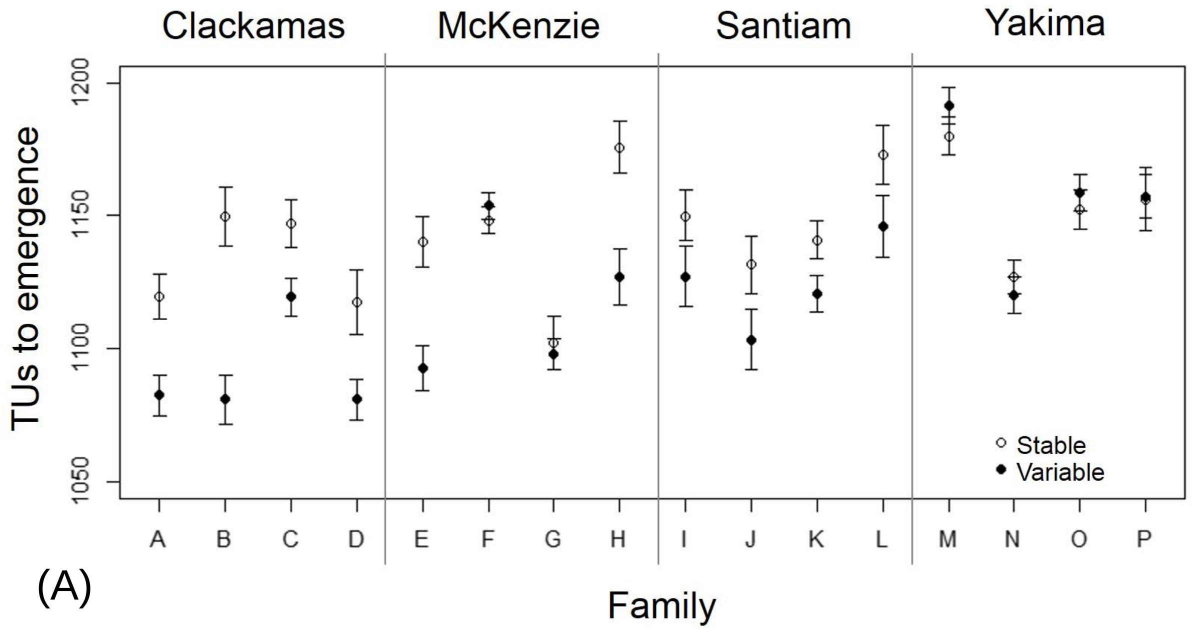
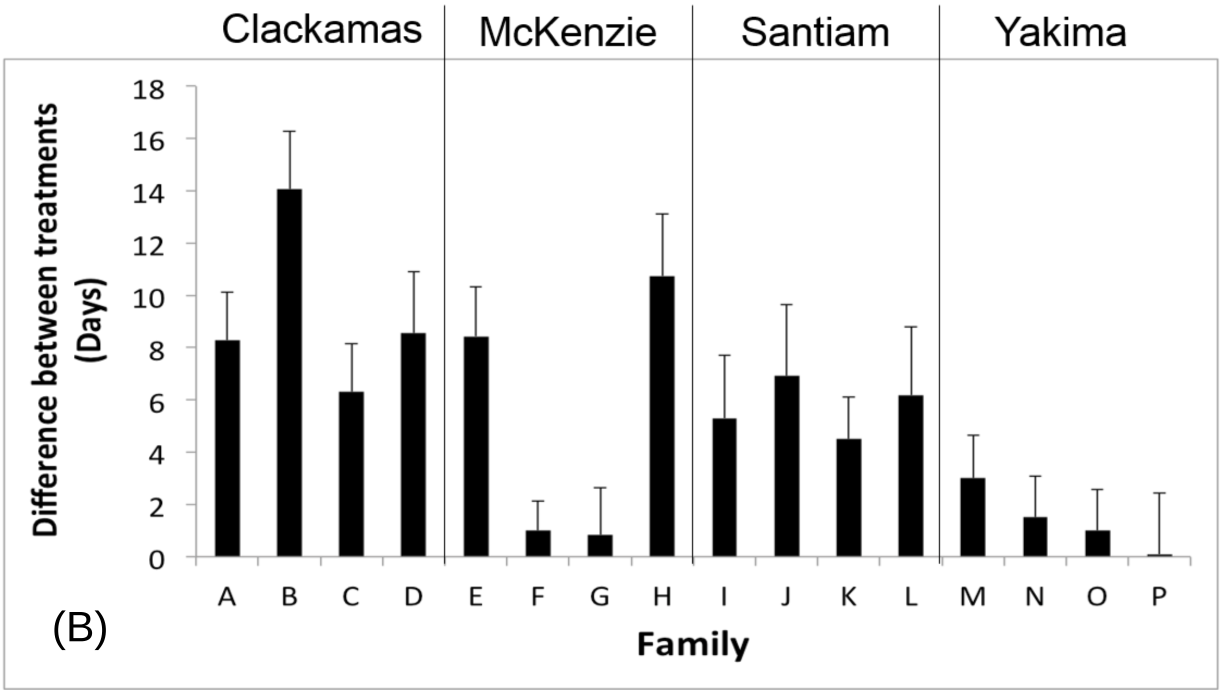


Fig. 11: A) Mean TUs to emergence for buttoned up fish from each family (error bars represent SE) from thermal treatments 1 (stable) and 2 (daily variation). **B)** Average treatment difference in TUs to emergence for each family translated into calendar days (at average temp of 5°C)



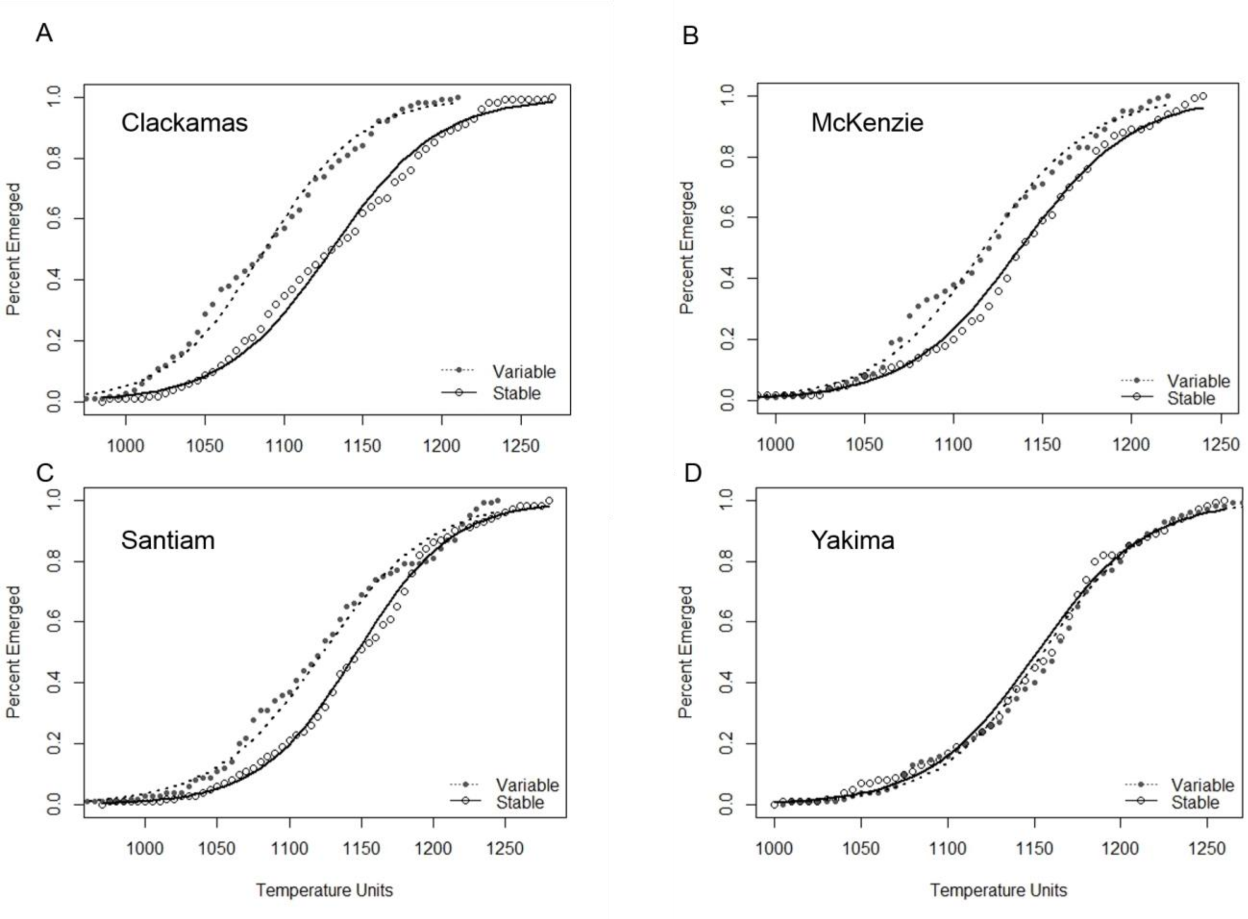


Fig. 12 a-d: Solid and dotted lines represent logistic regression fits for cumulative population emergence rate, separated by treatment. Open and closed circles represent actual data points for percent emergence from stable and variable treatments respectively.

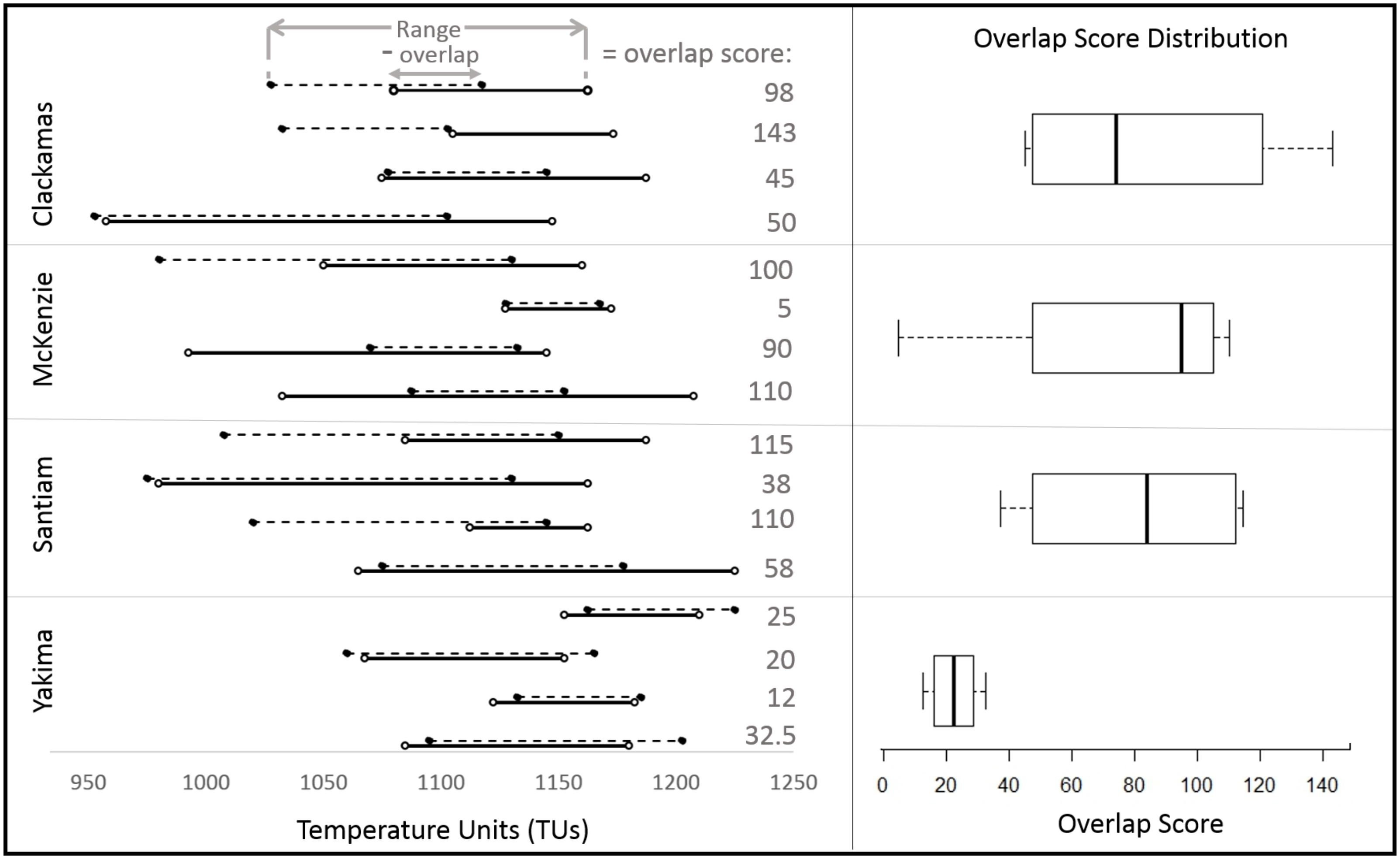


Fig. 13: Emergence period (from 20 to 80 percent emergence) for each family, split by treatments. Dashed line and closed circles represent variable treatment, solid line and open circles represent stable treatment. Mean and variance of overlap scores shown on right side. Overlap scores represent synchronicity of emergence period between treatments (lower overlap score = more synchronous). All family data represents means across replicates.

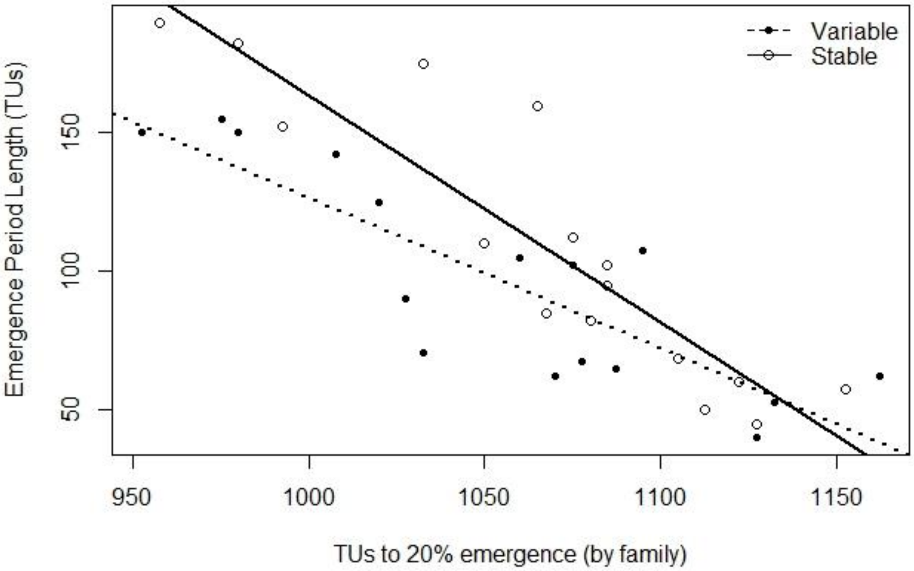


Fig. 14: Emergence period length is negatively correlated with TUs to 20% emergence. In addition, treatment effect was significant (P=0.0187, R^2 Variable=0.82, R^2 Stable=0.72).

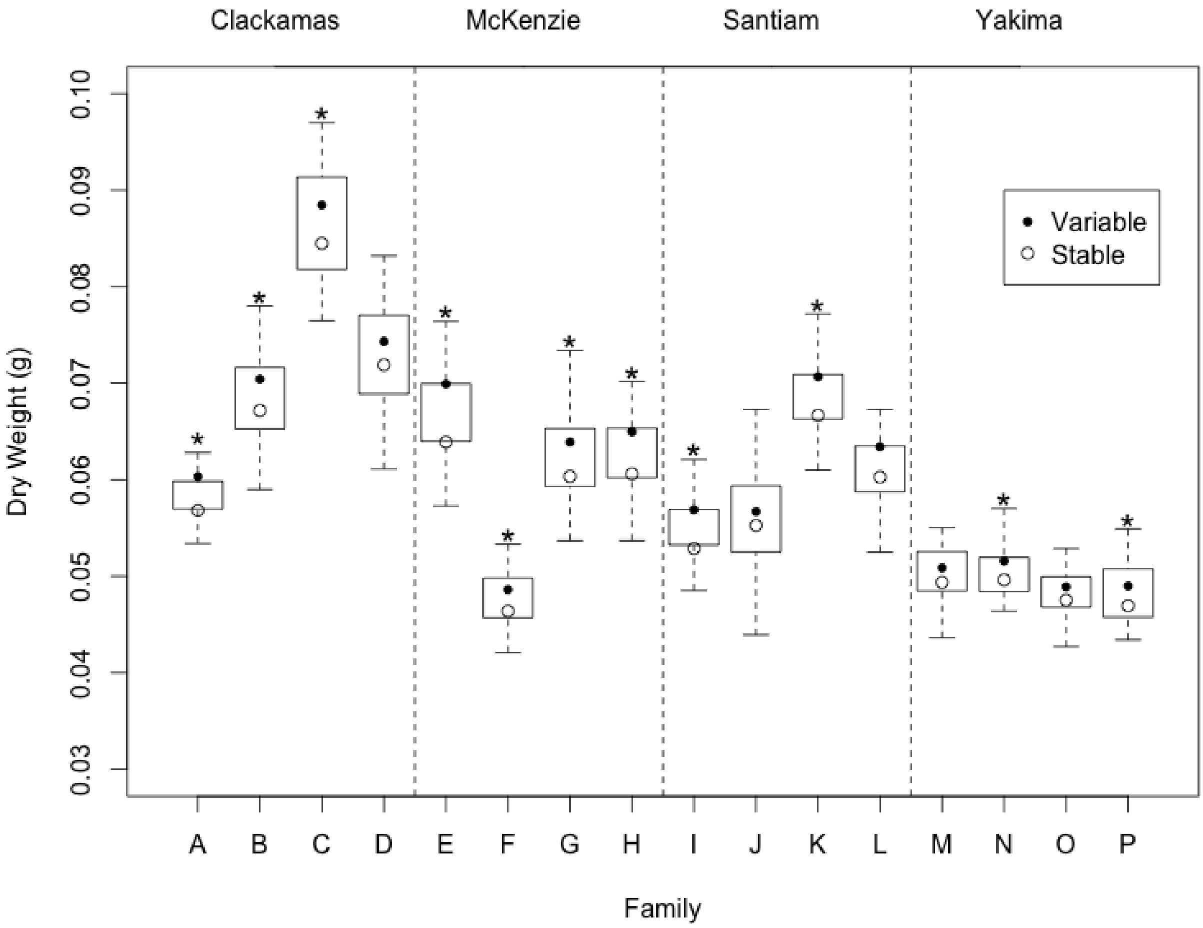


Fig. 15: Boxplot showing distribution of fry dry weights by family, with means by treatment indicated as open and filled circles. * indicates significant difference in means (t-test assuming equal variances) between reatments within families

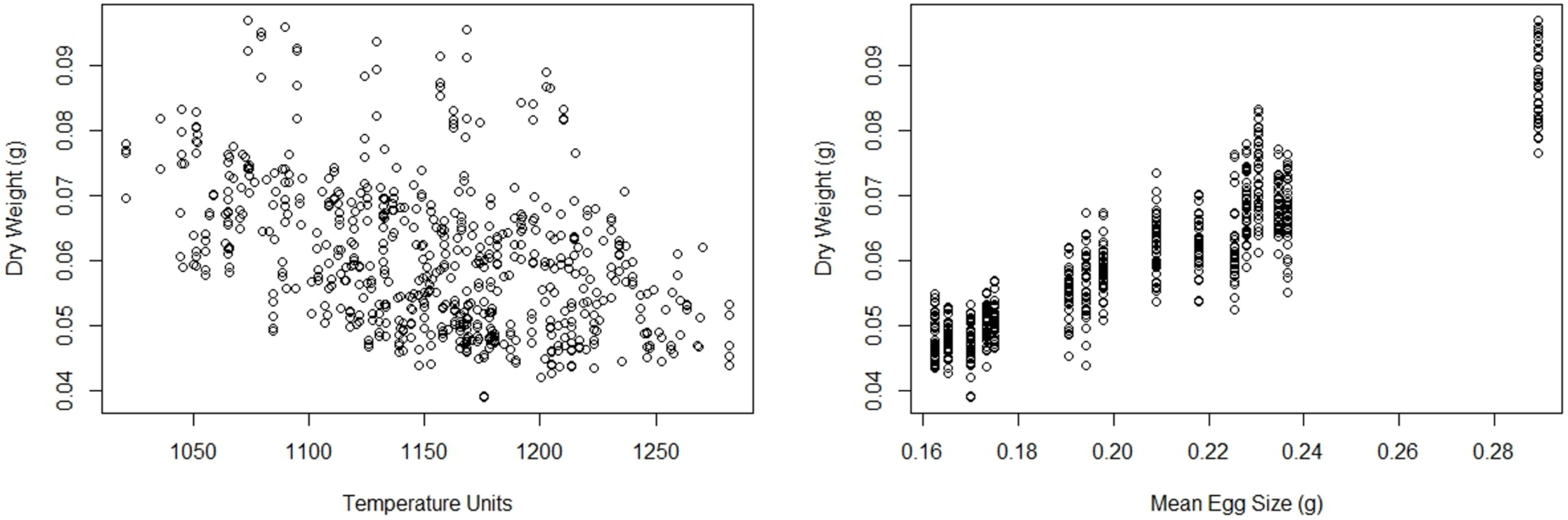


Fig. 16: Relationships between dry weight and TU at emergence (left), and dry weight at emergence and egg size (right).

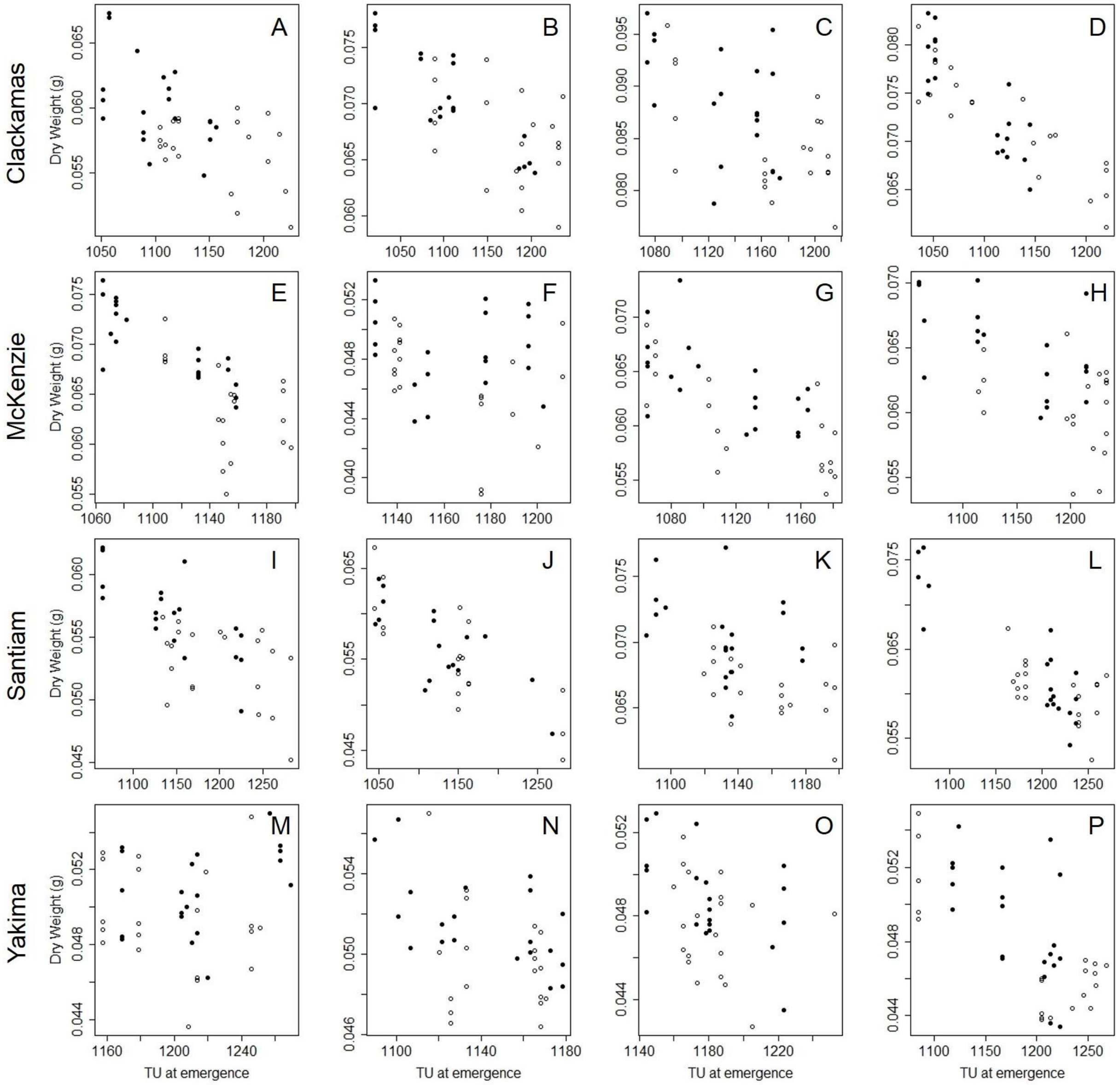


Fig 17: The relationship between dry weight and TU at emergence for each family, separated by treatment (stable ○, variable ●). Families A-P had varying degrees of response to temperature treatments.

Population	Family	Female FL (cm)	Total egg weight (g)	Egg weight mean (g) (n=10)	Fecundity (total # eggs)	% Survival to eyed stage (mean across treatments)
Clackamas	1 **(A)**	75.0	1072.5	0.198	5419	68.1
	2 **(B)**	83.0	1200.7	0.227	5282	81.7
	3	87.0	1377.4	0.311	4430	62.6
	4	72.5	870.0	0.259	3355	35.7
	5 **(C)**	80.5	1162.0	0.289	4018	84.2
	6 **(D)**	82.0	1079.8	0.230	4687	57.4
McKenzie	1 **(E)**	*	*	0.237	*	93.7
	2			0.264		85.3
	3 **(F)**			0.170		86.2
	4			0.249		75.2
	5 **(G)**			0.209		92.0
	6 **(H)**			0.218		85.5
South Santiam	1	84	1178.1	0.248	4750	75.5
	2	77	1087.3	0.241	4521	74.3
	3 **(I)**	76	904.8	0.191	4745	81.3
	4 **(J)**	77	967.7	0.194	4980	76.8
	5 **(K)**	77	1031.7	0.235	4396	80.0
	6 **(L)**	81	951.2	0.225	4222	85.9
Yakima	1	77	922.0	0.197	4675	45.2
	2 **(M)**	79	833.0	0.173	4804	63.5
	3	74	630.0	0.208	3033	74.3
	4 **(N)**	68	575.0	0.175	3283	85.2
	5 **(O)**	74	751.0	0.165	4560	89.2
	6 **(P)**	74	759.0	0.163	4668	83.7

Table 1. Family egg weight, fecundity, and survival to eyed stage. Four out of six families from each population were chosen to monitor for hatch and emergence timing based on % survival to eyed stage (Families A-P). If there was a choice between two families with lower survival, the family with egg size closer to the population mean was chosen (i.e. CK6 vs. CK3 and YK2 vs. YK3). * Individual values for McKenzie females were not available, but overall averages for 2012 broodstock were 78mm (fork length), and 4270 eggs per female (fecundity).

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