AN ABSTRACT OF THE THESIS OF

Julia R. Unrein for the degree of Master of Science in Fisheries Science presented on December 10, 2014.

Title: Early Self-Sorting Behavior in Chinook salmon is Correlated with Variation in Growth, Behavior and Morphology Later in Life.

Abstract approved: _____

Carl B. Schreck

Juvenile Chinook salmon (*Oncorhynchus tshawytscha*) exhibit an array of life history tactics in Oregon's Willamette River Basin, yet we do not know to what extent it is driven by phenotypic plasticity or whether it is predetermined and how conditions in the early rearing environment may affect phenotype expression. We have found hatchery-origin fry sort themselves into distinct surface and bottom oriented phenotypes within days of first feeding and this orientation persists after separation. Surface and bottom phenotypes demonstrated differences in head and body morphology at 2 months post-swim up across three brood years (BY). The surface phenotype exhibits a shorter head and deeper body compared to bottom phenotype. The BY 2012 surface phenotype spent 3 times longer, on average, interacting with their mirror image in an open arena than the bottom phenotype. Tests conducted with BY 2013 fish indicated that bottom-oriented fish engaged in swimming-against-mirror behavior 5 times more than the surface phenotype when the mirror was near gravel refuge. After 8 months of rearing, the BY 2012 surface phenotype was 10% larger than bottom fish and morphometric differences persisted. Surface and bottom phenotypes from BY 2013, were reared under two

temperatures and as either separate or combined phenotype groups. The two phenotypes grew at the same rate at 12°C, irrespective of separate or combined rearing, but at 7°C surface fish were significantly larger than bottom fish after three months until temperatures increased after which the two phenotypes converged. While equal in size, the morphologies of the BY 2013 orientation phenotypes were consistent with previous findings. These differences seen in body shape between the surface and bottom oriented groups are similar to differences exhibited between wild subyearling and yearling life history types in the basin. Such phenotypic differences may offer potential for predicting juvenile life history trajectory early in life.

EARLY SELF-SORTING BEHAVIOR IN CHINOOK SALMON IS CORRELATED
WITH VARIATION IN GROWTH, BEHAVIOR AND MORPHOLOGY
LATER IN LIFE

by
Julia R. Unrein

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APPROVED:

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Dean of the Graduate School

I understand that my thesis will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my thesis to any reader upon request.

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Julia R. Unrein, Author

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CONTRIBUTION OF AUTHORS

Carl Schreck, David Noakes were advisors and editors for the manuscript. Eric Billman contributed training and assistance for the morphometric analysis and manuscript editing. Rob Chitwood initially described the vertical sorting behavior and contributed to development of the experimental design and editing process.

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CHAPTER 1- GENERAL INTRODUCTION

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Phenotype Diversity and Life History Variation

Variation in migration timing can serve as a means of bet hedging for many migratory species, this may ensure that some individuals within a population survive when unfavorable conditions arise. Within a given species, phenotype variation may present itself in many ways including morphology, foraging tactics (McLaughlin et al. 1999), predator avoidance (Coleman and Wilson 1998), exploratory tendency (Armstrong et al. 1997), agonistic response (Taylor and Larkin 1986) and stress response (Koolhaas et al. 1999). Variation among individuals can manifest itself in a broad spectrum but can also exhibit a relatively bimodal structure (Conrad and Sih 2009). Animal personalities or behavioral syndromes are such an example, where human observers classify individuals as either bold or shy, proactive or reactive, dominant or subordinate (Sih et al. 2004). These attributes may be correlated with variation in morphology (Holtby et al. 1993; Wilson and McLaughlin 2010), growth and metabolic rate (Metcalfe et al. 1995; Metcalfe 1998), stress response, dominance and aggression (Pottinger and Carrick 2001; Schjolden et al. 2005).

While some personalities or coping styles may seem maladaptive in one environment they may be beneficial under different conditions (Wolf et al. 2007), for example an active risk-taking individual may benefit under conditions of high competition for limited resources while under conditions with lower competition and higher risk of predation a risk-adverse individual may have a competitive advantage. While behavior is often thought of as highly plastic, these individual tendencies may be established early and maintain themselves throughout an individual's life because of the

energetic costs associated with current maintenance requirements, any energy invested in morphological specialization and with acquiring new information (Dewitt et al. 1998). One of the most intriguing questions then is whether these behavioral differences can ultimately translate into distinct life-history tactics.

Many migratory species exhibit different life history strategies within or among populations, each strategy in turn experiences different risks and reproductive tradeoffs. In some cases individuals either migrate or become resident, termed partial migration (Jonsson and Jonsson 1993). For these populations, migrant and resident types may also express variation in physiological, behavioral and/or morphological traits. Morinville and Rasmussen (2003) found that while migrant brook trout (*Salvelinus fontinalis*) exhibited higher consumption rates than resident individuals, they had lower growth efficiencies and thus higher metabolic costs. The migrant and resident phenotypes of the roach (*Rutilus rutilus*) exhibit differences in boldness, where bolder individuals are more likely to adopt a migrant life history strategy (Chapman et al. 2011). Changes in morphological features such as silvering of the skin and a general streamlining of the body have long been associated with smoltification and outmigration in salmonids (Hoar 1976; Jonsson 1985).

We do not know the extent to which these correlated characteristics are genetically or environmentally based but characteristics such as adult migration timing in Chinook salmon (*Oncorhynchus tshawytscha*) have been shown to be at least partially heritable (Quinn 2005, Unwin and Kinnison 2000; O'Malley et al. 2007). Stress coping styles, are such an example, where proactive (low responding) and reactive (high

responding) lines have been produced through selective breeding in rainbow trout (*Oncorhynchus mykiss)* and Atlantic salmon (*Salmo salar*) (Pottinger and Carrick 1999b).

A study conducted with fall Chinook in the Snake River Basin suggests that the life history trajectory, including determination of migration timing, occurs soon after emergence (Perkins and Jager 2011). Through individual-based modeling, these diverging trajectories could be at least partially explained by small variation in an individual's size and growth opportunity. It is possible that in addition, early behavioral differences such as activity levels and position in the water column could determine which rearing habitats individuals occupy. Juveniles that remain to rear in natal tributaries and those that move down in to mainstem habitats may experience different temperature profiles, flow dynamics and growth opportunities. In addition to variation in emergence timing and/or size at emergence, variation in growth rate, resulting from conditions of the rearing environment, can lead to individuals attaining size thresholds and physiological status for out-migration at different time points during the year (Metcalfe 1998; Beckman et al. 2003).

Wild Chinook in the Upper Willamette Basin

Spring Chinook salmon in the Upper Willamette Basin display considerable variation in length of juvenile freshwater residence and in timing of ocean migration (Reimers 1973; Schroeder et al. 2007). Historically, it was thought that spring Chinook salmon displayed one of two possible juvenile life history tactics: ocean-type, migrating seaward as subyearlings, more common of coastal and southern populations and the

stream-type which migrate out as yearling smolts, and are typically found at higher elevations and Northern latitude systems (Quinn 2005). However, a greater number of life history tactics have been documented in some ecological regions of the Chinook salmon home range (Healey 1991). In the Willamette River Basin seven different life history tactics have been documented and as many downstream migratory phenotypes exist (Waples et al. 2001; Schroeder et al. 2007).

Spring Chinook salmon spawning typically occurs in mid-August and into early October (Healey 1991). The duration of the spawning period along with variations in temperature and discharge can have dramatic effects on incubating embryos. In spawning season, if temperatures remain warm (12°C) into late fall, emergence timing will take place in late winter and the young of the year may utilize yolk sacs before prey items become available (Beer and Anderson 2001; Murray and McPhail 1988). Alternatively, fry emerging later in the spring may be at risk of being washed out of redds or suffocated in high sediment loads during peak flows (Healey 1991). It is likely the variation in spawning, emergence and migration timing ensures a proportion of the population survives seasonal variations and climatic events.

Migratory patterns and survival of spring Chinook salmon are influenced by the environmental and habitat conditions encountered during their passage downstream through hydroelectric projects. In the 2008 Willamette River Basin Biological Opinion, monitoring of dam passage efficiency and survival of the ESA-listed spring Chinook was required as a Reasonable and Prudent Action (RPA) measure by the National Marine

Fisheries Service (NMFS). In the Upper Willamette basin, juvenile out-migration through flood control dam projects occurs during pool draw down in October–February. Typically fish migrate either as subyearling fall migrants or the following spring as yearling smolts, however large numbers of newly emerged fry have been detected migrating out of the upper Willamette River tributaries to rear in mainstem and flood plain habitats as early as January (Schroeder et al. 2007)

These various migratory pathways are more generally described as individuals that migrate out soon after emergence, some of which spend a few months up to a year rearing in mainstem habitats or in the reservoirs and migrate as subyearlings or yearling smolts and a small proportion of precocial males. We predict that the young fry of the subyearling, fall migrants and spring smolts likely exhibit physiological, behavioral or morphological differences which may lead them down these particular life history pathways, differences that may be detectable even in hatchery-origin fish from the same sub-basins.

Rationale for Development of a Wild Surrogate

Evaluation of wild juvenile salmon passage has previously relied on conventionally reared hatchery-origin fish to accommodate the large sample sizes required for passive integrated transponder (PIT) and juvenile salmonid acoustic telemetry (JSAT) tagging studies (Buchanan et al. 2010). However, morphological, physiological and behavioral differences between hatchery and wild fish may confound calculations of dam passage efficiency and survival. This has created a need for

surrogate wild juveniles for use in research concerning dam passage and survival of wild fish. At Oregon State University, we are developing and evaluating alternative rearing strategies used to raise hatchery-origin juveniles to exhibit morphological, physiological and migratory characteristics of naturally-reared juveniles in the Willamette River Basin. Methods include control of incubation and early rearing temperatures, rearing over substrate, and developing diets to modulate growth to more similarly mimic the growth rates and proximate composition of the wild fish rearing in the reservoir and upper tributaries.

One of the most obvious differences between wild and hatchery-origin Chinook is juvenile size and morphology at a given age (Zabel and Williams 2002; Wessel and Smoker 2006). In the Columbia River, hatchery and wild fish could be discerned with 88.9 – 100% accuracy for subyearlings and 90-100% accuracy for yearlings through gross morphological assessment alone; hatchery fish tended to exhibit larger body size, eye size and pupil diameter compared to their wild counterparts (Tiffan and Connor 2011). Internal anatomy also revealed Chinook reared in simplified habitats (i.e. without natural substrates, or structure) demonstrated reduced volumetric measurements of their olfactory bulb and telencephalon, structures important for olfaction and homing as well as spatial cognition and learning (Kihslinger et al. 2006).

Physiologically, hatchery fish tend to have higher lipid content. Increased rates of male precocity have been attributed to altered growth rates in the hatchery environment and higher lipid levels in commercially available feeds (Wood et al. 1957; Rowe et al. 1991; Larsen et al. 2004; Shearer et al. 2006). Because size is often correlated with

survival of out-migrant smolts (Lundqvist et al. 1994) due to increased ability to escape

predation and compete for resources such as foraging and refuge areas, hatchery

production aims to produce fish unimodal in size and large enough to increase chances of

survival after release. However, applying this rearing strategy can drive selection

towards increased aggression (Swain and Riddell 1990), fast growth and increased

incidence of male precocity (Larsen et al. 2006) in the hatchery rearing environment,

potentially exacerbating effects of hatchery and wild fish interactions.

Behavioral differences between natural and conventionally-reared salmonids have

been noted in a number of contexts such as reduced predator avoidance, increased

aggression towards conspecifics and tameness or boldness towards hatchery personnel

associated with hand feeding at the surface (Fritts et al. 2007; Chittenden et al. 2010).

Differences in smoltification and migration timing have also been documented in

hatchery fish, where typically the timing and duration of migratory window was shorter

than wild spring Chinook (Shrimpton et al. 1994; Jonsson et al. 1991; Kostow 2004).

Evidence and Persistence of Vertical Sorting Behavior

We have observed that recently emerged juvenile Chinook salmon exhibit

differences in vertical orientation in rearing tanks. The behavioral difference is marked

by a proportion of fry orienting to the top of the water column and actively feeding at the

surface, while others remain closely associated to the tank bottom, feeding in the mid-

column and near the bottom. This behavior occurs within the first week just after per *os*

feeding begins. Once juveniles are separated their orientation is maintained in new

holding tanks for several months. This self-sorting behavior has now been documented in three brood years, from two Willamette Basin stocks, Marion Forks' North Santiam stock and McKenzie Hatchery stock, and at two different research facilities the Fish Performance and Genetics Laboratory (hereafter, FPGL) and the Oregon Hatchery Research Center (hereafter, OHRC).

Other examples of differences in vertically positioning of juvenile salmonids have been described both in wild and cultured situations. Extensive work has been conducted to determine how vertically positioning of wild juvenile brook trout relates to growth (McLaughlin et al. 1999), body and brain morphology (McLaughlin et al. 1994; Wilson and McLaughlin 2010), agonistic response (McLaughlin et al. 1999), boldness and exploratory behavior (Farwell and McLaughlin 2009; Wilson and McLaughlin 2007) and stress coping strategies (Farwell et al. 2014). Results of their work suggests that this behavioral variation is correlated with variation in spatial use in a novel environment, foraging activity and growth rate potential. Individuals classified as using an active search strategy in the upper portion of the water column were generally more aggressive but displayed bimodal activity levels and higher growth rate potentials than those individuals that adopted a sedentary hovering strategy and occupied lower portion of the water column. These individuals were intermediately active, generally less aggressive and demonstrated lower growth rate potential.

In the captive rearing environment juvenile Atlantic salmon exhibit variation in tank position and feeding strategy. Some fish remain at all times nearest the feeding location, while others are generally associated with the bottom and feed on uneaten food

which settles down to them, still others have an intermediate tactic which involves darting in and obtaining food before returning below the aggressive surface fish (Adams et al. 1998). In addition fin damage is found to be much greater in the fish that compete for positions near food resources (Huntingford and Adams 2005).

Among brook trout (Vincent 1960) and masu salmon (*O. masou*) (Reinhart 2001), hatchery-reared fish exhibited surface orientation in comparison to wild fish which were observed to be more wary and occupied the bottom of the tanks and feed in the mid-column. In both cases this provided surface-oriented fish with greater food resources in a captive environment as evidenced by the larger size of the hatchery fish. Once released into the wild, however, this behavior is likely maladaptive by increasing susceptibility to predation. Vincent's (1960) findings demonstrated that once released into a local pond, the wild brook trout were recaptured more in greater numbers compared to both wild x domestic backcrosse and domestic fish. Based on his recapture data, survival was estimated to be 65% in wild fish, 62% in domestic x wild backcross and only 43% in the domestic groups. Interestingly, of the 105 domestic fish that were recaptured, growth and condition did not differ from those that remained in the hatchery environment but 12 were mature males, while none of the backcrosses or wild fish were found to be precocious.

Growth

In many vertebrate taxa, behavioral differences between individuals are thought to be driven by developmental and metabolic rates (Biro and Stamps 2010). It is difficult,

however, to distinguish whether the behavior or physiology is driving these differences among individuals, as correlations can be detected but direct and indirect causation are challenging to determine. Therefore, several studies have been conducted to determine if standard metabolic rate (SMR) or growth rates can be attributed to bold or aggressive behavior in response to increased energetic demands (Biro and Stamps 2010). Researchers have demonstrated that SMR is positively correlated with aggression levels and territoriality in a number of salmonid species (Cutts et al. 1998; Metcalfe 1998; Sloat et al. 2014).

Growth rate however, may not always show the same trend as metabolism increases when food is limited because decreased residence time in the gut leads to reduced conversion efficiency (Millidine et al. 2009).   At the same time, smaller or less aggressive subordinate individuals may have to spend more energy coping with stress and recovering from injuries received while competing with dominant individuals. Therefore, the best strategy for slower growing individuals may be to reduce movement and aggressive interaction, instead occupy interstitial areas in gravel or near the bottom to save energy (Metcalfe et al. 1986). Under poor growing conditions, the tables may also turn in favor of slower metabolic rates. In laboratory studies conducted with larval radiated shanny (*Ulvaria subbifurcata*), selection against high standard metabolic rates was detected during times of limited food availability (Bochdansky et al. 2005). Stochasticity in the early rearing environment can serve to balance the odds from year to year, further perpetuating the existence of individual variation in these life history traits.

Behavior

Observations of variation in vertical position in the water column have been documented in fish in both wild and cultured systems and in a number of salmonid and trout species. Variation in vertical distribution and foraging tactic, documented in wild brook trout (*Salvelinus fontinalis*), was correlated with differences in spatial use and tendencies for risk-taking behavior in laboratory trials (Farwell and McLaughlin 2009). Those individuals that exhibited an open search foraging tactic were more active and spent longer in an unfamiliar environment with a novel object than those that were more sedentary and adopted a sit-and-wait foraging tactic. In studies conducted with both wild and domesticated brook trout, hatchery individuals exhibited surface orientation, while wild individuals were more wary and remained closely associated with the tank bottom (Vincent 1960). Likewise, studies have identified a behavioral syndrome among cultured Atlantic salmon, where they describe aggressive fish positioning themselves near the surface feeders while others remain near or on the bottom, waiting for uneaten food to settle down to them (Huntingford and Adams 2005). We hypothesized that these vertical orientation behaviors in Chinook salmon may be associated with other characteristics including growth, agonistic and/or risk taking behavior and morphology.

Morphology

Variation in body form and head shape within and among closely related fish species often reflect adaptation to particular habitat characteristics, types of prey and predators as well as competitors they are likely to encounter. As the suites of abiotic and

biotic factors individuals experience become more dissimilar, other aspects of the life-history such as migration timing, and age at reproduction have the potential to change as a result. It follows, then, that comparisons of body shape and skull morphology are commonly utilized when comparing groups of fish thought to exhibit different life history strategies (Webb 1984). Variation in body form can arise from resource polymorphism as in many post-glacial lake fishes and between migrant and resident forms (Skulason and Smith 1995; Jonsson and Jonsson, 1993). In lacustrine habitats, morphological variation is correlated with spatial position in the water column and foraging tactics in several species including arctic char (*Salvelinus alpinus)* (Jonsson and Jonsson 2001), three-spined stickleback (*Gasterosteus aculeatus* L.) (Kristjansson et al. 2002), whitefish (*Coregonus clupeaformis*) (Bodaly 1979) and lake trout (*Salvelinus namaycush*) (Zimmerman et al. 2006).

Differences in body shape are often attributed to the flow conditions an individual experiences in a riverine environment (Langerhans et al. 2003; Keeley et al. 2007). Salmon that typically utilize a sit-and-wait feeding strategy to capture drifting invertebrates tend to exhibit deeper bodies and caudal peduncles that facilitate burst swimming in fast moving waters (Webb 1984a). However, flow conditions may influence body shape differently among species; for example, Atlantic and Chinook salmon reared in faster waters exhibited a deeper, more robust body shape, while brown trout rearing in the same velocity were more slender-bodied (Pakkasmaa and Piironen, 2000). Likewise, brook trout reared at higher velocities were more streamlined and had

deeper caudal peduncles and larger maximal caudal fin heights than those reared at lower velocities (Imre et al. 2002).

Little is known about juvenile Chinook vertical positioning in stream and river habitats, except that an ontogenetic shift occurs: young juveniles adhere to shallow stream margins with reduced velocities to avoid being swept downstream, and as they become more effective at maintaining position they move into deeper and faster-moving waters (Healey 1991). Early behavioral differences such as activity levels and orientation in the water column or flow could determine the types of habitats where these young fish subsequently rear. In the wild, vertical position in the water column may, to some extent, dictate downstream movement of juveniles into lower tributaries or mainstem habitats. Hoar (1953) describes downstream movement of salmon fry to be associated with surface orientation and increased activity at night.

As juveniles disperse downstream, they come to occupy areas with different flow dynamics, temperatures and growth opportunities, so it follows that variation in body shape and/or head morphology may be associated with habitat characteristics and/or foraging tactic. Wild Chinook juveniles collected from tributary and mainstem habitats of the in the Willamette River demonstrate differences in body shape (Billman et al. 2014). These differences in morphology have also shown to be a useful predictor of subyearling and yearling life history tactics in Chinook salmon on the Columbia River (Beeman et al. 1994; Tiffan et al. 2000) and body shape has also been correlated with migratory phenotypes of Chinook in the Willamette Basin (Billman et al. 2014).

Another source of morphological variation among species is the effect of domestication. Morphological differences attributed to the artificial rearing environment have been documented in several salmonid species (Wessel et al. 2006; Tiffan and Connor 2011). For example, smaller heads have been associated with fast growing individuals in a number of fish species including rainbow trout (Martin 1949) and brook trout (Vincent 1960). This decrease in head size, proportional to body size, was correlated with reduced osmoregulatory performance and decreased stamina in swimming performance tests conducted by Vincent (1960) on brook trout. His findings suggested that it was the smaller head size of the faster growing hatchery brook trout that resulted in decreased gill epithelial surface area to body size ratios and thus decreased oxygen intake and stamina. Trade-offs associated with an increased growth rate have also been noted in a strain of fast-growing pumpkinseed sunfish (*Lepomis gibbous*) including fragility of the scales (Arendt et al. 2001) and delayed ossification of cranial structures (Arendt and Wilson 2000). These examples demonstrates the potential for reduced capacity and/or early mortality trade-off that can be associated with higher growth rates (Stamps 2007).

Study Goals and Objectives

The goals of my Master's project were to determine if early vertically self-sorting behavior is correlated with persistent differences in growth, morphology and other behavioral contexts (boldness, exploratory tendency and/or agonistic response) later in life and to determine how aspects of the rearing environment may affect their expression.

The results of this work could inform hatchery personnel and researchers of a method for identifying fish of different phenotypes early in life. Additionally, alternative rearing strategies could be developed to better suit distinct phenotypic groups and insure wild Chinook surrogate release groups exhibit targeted attributes of the wild phenotype such as growth parameters, body condition, body composition, etc. The findings also serve to point direction for future research in whether these early phenotypes can be indicative of juvenile migratory tactics and explain how certain phenotypes may be selected for or against in the changing natural and conventional hatchery environments.

Objective 1: Determine if orientation types demonstrate differences in:

Growth

H_0 Surface and bottom oriented phenotypes will be the same size at the end of the rearing experiments.

H_1 The surface phenotype was generally more active and less wary when feeding therefore, I predict that the surface phenotype will be larger at the end of the rearing experiments.

Behavior

H_0 Surface and bottom phenotypes will demonstrate no differences in boldness, exploratory tendency or agonistic response.

H_1 The surface phenotype exhibited more active and bold (willingness to feed near surface with personnel present) therefore, I predict the surface phenotype will be bolder, more exploratory and/ or more aggressive than bottom fish.

Morphology

H_0 Surface and bottom phenotypes will demonstrate no differences in body or head shape

H_1 Given that the two phenotypes utilize different portions of the water column to feed, I predict that the surface and bottom phenotypes will demonstrate differences in body and/or head shape.

Objective 2: Determine the effect of aspects of the rearing environment:

Feed Delivery Location

H$_0$ Surface and subsurface feeding will demonstrate no differences in growth, behavior or morphology.

H$_1$ I predict that surface fed fish will be larger, more aggressive than subsurface fed fish as surface feeding will encourage surface orientation over time, fish will compete for this portion of the water column to feed.

Rearing Temperature

H$_0$ Rearing temperature will affect growth of surface and bottom phenotypes in similar ways.

H$_1$ I predict that the surface and bottom phenotypes will grow better under different temperature regimes: 7°C and 12°C. At the same ration levels, if surface fish have a faster growth rate than bottom fish, surface fish will be larger at the end of rearing experiment under the 7°C treatment.

Combined Phenotype Rearing

H$_0$ Combined vs. separated phenotype rearing will not affect fish growth or morphology.

H$_1$ If surface fish are able to consume more food given their vertical position nearest to the feeding location, the difference in size and morphology will be magnified between surface and bottom phenotypes when reared together vs. as a separate group.

Firstly, I aimed to determine whether the surface- and bottom-oriented phenotypes demonstrate morphological differences. To do this, I compared body shape from three consecutive brood years (BY 2011, 2012 and 2013) of vertically self-sorted juveniles. In addition, I analyzed additional landmarks on the head of juveniles from BY 2013 to determine specifically how head morphology varied among surface and bottom phenotypes. Once sorted, I assigned groups of surface and bottom phenotypes to various

rearing treatments to determine the effects of food delivery location (BY 2012) at the

FPGL and rearing temperature and separate versus combined phenotype rearing (BY

2013) at the OHRC on the growth, behavior and morphology of these phenotypes later in

juvenile life.

Literature Cited

Adams, C.E., Huntingford, F.A., Turnbull, J.F., and Beattie, C. (1998). Alternative competitive strategies and the cost of food acquisition in juvenile Atlantic salmon (*Salmo salar*). Aquaculture 167: 17–26.

Arendt, J.D., and Wilson, D.S. (2000). Population differences in the onset of cranial ossification in pumpkinseed (*Lepomis gibbosus*), a potential cost of rapid growth. Canadian Journal of Fisheries and Aquatic Sciences 57: 351–356.

Arendt, J., Wilson, D.S., and Stark, E. (2001). Scale strength as a cost of rapid growth in sunfish. Oikos, 93: 95–100.

Armstrong, J.D., Braithwaite, V.A., Huntingford, F.A. (1997). Spatial strategies of wild Atlantic salmon parr: Exploration and settlement in unfamiliar areas. The Journal of Animal Ecology 66: 203–211.

Beckman, B.R., Larsen, D.A., and Dickhoff, W.W. (2003). Life history plasticity in Chinook salmon: relation of size and growth rate to autumnal smolting. Aquaculture 222: 149–165.

Beeman, J.W., Rondorf, D.W., and Tilson, M.E. (1994). Assessing smoltification of juvenile springChinook salmon (*Oncorhynchus tshawytscha*) using changes in body morphology. Canadian Journal of Fisheries and Aquatic Sciences 51: 836–844.

Beer, W.N., and Anderson, J.J. (2001). Effect of spawning day and temperature on salmon emergence: interpretations of a growth model for Methow River chinook. Canadian Journal of Fisheries and Aquatic Sciences 58: 943–949.

Billman, E.J., Whitman, L.D., Schroeder, R.K., Sharpe, C.S., Noakes, D.L.G., and Schreck, C.B., (2014). Body morphology differs in wild juvenile Chinook salmon (*Oncorhynchus tshawytscha)* that express different migratory phenotypes in the Willamette River, Oregon, U.S.A. Journal of Fish Biology 85: 1097–1110.

Bochdansky, A.B., Grønkjær, P., Herra, T.P., and Leggett, W.C. (2005). Experimental evidence for selection against fish larvae with high metabolic rates in a food limited environment. Marine Biology 147:1413–1417.

Bodaly, RA. (1979). Morphological and ecological divergence within the lake whitefish (*Coregonus clupeaformis*) species complex in Yukon Territory. Journal of the Fisheries Research Board of Canada 36: 1214–1222.

Chapman, B.B., Hulthén, K., Blomqvist, D.R., Hansson, L.-A., Nilsson, J.-Å., Brodersen, J., Anders Nilsson, P., Skov, C., and Brönmark, C. (2011). To boldly go: individual differences in boldness influence migratory tendency. Ecology Letters 14: 871–876.

Chittenden, C.M., Biagi, C.A., Davidsen, J.G., Davidsen, A.G., Kondo, H., McKnight, A., Pedersen, O., Raven, P.A., Rikardsen, A.H., Shrimpton, J.M., Zuehlke, B., McKinley, R.S., and Devlin, R.H. (2010). Genetic versus rearing-environment effects on phenotype: hatchery and natural rearing effects on hatchery and wild born coho salmon. PLoS ONE 5: e12261.

Coleman, K., and Wilson, D.S. (1998). Shyness and boldness in pumpkinseed sunfish: individual differences are context specific. Animal Behaviour 56: 927–936.

Conrad, J.L., and Sih, A. (2009). Behavioural type in newly emerged steelhead *Oncorhynchus mykiss* does not predict growth rate in a conventional hatchery rearing environment. Journal of Fish Biology 75: 1410–1426.

Cutts, C.J., Metcalfe, N.B., and Taylor, A.C. (1998). Aggression and growth depression in juvenile Atlantic salmon: the consequences of individual variation in standard metabolic rate. Journal of Fish Biology 52: 1026–1037.

Farwell, M., Fuzzen, M.L.M., Bernier, N.J., and McLaughlin, R.L. (2014). Individual differences in foraging behavior and cortisol levels in recently emerged brook charr (*Salvelinus fontinalis*). Behavioral Ecology and Sociobiology 68: 781–790.

Farwell, M., and McLaughlin, R.L. (2009). Alternative foraging tactics and risk taking in brook charr (*Salvelinus fontinalis*). Behavioral Ecology 20: 913–921.

Fritts, A.L., Scott, J.L., and Pearsons, T.N. (2007). The effects of domestication on the relative vulnerability of hatchery and wild origin spring Chinook salmon (*Oncorhynchus tshawytscha*) to predation. Canadian Journal of Fisheries and Aquatic Sciences 64: 813–818.

Healey, M.C. (1991). Life history of Chinook salmon (*Oncorhynchus tshawytscha*). Pages 313–393 in Groot, C. and Margolis, L., editors. Pacific Salmon Life Histories. UBC Press, Vancouver, British Columbia.

Hoar, W.S. (1953). Control and timing of fish migration. Biological Reviews 28: 437–452.

Hoar, W.S. (1976). Smolt transformation: evolution, behavior, and physiology. Journal of the Fisheries Board of Canada 33: 1233–1252.

Holtby, L.B., Swain, D.P., and Allan, G.M. (1993). Mirror-elicited agonistic behavior and body morphology as predictors of dominance status in juvenile coho salmon (*Oncorhynchus kisutch*). Canadian Journal of Fisheries and Aquatic Sciences 50: 676–684.

Huntingford, F., and Adams, C. (2005). Behavioural syndromes in farmed fish: implications for production and welfare. Behaviour 142: 1213–1228.

Imre, I., McLaughlin, R.L., and Noakes, D.L.G. (2002). Phenotypic plasticity in brook charr: changes in caudal fin induced by water flow. Journal of Fish Biology 61: 1171–1181.

Jonsson, B. (1985). Life history patterns of freshwater resident and sea-run migrant brown trout in Norway. Transactions of the American Fisheries Society 114: 182–194.

Jonsson B and Jonsson, N. (1993). Partial migration: niche shift versus sexual maturation in fishes. Reviews in Fish Biology and Fisheries 3: 348–365.

Jonsson, B., Jonsson, N., Brodtkorb, E., and Ingebrigtsen, P.J. (2001). Life-history traits of brown trout vary with the size of small streams. Functional Ecology 15: 310–317.

Jonsson, B., Jonsson, N., and Hansen, L.P. (1991). Differences in life history and migratory behavior between wild and hatchery-reared Atlantic salmon in nature. Aquaculture 98: 69–78.

Keeley, E.R., Parkinson, E.A., and Taylor, E.B. (2007). The origins of ecotypic variation of rainbow trout: a test of environmental vs. genetically based differences in morphology. Journal of Evolutionary Biology 20: 725–736.

Kihslinger, R.L., Lema, S.C., and Nevitt, G.A. (2006). Environmental rearing conditions produce forebrain differences in wild Chinook salmon *Oncorhynchus tshawytscha*. Comparative Biochemistry and Physiology-Part A: Molecular and Integrative Physiology 145: 145- 151.

Koolhaas, J.M., Korte, S.M., De Boer, S.F., Van Der Vegt, B.J., Van Reenen, C.G., Hopster, H., De Jong, I.C., Ruis, M.A.W., and Blokhuis, H.J. (1999). Coping styles in animals: current status in behavior and stress-physiology. Neuroscience and Biobehavioral Reviews 23: 925–936.

Kristjansson, B.K., Skulason, S., and Noakes, D.L.G. (2002). Morphological segregation of Icelandic threespine stickleback (*Gasterosteus aculeatus* L). Biological Journal of the Linnean Society 76: 247–257.

Langerhans, R.B., Layman, C.A., Mona Shokrollahi, A., and DeWitt, T.J. (2004). Predator driven phenotypic diversification in *Gambusia affinis*. Evolution 58: 2305–2318.

Larsen, D.A., Beckman, B.R., Cooper, K.A., Barrett, D., Johnston, M., Swanson, P., and Dickhoff, W.W. (2004). Assessment of high rates of precocious male maturation in a spring Chinook salmon supplementation hatchery program. Transactions of the American Fisheries Society 133: 98–120.

Larsen, D.A., Beckman, B.R., Strom, C.R., Parkins, P.J., Cooper, K.A., Fast, D.E., and Dickhoff, W.W. (2006). Growth modulation alters the incidence of early male

maturation and physiological development of hatchery-reared spring Chinook salmon: a comparison with wild fish. Transactions of the American Fisheries Society 135: 1017–1032.

Lundqvist, H., McKinnell, S., Fängstam, H., and Berglund, I. (1994). The effect of time, size and sex on recapture rates and yield after river releases of *Salmo salar* smolts. Aquaculture 121: 245–257.

Martin, W.R. (1949). The mechanics of environmental control of body form in fishes. Publications of the Ontario Fisheries Research Laboratory, University of Toronto Studies: Biology Series 70: 1–72.

McLaughlin, R.L., Ferguson, M.M., and Noakes, D.L.G. (1999). Adaptive peaks and alternative foraging tactics in brook charr: Evidence of short-term divergent selection for sitting-and waiting and actively searching. Behavioral Ecology and Sociobiology 45: 386–395.

McLaughlin, R.L., Grant, J.W.A., and Kramer, D.L. (1994). Foraging movements in relation to morphology, water-column use, and diet for recently emerged brook trout (*Salvelinus fontinalis*) in still-water pools. Canadian Journal of Fisheries and Aquatic Sciences 51: 268–279.

Metcalfe, N.B. (1998). The interaction between behavior and physiology in determining life history patterns in Atlantic salmon (*Salmo salar*). Canadian Journal of Fisheries and Aquatic Sciences 55: 93–103.

Metcalfe, N.B., Huntingford, F.A., and Thorpe, J.E. (1986). Seasonal changes in feeding motivation of juvenile Atlantic salmon (*Salmo salar*). Canadian Journal of Zoology 64: 2439–2446.

Metcalfe, N.B., Taylor, A.C., and Thorpe, J.E. (1995). Metabolic rate, social status and life history strategies in Atlantic salmon. Animal Behaviour 49: 431–436.

Millidine, K.J., Armstrong, J.D., and Metcalfe, N.B. (2009). Juvenile salmon with high standard metabolic rates have higher energy costs but can process meals faster. Proceedings of the Royal Society B: Biological Sciences 276: 2103–2108.

Morinville, G.R., and Rasmussen, J.B. (2003). Early juvenile bioenergetic differences between anadromous and resident brook trout (*Salvelinus fontinalis*). Canadian Journal of Fisheries and Aquatic Sciences 60: 401–410.

Murray, C.B., and McPhail, J.D. (1988). Effect of incubation temperature on the development of five species of Pacific salmon (*Oncorhynchus*) embryos and alevins. Canadian Journal of Zoology 66: 266–273.

O'Malley, K.G., Camara, M.D., and Banks, M.A. (2007). Candidate loci reveal genetic differentiation between temporally divergent migratory runs of Chinook salmon (*Oncorhynchus tshawytscha*). Molecular Ecology 16: 4930–4941.

Pakkasmaa, S and Piironen, J. (2000). Water velocity shapes juvenile salmonids. Evolutionary Ecology 14: 721–730.

Perkins, T.A., and Jager, H.I. (2011). Falling behind: delayed growth explains life-history variation in Snake River fall Chinook salmon. Transactions of the American Fisheries Society 140: 959–972.

Pottinger, T.G., and Carrick, T.R. (1999). Modification of the plasma cortisol response to stress in rainbow trout by selective breeding. General and comparative endocrinology 116: 122-132.

Pottinger, T.G., and Carrick, T.R. (2001). Stress responsiveness affects dominant subordinate relationships in rainbow trout. Hormones and Behavior 40: 419–427.

Quinn, T.P. (2005). The Behavior and Ecology of Pacific Salmon and Trout, University of Washington Press, Seattle, Washington.

Quinn, T.P., Unwin, M.J., and Kinnison, M.T. (2000). Evolution of temporal isolation in the wild: genetic divergence in timing of migration and breeding by introduced Chinook salmon populations. Evolution 54: 1372–1385.

Reimers, P.E. (1973). The length of residence of juvenile fall Chinook salmon in Sixes River, Oregon. Research reports of the fish commission of Oregon 4, 1-43.

Rowe, D.K., Thorpe, J.E., and Shanks, A.M. (1991). Role of fat stores in the maturation of male Atlantic salmon (*Salmo salar*) parr. Canadian Journal of Fisheries and Aquatic Sciences 48: 405–413.

Schjolden, J., Stoskhus, A., and Winberg, S. (2005). Does individual variation in stress responses and agonistic behavior reflect divergent stress coping strategies in juvenile rainbow trout? Physiological and Biochemical Zoology 78: 715–723.

Schroeder, R.K., Kenaston, K.R., and McLaughlin, L.K. (2006). Spring Chinook salmon in the Willamette and Sandy Rivers. (Annual Progress Report No. F-163-R 11/12). Oregon Department of Fish and Wildlife Salem, Oregon.

Scott, L. E., and Johnson, J. B. (2010). Does sympatry predict life history and morphological diversifications in the Mexican livebearing fish *Poeciliopsis baenschi*? Biological Journal of the Linnean Society 100: 608-618.

Shearer, K., Parkins, P., Gadberry, B., Beckman, B., and Swanson, P. (2006). Effects of growth rate/body size and a low lipid diet on the incidence of early sexual maturation in juvenile male spring Chinook salmon (*Oncorhynchus tshawytscha*). Aquaculture 252: 545–556.

Shrimpton, M.J., Bernier, N.J., and Iwama, G.K. (1994). Differences in measurements of smolt development between wild and hatchery-reared juvenile coho salmon (*Onchorhynchus kisutch*) before and after saltwater exposure. Canadian Journal of Fisheries and Aquatic Sciences 51: 2170–2178.

Sih, A., Bell, A.M., Johnson, J.C., and Ziemba, R.E. (2004). Behavioral Syndromes: An Integrative Overview. The Quarterly Review of Biology 79: 241–277.

Skulason, S., and Smith, T.B. (1995). Resource polymorphisms in vertebrates. Trends in Ecology and Evolution. 10: 366–370.

Sloat, M.R., Reeves, G.H., and Jonsson, B. (2014). Individual condition, standard metabolic rate, and rearing temperature influence steelhead and rainbow trout (*Oncorhynchus mykiss*) life histories. Canadian Journal of Fisheries and Aquatic Sciences 71: 491–501.

Stamps, J.A. (2007). Growth-mortality tradeoffs and personality traits in animals. Ecology Letters 10: 355–363.

Swain, D.P., and Riddell, B.E. (1990). Variation in agonistic behavior between newly emerged juveniles from hatchery and wild populations of coho salmon, *Oncorhynchus kisutch*. Canadian Journal of Fisheries and Aquatic Sciences 47: 566–571.

Tiffan, K.F., and Connor, W.P. (2011). Distinguishing between natural and hatchery Snake River fall Chinook salmon subyearlings in the field using body morphology. Transactions of the American Fisheries Society 140: 21–30.

Tiffan, K.F., Rondorf, D.W., Garland, R.D., and Verhey, P.A. (2000). Identification of juvenile fall versus spring Chinook salmon migrating through the Lower Snake River based on body morphology. Transactions of the American Fisheries Society 129: 1389–1395.

Vincent, R.E. (1960). Some influences of domestication upon three stocks of brook trout (*Salvelinus fontinalis* Mitchill). Transactions of the American Fisheries Society 89: 35–52.

Waples, R.S. et al. (2001). Characterizing diversity in salmon from the Pacific Northwest. Journal of Fish Biology 59: 1–41.

Webb, PW. (1984). Body form, locomotion, and foraging in aquatic vertebrates. American Zoologist 24: 107-120.

Wessel, M.L., Smoker, W.W., and Joyce, J.E. (2006). Variation of morphology among juvenile Chinook salmon of hatchery, hybrid, and wild origin. Transactions of the American Fisheries Society 135: 333–340.

Wilson, A.D.M., and McLaughlin, R.L. (2007). Behavioural syndromes in brook charr, *Salvelinus fontinalis*: prey-search in the field corresponds with space use in novel laboratory situations. Animal Behaviour 74: 689–698.

Wilson, A.D., and McLaughlin, R.L. (2010). Foraging behaviour and brain morphology in recently emerged brook charr, *Salvelinus fontinalis*. Behavioral Ecology and Sociobiology 64: 1905–1914.

Wolf, M., Van Doorn, G.S., Leimar, O., and Weissing, F.J. (2007). Life-history trade-offs favour the evolution of animal personalities. Nature 447: 581–584.

Wood, E.M., Yasutake, W.T., Woodall, A.N., and Halver, J.E. (1957). The nutrition of salmonid fishes. I. chemical and histological studies of wild and domestic fish. Journal of Nutrition. American Society of Nutrition 61: 465–478.

Zabel, R.W., and Williams, J.G. (2002). Selective mortality in Chinook salmon: what is the role of human disturbance? Ecological Applications 12: 173–183.

Zimmerman, M.S., Krueger, C.C., Eshenroder, R.L. (2006). Phenotypic diversity of lake trout in Great Slave Lake: differences in morphology, buoyancy, and habitat depth. Transactions of the American Fisheries Society 135, 1056–1067.

CHAPTER 2-EARLY SELF-SORTING BEHAVIOR IN CHINOOK SALMON IS CORRELATES WITH VARIATION IN GROWTH, BEHAVIOR AND MORPHOLOGY LATER IN LIFE

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Abstract

Juvenile Chinook salmon (*Oncorhynchus tshawytscha*) exhibit an array of life history tactics in Oregon's Willamette River Basin, yet we do not know to what extent it is driven by phenotypic plasticity or whether it is predetermined and how conditions in the early rearing environment may affect phenotype expression. We have found hatchery-origin fry sort themselves into distinct surface and bottom oriented phenotypes within days of first feeding and this orientation persists after separation.   Surface and bottom phenotypes demonstrated differences in head and body morphology at 2 months post-swim up across three brood years (BY). The surface phenotype exhibits a shorter head and deeper body compared to bottom phenotype. The BY 2012 surface phenotype spent 3 times longer, on average, interacting with their mirror image in an open arena than the bottom phenotype. Tests conducted with BY 2013 fish indicated that bottom-oriented fish engaged in swimming-against-mirror behavior 5 times more than the surface phenotype when the mirror was near gravel refuge. After 8 months of rearing, the BY 2012 surface phenotype was 10% larger than bottom fish and morphometric differences persisted.   Surface and bottom phenotypes from BY 2013, were reared under two temperatures and as either separate or combined phenotype groups. The two phenotypes grew at the same rate at 12°C, irrespective of separate or combined rearing, but at 7°C surface fish were significantly larger than bottom fish after three months until temperatures increased after which the two phenotypes converged. While equal in size, the morphologies of the BY 2013 orientation phenotypes were consistent with previous findings. These differences seen in body shape between the surface and bottom oriented

groups are similar to differences exhibited between wild subyearling and yearling life history types in the basin. Such phenotypic differences may offer potential for predicting juvenile life history trajectory early in life.

Introduction

There exists a complex array of spring Chinook salmon (*Oncorhynchus tshawytscha*) juvenile life history types in the Willamette River Basin, yet we do not yet understand how and when these different life history trajectories take place. Timing of smoltification is greatly influenced by environmental factors such as photoperiod, and temperature regime as well as the physiological status, and growth potential of the individual (Patiño et al. 1986; Metcalfe 1998; Beckman 2002). At the same time, migration timing is also driven, at least in part, by genetics (Quinn 2005, Unwin and Kinnison 2000; O'Malley et al. 2007). This suggests juvenile life history patterns that occur over generations may have a genetic basis, but that conditions in the early rearing environment may affect their expression.

Variation among Chinook has been documented both between and within populations throughout their range (Waples et al. 2001; Quinn 2005). The divergence of ocean and stream-type Chinook are thought to be the first separation between inland and coastal populations considered two races of the Chinook species (Gilbert 1912; Quinn 2005; Healey 1991). More recent work, by Waples *et al.* (2001) and Bottom *et al.* (2005) have described many more juvenile life history patterns among and within populations. The Chinook of the Upper Willamette Basin are genetically distinct from populations in the Lower Columbia and while having a large yearling smolt contribution (Waples et al. 2004), large numbers of fry migrate out of tributaries to rearing in productive floodplain habitats and these subyearling migrants can make up a large percentage of returning adults in some years (Schroeder et al. 2007).

There is a critical need to understand when these juvenile life history tactics are determined and how these phenotypes could be affected by changes to their incubation and rearing habitats. Evidence from fall Chinook in the Snake River Basin, for example, suggest these life history trajectories are made soon after emergence due to relatively small differences in size and growth rate (Perkins and Jager 2011). Behavioral traits such as activity levels, exploratory tendency, dominance and boldness (Rasmussen and Belk 2012; Metcalfe and Thorpe 1992; Chapman et al. 2011) that have been associated with migratory individuals in other species may also be important factors in determining juvenile life history tactics in Chinook salmon.

Taylor and Larkin (1986) found in recently emerged Chinook salmon from the Fraser River system that ocean-type individuals expressed negative rheotaxis and reduced aggression towards conspecifics when compared to their stream-type counterparts. Within the Upper Willamette Basin, however, juveniles express a number of migratory pathways including a fry migrant, summer and fall subyearling migration as well as a yearling spring migration (Schroeder et al. 2007). In the Willamette Basin, some of these different life history trajectories can even be differentiated through body morphology, where mainstem rearing juveniles resemble fall migrants exhibiting relatively shorter heads and deeper bodies compared to those individuals rearing near natal tributaries and spring yearling smolts which are generally more streamlined (Billman et al. 2014). This suggests that differences in behavior, activity levels and downstream movement likely result in individuals rearing in environments with very different flow conditions,

temperatures and growth opportunities. Potentially, the timing for when juvenile life history tactics are determined occurs much earlier than previously thought.

We have observed that within days after *per os* feeding begins, hatchery-origin Chinook salmon exhibit differences in their vertical orientation within the water column. We hypothesize that if Chinook salmon display self-sorting behaviors early on in life, this may also indicate *physiological, morphological and behavioral* differences in other contexts (i.e. growth and metabolic rate, exploration and aggression).

The goal of this project was to identify early indicators of growth, morphological and behavioral differences in Chinook salmon juveniles and determine how aspects of the rearing environment may affect the expression of particular phenotypes. Our hypotheses were that 1) Surface and bottom phenotypes would exhibit differences in growth, body and or head morphology and in various behavioral contexts such as exploratory tendencies, boldness and agonistic behavior and 2) Abiotic and biotic conditions in the rearing environment, i.e. location of food delivery, water temperature and rearing as either a separate or combined phenotype group would affect the expression and/or magnitude of differences among these traits.

Methodology

Embryo Acquisition and Incubation

Eyed embryos were obtained from nine adult pairs of North Santiam spring Chinook stock, spawned at the Oregon Department of Fish and Wildlife (ODFW)

hatchery in Marion Forks, Oregon for brood years 2011, 2012 and 2013. Eyed embryos were transported to Oregon State University's Fish Performance and Genetics Laboratory (hereafter, FPGL) in Corvallis, OR where they were incubated in Heath trays at a constant 6°C with pathogen-free well water. Approximately 1 week prior to first feeding, the emerging fry were ponded (transferred from Heath trays) to circular (1.8 m diameter) rearing tanks supplied with single-pass flow-through well water at 12°C (unless otherwise noted). Onset of feeding followed confirmation that the gut tract of the young fish was formed, a stage referred to as "buttoned up". The fish were then fed a commercially available starter diet (BioVita, BioOregon) 6-8 times a day to satiation.

Vertical Sorting Protocols

Within days after first feeding commenced, differences in vertical orientation were readily apparent and the separation of surface and bottom oriented phenotypes could be performed after introducing feed to the stock tanks. The surface oriented juveniles aggressively fed at the surface while bottom oriented individuals remained closely associated with the tank bottom and fed in this vicinity. At this time, we separated the two layers from each other by carefully dip-netting the surface oriented fish out of the tanks and placing them into 0.9 m diameter tanks with gravel substrate at a density of 100 fish per tank. The bottom-oriented fish were then similarly removed and placed into separate tanks as above. Rearing conditions within brood years were identical between surface and bottom phenotype groups. Flow rates were standardized to approximately 6 L x min^{-1}. Fish were fed at a ration of 2% body weight x day^{-1}.

Growth Experiment

The first growth experiment was conducted in 2013 at the FPGL, where four separate groups of ~ 100 fish, approximated by wet weight, of the surface and bottom phenotypes had been transferred to 8, 0.9 m tanks from their common stock tanks. Each phenotype, therefore, had four replicate tanks these were then split into two feeding location treatments: surface and mid-column. Both the surface and mid-column feeding methods distributed pellets in a random distribution by hand or by injecting feed mixed with water into the mid-column using a large syringe. Fish were fed a commercial starter diet 4- 6 times per day at a ration of 2.0% body weight x day^{-1} and transitioned feeding a fry diet (1.2 mm) at a daily ration of 1.2 % body weight x day^{-1} twice a day, once fish were on average 5.0 grams, as specified by the manufacturer. Monthly subsampling of 20 fish per tank was conducted to monitor growth and condition and to adjust weekly feed rations.

The final size of fish among the phenotypes and feeding treatments was compared in October 2013, 10 months post-emergence and 8 months of rearing under respective treatments. Distributions of fork length, weight, and condition factor among the treatments were first tested for normality using Shapiro-Wilk normality test and for equal variance using a two-sample equal variance test (R core team 2014, package: stats, function: shapiro.test, t.test). If assumptions of normality were violated, a Mann-Whitney test was performed on two-samples or a Kruskal-Wallis Test One Way ANOVA on Ranks (R core team 2014, package: stats, function: wilcox.test, kruskal.test) was used for multiple comparisons followed by post-hoc analysis using R version 3.1.0 package:

multcomp function: mcp for multiple comparisons with Tukey's HSD test (Hothorn, Bretz and Westfall 2008), to determine if there were differences among the replicate rearing tanks. If the replicate tanks were not significantly different, then replicate treatments were averaged and comparisons among surface and bottom phenotypes were made using a Mann-Whitney Test. If the treatments had unequal variance but otherwise were normally distributed, a Welch's t-test was performed which does not require assumption of equal variance. If no differences were attributed to the rearing tanks, or rearing treatments, then a direct comparison of surface vs. bottom phenotype (i.e. pooled feeding treatments) was performed and statistical significance was reported when $p <$ 0.05.

To determine if the different phenotypes would grow differently if they were held together or apart, a second growth experiment was conducted the following year at the Oregon Hatchery Research Center near Alsea, Oregon (Hereafter, OHRC) (See Table 1) for experimental timeline). Phenotype groups were first separated at the FPGL as described previously. Two weeks after separation, the two phenotypes were transported to the OHRC and held in two 0.9 m holding tanks. Fish were given a week recovery from transportation before given a Visual Implant Elastomer (VIE) tag to identify the two phenotypes. Individuals belonging to both surface and bottom phenotypes were marked by injecting elastomer along the anal fin following protocols described by Leblanc and Noakes (2012). After tagging, fish were transferred to fry baskets in their respective separate or combined phenotype groups to monitor for any post-tagging mortality. After 3 days of recovery and observation, groups of 100 fish from surface and bottom

phenotypes were stocked as either a separate group (100 fish same phenotype) or combined in the same tank (50/50 surface/bottom) in duplicated 0.9 m diameter tanks. The rearing treatments consisted of both separate and combined (separated initially and recombined) rearing of the phenotypes to establish differences in growth among the self-sorted phenotypes seen in BY 2012, and determine if rearing the two phenotypes together created an environment where competitive interaction would affect the growth of surface or bottom phenotypes.

In addition the separate and combined phenotype groups were reared at two temperatures, 7°C and 12°C, to determine whether temperature influenced performance of the two orientation phenotypes. Heating and chilling of respective treatments began the day after adding fish to the tanks. Surface water from Fall Creek was delivered through a surface inflow and drained through the bottom via a center standpipe; flow rates in the tanks were approximately 6 L x min^{-1}. Rations and diets followed methodology used in the previous year's experiment, with the exception that fish were fed only at the surface.

Fish 12°C were reared until the end of June, (4 months of rearing) at which time final length and weight were recorded. Also at this time, the 7°C treatments were transitioned to ambient OHRC water conditions (10°C) because of chiller constraints. Length, weight and digital images were obtained from a subset of 50 individuals (25/replicate tank) from each of the 7°C separate and combined rearing treatments in August to match closely the size of the fish from the 12°C treatments for morphometric comparisons. Body condition factor was calculated for each individual using Fulton's K

formula: weight/length^3 x 10^5 (Fulton 1904). Size and condition comparisons were analyzed using statistical methodology described above.

Behavioral Assessments

Behavioral trials were conducted on the BY 2012 vertically-self sorted (surface and bottom oriented) phenotypes and feeding treatments (fed at the surface vs. mid-column) after groups had undergone 6 months of rearing under their respective treatments at the FPGL. For assessing exploratory tendencies and boldness among individuals we used an approach similar to that of Brown et al. (2007), Wilson and McLaughlin (2007), and Chapman et al. (2011). In addition, we used mirror image stimulation to assess agonistic response, an approach used to characterize aggressiveness in several juvenile salmonids including steelhead (*O. mykiss*) (Berejikian et al. 1996), coho (*O. kisutch*) (Holtby et al. 1993) and Chinook salmon (Taylor and Larkin 1986).

Forty fish were PIT tagged from each rearing group representing 20 individuals per replicate tank, so behaviors could be linked back to their rearing treatment as well as provide ability to track growth and record the individual's sex. No mortality was experiences in any of the tagged groups and fish resumed feeding within 24 hours after the tagging procedure. All fish underwent behavioral assessments after at least a 48-hour in two 0.9 m holding tanks for recovery from tagging.

For each trial, one fish was netted from one of the two holding tanks and placed into a labeled container with water and scanned for its PIT tag code. Each fish was transferred from its container to one of the two replicate behavior tanks. Each flow

through tank was equipped with a partition separating a gravel refuge area from the open arena and mirror. Water temperature ranged from 12.9 – 13.3 °C during the trials. Flows were 2 L x min-[1] for each of the two tanks and water was delivered through a bottom spray bar that extended in each direction along the corners of the refuge area, that provided multi-directional flow.

To assess boldness, exploratory tendency and agonistic behavior each individual was given a 15 minute settling period in the refuge area, after which, the partition was removed and the fish were allowed to volitionally explore a barren, environment that was delineated in 4, 30.5 cm marked sections. Across the tank at the opposite end of the refuge area, a mirror was suspended to provide mirror image stimulation for assessing agonistic response. Trials were recorded for 30 minutes with a digital video camera for later analysis. The behavioral metric used to assess boldness was latency to emerge from the refuge. Exploratory tendencies were assessed by counting the number of marked grid sections that were crossed (longitudinal movement). Agonistic behavior was assessed by recording both the number of approaches to the mirror and the total time individual fish interacted with its mirror image. Videos were analyzed in such a way that the phenotype, rearing tank and the treatment it belonged to was not known until after quantification of the metrics assessed.

We modeled the effect of orientation phenotype for both the number of mirror encounters (separate periods of contact with the mirror) as well as the total time an individual spent interacting with its mirror image to quantify agonistic response. The number of mirror approaches an individual performed was only compared among

individuals that emerged from the refuge (n = 129 from a total of 150 trials, bottom = 64, surface = 65) and analyzed using a mixed model negative binomial regression with a log link function. Negative binomial regression was performed as count data was over dispersed, i.e. the variance was larger than the mean. Analyses were conducted using R version 3.1.0 package: MASS using function: glm.nb for binomial regression (Venables and Ripley 2002). For the time spent interacting with their mirror image we used a generalized linear model (GLM) with a gamma distribution to model the continuous variable of time analyzed using the glmmadmb function in R 3.1.0 package: glmmADMB (Fournier et al. 2012; Skaug et al. 2014). This analysis was performed only for the fish that approached the mirror (i.e. > 0 seconds with the mirror) (56 out of a total of total 150 trials, bottom n = 26, surface = 30) because gamma models allow for only a positive continuous data structure.

Initially, we proposed hypotheses for which explanatory variables would likely be driving the variation in behaviors, we then explored how these models of interest compared to other parameter combinations using the all subset selection method with the R version 2.9 package: leaps, function: regsubsets. Three to four models with the lowest AIC (Akaike Information Criterion) and BIC (Bayesian Information Criterion) values were then used to compare the best fit. I compared hierarchical models using both Likelihood-ratio tests and the second order Akaike Information Criterion (AICc) values to select the final model. Second order AIC values were used as my sample size was relatively small (i.e, n < 10 times the number of parameters) (Burnham and Anderson 2002).

Since we used the full data set to select the models of interest, a cross validation procedure was performed to account for potential bias. Cross validation was accomplished using R package: boot, function: glm.cv, this procedure uses a single observation from the entire dataset in each model and is tested against the remaining dataset to determine which of the models predicts the best fit (Davidson and Hinkley 1997; Canty and Ripley 2014). Possible parameters in the global model included main effects of phenotype, feeding treatment, fish size, behavioral tank, trial date and the order in which each trial was run. In addition a random effect of rearing tank was incorporated in the GLM. The final model used to compare the number of bouts with the mirror is shown below.

$$Y_{ijk} = \beta_i + \beta_j + b_k + \varepsilon_{ik} + \varepsilon_{jk}$$

Where the response variable Y_{ijk} is the count of mirror approaches by the feeding treatment (surface or subsurface) (i), in the behavioral tank (j), from rearing tank (k) as a random effect term. The last terms ε_{ik} and ε_{jk} are random variables representing the deviation in mirror approaches by the individual's rearing tank to the effect of the individual's feeding treatment and the behavioral tank an individual's trial took place in. Similarly, the final model used compare time spent interacting with the mirror is shown below.

$$Y_{ijk} = \beta_i + \beta_j + b_k + \varepsilon_{ik} + \varepsilon_{jk}$$

Where the response variable Y_{ijk} is the time spent interacting with the mirror by phenotype (i) in behavior tank (j) and rearing tank (k) as a random effect term. The last

terms εik and εjk are random variables representing the deviation in time spent interacting with the mirror by the individual's rearing tank to the effect of phenotype and behavioral tank, respectively (Table 5).

After four months of rearing at the OHRC, 24 individuals from the surface and bottom (12 from each replicate tank, from the separately reared phenotype groups only) were each given a mirror image stimulation test. We did not assess boldness or exploratory tendency in this test, but simply the total time spent actively swimming or holding in the water column as a measure of activity level and the number of distinct swimming against mirror (SAM) behaviors performed during the 15-minute trial. The SAM metric has been used to measure individual agonistic response in many fish species from zebrafish (*Danio rerio*) (Blaser and Gerlai 2006) to coho salmon (Rosenau and McPhail 1987; Holtby et al. 1993) and between ocean and stream-type Chinook populations (Taylor and Larkin 1986).

Trials were conducted in 4 replicate 38.1cm x 17.8 cm x 30.5 cm arenas filled to 20.3 cm depth. A partition was mounted to a pulley system to reveal a mirror behind it. Individual fish were netted at random from their stock tanks placed into a smaller container with water and randomly assigned to one of the four arenas. Fish were given a 10-minute settling period before the partitions were lifted remotely behind a blind. An individual's behavior was then recorded with a digital video camera for 15 minutes. At the end of each trial, fish were removed, weighed, measured and held in temporary holding tanks until the end of the day to prevent reusing any individuals. Partial water changes were performed between trials to ensure water temperatures were within 1°C

during the 12 trials conducted between the time of 0800 and 1700 hours. Videos were

analyzed in such a way that the phenotype and the rearing tank it came from was not

known until after quantification of the metrics assessed.

Statistical comparisons were done using generalized linear mixed models. For

determining the effect of phenotype on activity levels we used a Generalized Linear

Model with a Gamma distribution as the data exhibited a positive continuous over-

dispersed distribution (Table 6a). For the number of SAM behaviors performed by the

surface and bottom phenotypes, we used Negative Binomial Linear Regression (Table

6b). Model selection and cross validation procedures were conducted in a manner

described in the first year's behavioral experiment.

Morphology

When fish averaged 55 mm, approximately 2 months post-emergence, a

subsample of fish (see Table 2 for details) from each group were anesthetized with

Tricaine Methanesulfonate (hereafter, MS-222, buffered to pH 7.0 with Sodium

Bicarbonate) for all three brood years. The fork lengths (± 1mm) were measured and a

lateral image of the left side of each fish was obtained.   Fish size was compared between

the two phenotypes using a two-sample *t*-test, after confirming normality and equal

variance. Body morphology between the surface and bottom phenotypes was compared

using landmark-based geometric morphometric analysis. Quantification of body shape

was conducted using fifteen landmarks modified from Beeman *et al.* (1994) and Tiffan

and Connor (2011) and digitized using tpsDig (Rohlf, 2010a) (Figure 2a). Variation in

shape from digital landmarks was summarized into relative warps using tpsRelw (Rohlf, 2010b). This procedure accounts for variation in position, orientation, and scale of the specimen in each image, and then uses affine and non-affine shape components from aligned specimens in a principal components analysis to calculate relative warp scores (Rohlf and Slice 1990). The first fifteen relative warps, which combined, explained more than 95% of the shape variation, were used as shape variables in subsequent analyses.

To better elucidate differences in head shape and jaw length and position digital imaged were obtained from formalin fixed specimens using a laboratory imaging studio to include landmarks placed on the head (BY 2013 only). Fish were euthanized with an overdose of MS-222 and fixed in 10% formalin buffered to pH 7.0 for 10 days before rinsing and preserving in 70% ETOH. Ten landmarks were used for the analysis of head morphology; these included five homologous landmarks and five semi-landmarks to define head shape as well as eye size and positioning (Figure 2b). From these landmarks relative warps were generated using tps Relw (Rolf 2010b) and the first 8 relative warps which explained 95% of the variation were used for subsequent statistical analyses.

Mixed model multivariate analysis of variance (MANOVA) on repeated measures in SAS, version 9.2 (proc MIXED; SAS Institute, 2008) was conducted to test for shape differences between all three brood years. Response variables were the relative warp scores; centroid size (i.e. the square root of summed squared distances from each landmark to the center of each configuration) was a covariate in the MANOVA. The analysis of head morphology (BY 2013 only) was conducted separately using a similar

analysis. In both head and body shape comparisons phenotype (levels = surface and bottom) was the main effect.

Relative warps are orthogonal and ordered according to the amount of variation they explain; therefore, they can be treated as repeated measures on the same individual with the use of an index variable used in repeated statement for mixed model analyses. The index variable reflects the order, not the value, of relative warps. We included the index variable as a fixed categorical effect in the model along with its interactions with phenotype (Scott and Johnson, 2010; Wesner, Billman, Meier and Belk 2011; Hassell, Meyers, Billman, Rasmussen and Belk 2012). Because relative warps are orthogonal axes of shape variation that account for decreasing amounts of the total variation, the magnitude and direction of differences between levels of main effect on one relative warp have no influence on differences between levels on other relative warps. Therefore, interaction between the index variable and the main effect tests for variation in shape on each relative warp independently and thus the interaction can be significant even if the main effect alone is not.

For visualization of the body shape and head shape differences between surface and bottom phenotypes, a canonical axis was derived from the main effect (phenotype) of the MANOVA. Correlations between superimposed landmark coordinates and the main effect were generated using proc CANCOR (SAS Institute, 2008). Thin-plate spline transformations were then generated in tpsRegr (Rohlf, 2003) using the canonical scores as the independent variable and superimposed landmark coordinates as the response variable (Langerhans et al., 2004; Hassell et al., 2012).

Body morphology comparisons were conducted again after the phenotypes had been rearing for 8 months under their respective feeding treatments in 2013. Digital images of the left lateral side were obtained from a subsample of twenty anesthetized fish from each group. In addition to comparing the effects of phenotype and feeding location on body shape, we sought to determine if the differences seen at the 2-month post-emergence stage persisted with fish age, to do this we used the landmark based geomorphic analysis as described above.

Body shape comparisons were made for the second year's growth experiment using separately reared phenotype groups from the 12°C treatments in June (4 months rearing) and in August (6 months rearing) for the 7°C treatment in an attempt to size match the groups. In addition, the effect of separate vs. combined phenotype rearing was compared for the 7°C treatment. Digital images were obtained from 50 fish (25/replicate tank) of each phenotype. Shape analysis followed digital landmarking and statistical methods described for the previous year's experiment.

Results

Growth

We found no evidence that the feed delivery location had any effect on final fish size at age for either phenotype (ANOVA; $F_{2,147} = 11.42$, treatment: $p = 0.571$); therefore, feeding treatments were combined for a two-sample t-test to compare surface and bottom phenotype groups (Figure 1a). Surface fish were significantly larger than bottom fish at the end of the rearing experiment (Welch's t-test; $t_{134} = -4.707$; $p < 0.0001$). Surface fish

were on average 119.7 mm (SE ± 1.0, n = 72) and bottom fish were 108.9 mm (± 1.4

mm, n = 78) in October sampling period. Condition factor did not vary significantly

among surface and bottom phenotypes (Mann-Whitney Rank Sum; $W = 3035$, $p = 0.394$),

which were 1.15 (± 0.01) and 1.17 (± 0.01), respectively.

Average size at age (Figure 1b) exhibited by the surface and bottom phenotypes

during their first four months of rearing at 12°C at the OHRC, either separately or

combined, did not differ (Mann-Whitney test; $W = 1114$; $p = 0.350$) or weight (Mann-

Whitney test; $W = 1244.5$; $p = 0.973$). Mean fork lengths of surface and bottom fish on

were 86.9 mm (± 1.28) and 85.0 mm (± 1.13), respectively. Mean weights were 7.017 g

(± 0.264) and 6.970 (± 0.323) for surface and bottom phenotypes respectively. Condition

factor, however, was significantly greater in the bottom phenotype group (Mann-Whitney

test; $W= 1829$; $p < 0.0001$). Condition factor averaged 1.04 (± 0.010) for surface fish

and 1.09 (± 0.008) for bottom fish.

Growth trajectories among surface and bottom phenotypes at 7°C differed from

the 12°C (Figure 1c). The mean fork length (± SE) of the two phenotypes did not differ

significantly after the first month of rearing but began to diverge after 3 months and

surface fish were significantly larger after 4 months, than the bottom phenotype,

regardless of rearing separately or with the bottom fish in combined phenotype group

(ANOVA; $F_{6,113} = 6.35$, phenotype: $p < 0.001$; treatment: $p = 0.328$). Surface fish were

estimated to be on average 7.2 mm (± 1.94) larger than the bottom phenotype. After four

months of rearing, the 7°C treatments had been transferred to ambient water conditions at

the OHRC (10°C) and a month later the size differences remained but were beginning to

contract (ANOVA; $F_{6,113}$ = 4.49; phenotype: p = 0.006; treatment: p = 0.544).   There was

no significant difference in size between the two phenotypes at the final sampling period,

after six months of rearing (ANOVA; $F_{3,196}$ = 2.56; p = 0.827).   Surface and bottom

phenotypes mean fork lengths were 86.2 mm ($\pm$ 0.80) and 85.5 mm ($\pm$ 0.77). I did detect

a significant, positive, effect of combined rearing on fish size in the 7°C (ANOVA, $F_{3,196}$

= 2.56, p = 0.015). Upon further analysis, it was the bottom phenotypes that differed

significantly in size between the combined and separately reared treatments (t-test, t_{98} =

2.52, p = 0.012). Mean fork lengths of bottom phenotype reared in combined and

separate groups were 87.3 mm ($\pm$ 1.09) and 84.4 mm ($\pm$ 1.04), respectively. Surface fish,

however, reared together with bottom fish were not significantly different in size from

those reared as a separate phenotype group, averaging 87.1 mm ($\pm$ 1.08) and 85.3 mm ($\pm$

1.18), respectively (t-test, t_{98} = 1.12, p = 0.267).

Behavioral Assessments

While the feeding location treatment did not demonstrate effects on growth or

morphology, fish fed at the surface had about twice as many mirror encounters as those

fed in the mid-column (Negative Binomial Regression, p = 0.0219, n = 138) (Figure 2a).

We found evidence from AICc weight comparisons that phenotype was an important

parameter to include in our final model. (Gamma Regression, n = 56, see Table 2 for

AICc weight comparisons). The effect of surface phenotype on time spent with the

mirror was estimated to be 3.5 times (95% CI [1.5, 8.3] times) greater than the bottom

phenotype holding the effect of the behavioral tank constant. On average, surface fish

spent 166 seconds (SE ± 50.5) while bottom fish spent 57 seconds (± 28.6) (Figure 2b).

The standard deviation associated with the random effect of rearing tank on the time an

individual spent interacting with the mirror was ± 1.0 seconds. We also found evidence

for an additive effect of the behavioral tank the fish's trial took place in (Table 2). Fish

of both phenotypes run in the second behavioral tank spent an estimated 4.5 times (95%

CI [1.9, 10.6] times) longer interacting with the mirror compared to the first behavioral

tank. This might be explained by the somewhat darker color of tank sides and slight

differences in ambient light levels between the two tanks. Potentially, this could affect

how individuals perceived risk of exploring the open arena, in the darker tank the contrast

between the fish and its white background may have been less extreme than in the lighter

colored tank. We did not detect a difference in the latency to emerge from the refuge

between the two phenotypes (Analysis of Deviance for Gamma models, $p = 0.9812$) or in

the number of grid section crossings performed (Log Likelihood Ratio test of Binomial

models, $p = 0.3495$). Feeding location also did not have a significant effect on latency to

emerge from the refuge (Analysis of Deviance for Gamma models, $p = 0.8875$ or the

number of compartment crossing performed (Log Likelihood Ratio test of Binomial

models, $p = 0.6726$).

BY 2013 surface fish were significantly larger, on average than bottom fish at the

time of the OHRC behavioral trials, (Mann-Whitney test; $W= 177.5$, $p = 0.023$, n = 48).

Mean fork length of surface fish was 76.2 mm (SE ± 0.92) and bottom fish were 72.0 mm

(± 1.27 mm). The time the two phenotypes spent actively swimming in their behavioral

test tank, however was not significantly different (Analysis of Deviance of Gamma

models comparing null to alternative model with phenotype as a fixed effect, $p = 0.7856$,

n = 48). The behavioral assessment demonstrated that bottom type fish exhibited

significantly more swimming against mirror (SAM) behavior (number of distinct periods

of physical contact) with the mirror compared to the surface phenotype (Figure 2c). On

average the bottom oriented phenotype performed 5.4 times (95% CI [1.5, 19.3] times)

the number of mirror bouts than the surface type (Negative Binomial Regression, n = 48,

see Table 3 for AICc weight comparisons).

Morphology

Mean fish size and weight between surface and bottom oriented fish did not vary

significantly at the time the 2 month morphometric comparisons were made (Table 4).

Body shape comparisons between surface and bottom oriented phenotypes demonstrated

significant differences that were consistent among brood years (Table 5a, Figure 4).

Surface fish exhibited deeper, shorter heads, were deeper bodied and had a deeper caudal

peduncle compared to the bottom phenotype (Figure 4). Head shape variation was also

significantly different between the BY 2013 surface and bottom oriented phenotypes

(Table 5b). Specifically, the surface oriented phenotype exhibited shorter, more rounded

snouts but deeper heads and the mouth position was angled slightly upward compared to

the bottom oriented individuals (Figure 4).

We did not detect any differences in body shape between phenotypes fed at the

surface or mid-column (MANOVA, $p = 0.339$, n = 153). Body shape differed

significantly between surface and bottom oriented phenotypes after 8 months of rearing at

the FPGL when fed at the surface (MANOVA, $p = 0.0277$, n = 70) (Table 6a).   The

surface phenotype on average exhibited shorter heads, a deeper body and caudal peduncle

while bottom phenotype exhibited a more streamlined shape (Figure 6a). Morphological

differences detected in surface and bottom phenotypes were consistent with the shape

differences observed in earlier life stages (see Figure 4 for comparison).

Although no differences in size were detected between the two orientation

phenotypes reared at the OHRC in 2014, surface and bottom fish exhibited differences in

body shape when raised at 12°C (Table 6b, $p = 0.0291$, n = 66). Body and head shape

comparisons of surface and bottom phenotypes were consistent with previous brood year

analyses in that surface fish had shorter heads and slightly deeper caudal peduncles than

bottom fish (Figure 6b, see Figures 4 and 6a. for comparison). Contrary to previous

comparisons, the bottom fish exhibited a deeper body shape, consistent with bottom fish

demonstrating a significantly higher condition factor, when raised at 12°C.

We found that the phenotype, the combined vs. separate rearing treatment and the

interaction between phenotype and rearing all demonstrated significant differences in

body shape (Table 6c). Consistent with previous findings, the surface phenotype had

shorter heads than the bottom phenotype in fish reared in the 7°C treatment (Figure 6c).

The individuals of either phenotype that were reared together appeared to have deeper

bodies and deeper caudal peduncles compared to the more streamline shape of the two

phenotypes that developed when fish layers were reared as a separate phenotype group.

Discussion

We have discovered a new phenomenon concerning expression of early life history traits in Chinook salmon. Chinook sort themselves into surface and bottom-oriented groups in rearing tanks within days after first feeding. This sorting behavior appears to be rather general for we found it displayed by two Willamette River hatchery stocks, across three brood years and between two different rearing facilities. Such sorting may also be a common expression of phenotypic variation in other salmonids, as we recently have observed in recently emerged steelhead. Not only is this self-sorting behavior noted consistently and persisting for many months, these orientation phenotypes are correlated with variation in body shape and head morphology, growth and agonistic behavior.

This association with the tank bottom, for the bottom phenotype, cannot be explained simply by the presence of fish above, as once separated, the two phenotypes maintain their respective orientation for several months. We employed a feeding location treatment in order to determine whether the two phenotypes would adapt to feeding at the surface or mid-column. When fed mid-column, surface fish quickly adapted to feed near the location where the food was delivered, however, bottom fish generally still fed from the mid-column when fed at the surface. Further, we did not find evidence that the method of food delivery affected growth or morphology.

Generalizations about growth between the two phenotypes are confounded because of differences between facilities and temperatures. The surface fish grew faster

than bottom fish at the FPGL, but not at the OHRC. The growth trajectories, however, differed between phenotypes reared at the OHRC at the two temperatures. At 12°C, the two phenotypes grew the same, but 7°C, the surface phenotype was larger after 3 months of rearing than the bottom fish. However, when the temperature was increased to 10°C the bottom phenotype caught up in size over the next two months. We speculate that this could be because the scope of growth (Warren and Davis 1967; Preide 1985) increased for the bottom fish because surface fish were already growing faster at the lower temperature and once the temperature increased without increase in rations more energy was needed for standard metabolism.

There were also behavioral differences between the phenotypes. The surface phenotype spent more time interacting with their mirror image than the bottom phenotype when the mirror was placed at the opposite end of the behavioral tank from the gravel refuge. However, when the mirror was placed near the fish, the bottom phenotype engaged in more swim-against-mirror behavior. Spending more time out in the open to interact with the mirror could be interpreted as boldness; presumably because this could increase risk of predation in a natural setting. Once fish were provided with cover and gravel near the mirror, it was the bottom phenotype that performed more than five times the aggressive contacts with the mirror than surface fish, even though both phenotypes spent the same amount of time actively swimming. This might explain the seemingly opposing results between behavioral tests where surface fish interacted with the mirror more when the mirror was further away from the gravel refuge and bottom fish interacted more when it was close.

Our findings could be indicative of the bottom fish engaging in territorial behavior while surface fish may be exhibiting reduced aggressive behavior or even schooling, as Iwata (1995) found aggressive territorial behavior to diminish across salmonids at the time of smoltification. In addition, several studies have used the mirror image stimulation test to demonstrate correlations between agonistic behavior and juvenile life history patterns. Hoar (1953) described marked difference in agonistic behavior between juvenile salmonids that demonstrate a propensity for downstream movement and those that will become territorial later in life and rearing in their natal streams. Holtby *et al.* (1993) demonstrated with juvenile coho that both agonistic response to a mirror image and depth of body might also predict dominance status and length of freshwater residency. Taylor and Larkin (1986) also found that stream-type Chinook spent significantly more time engaging in SAM behavior compared to Chinook from an ocean-type life history.

Feeding location also appeared to affect both phenotypes. Fish fed at the surface, regardless of phenotype made more mirror approaches than those fed in the mid-column, however, for the bottom fish, it did not have an effect on the time spent in the open. Interestingly, in both of our behavioral experiments fish length did not correlate with an increase in activity or agonistic behavior, suggesting these traits are more strongly associated with the orientation phenotype itself. This begs the question of whether these behavioral and morphological traits might be tightly controlled genetically. We do not yet know to what extent these traits are genetic, but future research is underway in hopes of answering these questions.

Morphology, head shape in particular, were different between the two phenotypes at two months post-swim up; and these differences were consistent across three brood years tested and were conserved later in life. This was so even though there were no differences in size at the end of the 12°C and 7°C rearing experiments at the OHRC. . None of the differences between phenotypes can be explained by sexual dimorphism, as the sexes were equally split between the two types (Chi-squared test, $p = 0.2735$, surface n = 41 ♀, n = 32 ♂ and bottom n = 40 ♀, n = 40 ♂). In general, the surface phenotype had shorter heads and a less streamlined body shape compared the bottom phenotype.

The difference in head shape may be associated with the increased growth rate the surface phenotype groups exhibited at the FPGL and in the 7°C OHRC treatment. Studies on cultured rainbow trout and Atlantic salmon also demonstrated that a smaller head shape was associated with faster growth rate (Martin 1949; Vincent 1960). The morphological differences we found between fish reared separately or together with the other phenotype also could be explained by differences in how growth and morphology interact during development. The positive effect of combined rearing on bottom fish size may explain the deeper bodied morphology of the phenotypes when they were reared together. Potentially those fish reared separately had more competition in portion of the water column where they feed, whereas, by rearing the two phenotypes together, they may partition the water column feeding near the surface or bottom.

These differences in body and head morphology between the two orientation phenotypes are especially intriguing in light of the morphological variation seen in life history tactics of their wild counterparts in the Willamette River Basin by Billman *et al.*

(2014). The morphology of the surface phenotype are similar to subyearling fall smolts and those rearing in mainstem habitats, while the bottom phenotype is more streamlined like the tributary rearing juveniles and spring smolts. It is interesting to speculate from this that the fall and spring migrant life history types potential may have already been differentiated near the time of onset of feeding.

Our work suggests that the potential for these two orientation phenotypes (and likely more) may be present and they may or not be observed or expressed under artificial rearing conditions. This phenomenon may be overlooked because of density effects on behavior. Under conventional hatchery conditions, where densities are typically very high, the separation may be difficult to see or, potentially, the crowding may not allow enough room to accommodate two distinct layers. While at very low densities and without substrate, anecdotally, we observed that the fish tended to school together. Flow, likely velocity, also affects expression of the sorting behavior; we noted that under increased flow the differences in orientation broke down.

Regardless of the conditions under which this separation occurs, these orientation phenotypes may be present in a hatchery environment, and if simply ignored these phenotypes may be selected against through conventional rearing and release strategies. For example, a bimodal size distribution has been observed in some hatchery stocks and when fish are released in the fall and assumed to be a uniform group, some proportion may not have reached the size thresholds and the physiological status to migrate out of the system, which may lead to the unintentional loss of those individuals through predation (Cameron Sharpe pers. com).

The novelty of our finding is that these two behavioral tactics occur within a population and are expressed shortly after *per os* feeding occurs. Other forms of behavioral variation among Chinook that have been described tend to be noted between populations or later in life such as timing of juvenile outmigration and adult return (see Gilbert 1912; Quinn 2005; Waples et al. 2001; Bottom et al. 2005; Schroeder et al. 2007). Morphological and behavioral variation have been identified relatively early in life among different populations and races of Chinook salmon (Taylor and Larkin 1986). Even within population morphological differences have been described by Beeman *et al.* 1994 and Billman *et al.* 2014 for the Willamette River Basin stocks, but these differences are generally noted at the time of smoltification or well into their freshwater rearing. The implications for our discovery are that surface and bottom phenotypes may be expressing different juvenile life history tactics as early as *per os* feeding. This follows results from a study on fall Chinook salmon in the Snake River Basin by Perkins and Jager (2011) that offers that the decision to migrate out as a subyearling or yearling occurs shortly after emergence and can be attributed to small differences in fry size and growth opportunity.

Other early determinants of juvenile migration timing that have been proposed in Atlantic salmon include timing and embryo size at first feeding (Metcalfe and Thorpe 1992). We, however, did not see evidence that surface and bottom fish were significantly different in size when separated and both phenotypes were feeding at their respective tank orientations. In addition, sex is another example of an early determinant for life history expression that has been demonstrated in coho salmon in which the males and females exhibit habitat partitioning as well as differences in morphology and parasite

load (Rodnick et al. 2008). The orientation phenotypes we have described may indicate another type of habitat partitioning in the captive environment that is not driven by sexual dimorphism. Our discovery creates a paradigm shift in thinking about when life history tactics are determined and how these may in turn affect timing of smoltification and outmigration. Early self-sorting behavior could provide a means of identifying life history trajectories soon after emergence and opportunities for studying how environmental factors may affect their expression.

Acknowledgments

Literature Cited

Adams, C.E., Huntingford, F.A., Turnbull, J.F., and Beattie, C. (1998). Alternative competitive strategies and the cost of food acquisition in juvenile Atlantic salmon (*Salmo salar*). Aquaculture 167: 17–26.

Angilletta Jr, M.J., Ashley Steel, E., Bartz, K.K., Kingsolver, J.G., Scheuerell, M.D., Beckman, B.R., and Crozier, L.G. (2008). Big dams and salmon evolution: changes in thermal regimes and their potential evolutionary consequences. Evolutionary Applications 1: 286–299.

Beckman, B.R. (2002). Growth and the plasticity of smolting in Chinook salmon. Ph.D. Dissertation, University of Washington, Seattle, WA.

Beeman, J.W., Rondorf, D.W., and Tilson, M.E. (1994). Assessing smoltification of juvenile spring Chinook salmon (*Oncorhynchus tshawytscha*) using changes in body morphology. Canadian Journal of Fisheries and Aquatic Sciences 51: 836–844.

Berejikian, B.A., Mathews, S.B., and Quinn, T.P. (1996). Effects of hatchery and wild ancestry and rearing environments on the development of agonistic behavior in steelhead trout (*Oncorhynchus mykiss*) fry. Canadian Journal of Fisheries and Aquatic Sciences 53: 2004–2014.

Billman, E.J., Whitman, L.D., Schroeder, R.K., Sharpe, C.S., Noakes, D.L.G., and Schreck, C.B., (2014). Body morphology differs in wild juvenile Chinook salmon (*Oncorhynchus tshawytscha)* that express different migratory phenotypes in the Willamette River, Oregon, U.S.A. Journal of Fish Biology 85: 1097–1110.

Blaser, R., and Gerlai, R. (2006). Behavioral phenotyping in zebrafish: comparison of three behavioral quantification methods. Behavior Research Methods 38: 456–469.

Brown, C., Burgess, F., and Braithwaite, V.A. (2007). Heritable and experiential effects on boldness in a tropical poeciliid. Behavioral Ecology and Sociobiology 62: 237–243.

Buchanan, R.A., Skalski, J.R., and Giorgi, A.E. (2010). Evaluating surrogacy of hatchery releases for the performance of wild yearling Chinook salmon from the Snake River Basin. North American Journal of Fisheries Management 30: 1258–1269.

Burnham, K.P., and Anderson, D.R. (2002). Model selection and multimodel inference: a practical information-theoretic approach, 2nd edition. Springer, New York.

Canty, A., and Ripley, B. (2014). boot: Bootstrap R (S-Plus) Functions. R package version 1.3–11.

Crozier, L.G., Hendry, A.P., Lawson, P.W., Quinn, T.P., Mantua, N.J., Battin, J., Shaw, R.G., and Huey, R.B. (2008). Potential responses to climate change in organisms with complex life histories: evolution and plasticity in Pacific salmon: Evolutionary responses to climate change in salmon. Evolutionary Applications 1: 252–270.

Currens, K.P., Sharpe, C.S., Hjort, R., Schreck, C.B., and Li, H.W. (1989). Effects of different feeding regimes on the morphometrics of Chinook salmon (*Oncorhynchus tshawytscha*) and rainbow trout (*O. mykiss*). Copeia 1989: 689–695.

Davison, A.C., and Hinkley, D.V. (1997). Bootstrap Methods and Their Applications. Cambridge University Press, Cambridge.

Finstad, A.G., Forseth, T., Ugedal, O., and NæSje, T.F. (2007). Metabolic rate, behavior and winter performance in juvenile Atlantic salmon. Functional Ecology 21: 905–912.

Fournier D.A., Skaug H.J., Ancheta J., Ianelli J., Magnusson A., Maunder M., Nielsen, A., and Sibert J. (2012). "AD Model Builder: using automatic differentiation for statistical inference of highly parameterized complex nonlinear models." Optimization Methods and Software 27: 233–249.

Fulton, T. W. (1904). The rate of growth of fishes. Twenty-second Annual Report, Part III. Fisheries Board of Scotland, Edinburgh, 141–241

Gilbert, C. H. (1912). Age at maturity of the Pacific coast salmon of the genus *Oncorhynchus*. Bulletin of the Bureau of Fisheries (U. S.) 32: 1–22.

Hassell, E.M.A., Meyers, P.J., Billman, E.J., Rasmussen, J.E., and Belk, M.C. (2012). Ontogeny and sex alter the effect of predation on body shape in a livebearing fish: sexual dimorphism, parallelism, and costs of reproduction. Ecology and Evolution 2: 1738–1746.

Healey, M.C. (1991). Life history of Chinook salmon (*Oncorhynchus tshawytscha*). Pages 313–393 in Groot, C. and Margolis, L., editors. Pacific Salmon Life Histories. Vancouver, British Columbia: UBC Press.

Hoar, W.S. (1953). Control and timing of fish migration. Biological Reviews 28: 437–452.

Holtby, L.B., Swain, D.P., and Allan, G.M. (1993). Mirror-elicited agonistic behavior and body morphology as predictors of dominance status in juvenile coho salmon (*Oncorhynchus kisutch*). Canadian Journal of Fisheries and Aquatic Sciences 50: 676–684.

Hothorn, T. Bretz, F., and Westfall, P. (2008). Simultaneous inference in general parametric models. Biometrical Journal 50: 346–363.

Iwata, M. (1995). Downstream migratory behavior of salmonids and its relationship with cortisol and thyroid hormones: a review. Aquaculture 135: 131–139.

Konietschke, F. (2012). nparcomp: Perform multiple comparisons and compute simultaneous confidence intervals for the nonparametric relative contrast effects. R package version 2.0. http://CRAN.R-project.org/package=nparcomp.

Kristjansson, B.K., Skulason, S., and Noakes, D.L.G. (2002). Morphological segregation of Icelandic threespine stickleback (*Gasterosteus aculeatus* L). Biological Journal of the Linnean Society 76: 247–257.

Langerhans, R.B., Layman, C.A., Langerhans, A.K., and Dewitt, T.J. (2003). Habitat associated morphological divergence in two Neotropical fish species. Biological Journal of the Linnean Society 80: 689–698.

Langerhans, R.B., Layman, C.A., Mona Shokrollahi, A., and DeWitt, T.J. (2004). Predator driven phenotypic diversification in *Gambusia affinis*. Evolution 58: 2305–2318.

Leblanc, C.A., and Noakes, D.L. (2012). Visible Implant Elastomer (VIE) tags for marking small rainbow trout. North American Journal of Fisheries Management 32: 716–719.

Lumley, T. using Fortran code by Alan Miller (2009). leaps: regression subset selection. R package version 2.9. http://CRAN.R-project.org/package=leaps

Maynard, D.J., Crewson, M., Tezak, E.P., McAuley, W.C., Schroder, S.L., Knudsen, C., Flagg, T.A., and Mahnken, C.V.W. (1996b). The postrelease survival of Satsop River fall Chinook salmon reared in conventional and seminatural raceway habitats, 1994. Pages 78–97 in D.J. Maynard, T.A. Flagg, and C.V.W. Mahnken, editors. Development of a Natural Rearing System to Improve Supplemental Fish Quality 1991-1995. Bonneville Power Administration, Portland, Oregon.

Maynard, D.J., Hackett, G.J.L., Wastel, M., LaRae, A.L., McDowell, C., Flagg, T.A., and Mahnken, C.V.W. (2001b). Effect of automated sub-surface feeders on behavior and predator vulnerability of fall Chinook salmon. Pages 6–19 in D.J. Maynard, B.A. Berejikian, T.A. Flagg, C.V.W. Mahnken, editors. Development of a natural rearing system to improve supplemental fish quality 1996-1998. Bonneville Power Administration, Portland, Oregon.

McLaughlin, R.L., Grant, J.W.A., and Kramer, D.L. (1994). Foraging movements in relation to morphology, water-column use, and diet for recently emerged brook trout (*Salvelinus fontinalis*) in still-water pools. Canadian Journal of Fisheries and Aquatic Sciences 51: 268–279.

Metcalfe, N.B., and Thorpe, J.E. (1992). Early predictors of life-history events: the link between first feeding date, dominance and seaward migration in Atlantic salmon, *Salmo salar* L. Journal of Fish Biology 41: 93–99.

National Marine Fisheries Service (NMFS). (2008). Willamette River Basin Flood Control Project. Biological Opinion. July 11, 2008, NMFS, Northwest Region, Seattle,Washington.

O'Malley, K.G., Camara, M.D., and Banks, M.A. (2007). Candidate loci reveal genetic differentiation between temporally divergent migratory runs of Chinook salmon (*Oncorhynchus tshawytscha*). Molecular Ecology 16: 4930–4941.

Patiño, R., Schreck, C.B., Banks, J.L., and Zaugg, W.S. (1986). Effects of rearing conditions on the developmental physiology of smolting coho salmon. Transactions of the American Fisheries Society 115: 828–837.

Perkins, T.A., and Jager, H.I. (2011). Falling behind: delayed growth explains life-history variation in Snake River fall Chinook salmon. Transactions of the American Fisheries Society 140: 959–972.

Priede, I.G. (1985). Metabolic scope in fishes. Pages 33-64 in Tytler, P., and Calow, P., editors. Fish Energetics: New Perspectives, London: Croom Helm.

Quinn, T.P. (2005). The Behavior and Ecology of Pacific Salmon and Trout, University of Washington Press, Seattle, Washington.

R Core Team (2014). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.Rproject.org/.

Rasmussen, J.E., and Belk, M.C. (2012). Dispersal behavior correlates with personality of a North American fish. Current Zoology 58: 260–270.

Reinhardt, U.G. (2001). Selection for surface feeding in farmed and sea-ranched masu salmon juveniles. Transactions of the American Fisheries Society 130: 155–158.

Rodnick, K.J., St.-Hilaire, S., Battiprolu, P.K., Seiler, S.M., Kent, M.L., Powell, M.S., and Ebersole, J.L. (2008). Habitat selection influences sex distribution, morphology, tissue biochemistry, and parasite load of juvenile coho salmon in the West Fork Smith River, Oregon. Transactions of the American Fisheries Society 137: 1571–1590.

Rohlf, F.J. (2003). tpsRegr, shape regression, version 1.28. Department of Ecology and Evolution, State University of New York at Stony Brook.

Rohlf, F.J. (2010a). tpsDig, version 2.16. Department of Ecology and Evolution, State University of New York at Stony Brook.

Rohlf, F.J. (2010b). tpsRelw, relative warps, version 1.49. Department of Ecology and Evolution, State University of New York at Stony Brook.

Rohlf, F.J., and Slice, D.E. (1990). Extensions of the procrustes method for the optimal superimposition of landmarks. Systematic Biology 39: 40–59.

SAS Institute. (2008). SAS 9.2 help and documentation. Cary, NC.

Sheer, M.B., and Steel, E.A. (2006). Lost watersheds: barriers, aquatic habitat connectivity, and salmon persistence in the Willamette and Lower Columbia River Basins. Transactions of the American Fisheries Society. 135: 1654–1669.

Schroeder, R.K., Kenaston, K.R., and McLaughlin, L.K. (2006). Spring Chinook salmon in the Willamette and Sandy Rivers. (Annual Progress Report No. F-163-R 11/12). Oregon Department of Fish and Wildlife Salem, Oregon.

Scott, L.E., and Johnson, J.B. (2010). Does sympatry predict life history and morphological diversifications in the Mexican livebearing fish *Poeciliopsis baenschi*? Biological Journal of the Linnean Society 100: 608-618.

Skaug H, Fournier D, Bolker B, Magnusson A and Nielsen A (2014). Generalized Linear Mixed Models using AD Model Builder_. R package version 0.8.0.

Sosiak, A.J., Randall, R.G., and McKenzie, J.A. (1979). Feeding by hatchery-reared and wild Atlantic salmon (*Salmo salar*) parr in streams. Journal of Fisheries Research Board Canada 36: 1408–1412.

Taylor, E.B., and Larkin, P.A. (1986). Current response and agonistic behavior in newly emerged fry of Chinook salmon, *Oncorhynchus tshawytscha*, from ocean-and stream-type populations. Canadian Journal of Fisheries and Aquatic Sciences 43: 565–573.

Tiffan, K.F., and Connor, W.P. (2011). Distinguishing between natural and hatchery Snake River fall Chinook salmon subyearlings in the field using body morphology. Transactions of the American Fisheries Society 140: 21–30.

Toms, C.N., Echevarria, D.J., and Jouandot, D.J. (2010). A methodological review of personality related studies in fish: focus on the shy-bold axis of behavior. International Journal of Comparative Psychology 23: 1–25.

Venables, W. N., and Ripley, B. D. (2002) Modern Applied Statistics with S. Fourth Edition. Springer, New York.

Waples, R.S. et al. (2001). Characterizing diversity in salmon from the Pacific Northwest. Journal of Fish Biology 59: 1–41.

Waples, R.S., Teel, D.J., Myers, J.M., and Marshall, A.R. (2004). Life-history divergence in Chinook salmon: historic contingency and paralled evolution. Evolution 58: 386–403.

Warren, C.E., and Davis, G.E. (1967). Laboratory studies on the feeding bioenergetics, and growth of fish. Pages 175–214 in Gerking, S.D., editor. The biological basis of fresh water fish production, Blackwell Scientific Publications, Oxford, UK.

Wesner, J. S., Billman, E. J., Meier, A., and Belk, M. C. (2011). Morphological convergence during pregnancy among predator and nonpredator populations of the livebearing fish Brachyrhaphis rhabdophora (Teleostei: Poeciliidae). Biological Journal of the Linnean Society 104: 386–392.

Wilson, A.D.M., and McLaughlin, R.L. (2007). Behavioural syndromes in brook charr, *Salvelinus fontinalis*: prey-search in the field corresponds with space use in novel laboratory situations. Animal Behaviour 74: 689–698.

Zimmerman, M.S., Krueger, C.C., and Eshenroder, R.L. (2006). Phenotypic diversity of lake trout in Great Slave Lake: differences in morphology, buoyancy, and habitat depth. Transactions of the American Fisheries Society 135: 1056–1067.

Table 1a. Timeline for BY 2012 experiment: growth sampling, behavioral trial and morphological comparisons. Surface and bottom fish were separated and began rearing experiments in early February 2013. The behavioral assessments began in early August. The final sampling for size and morphometric comparison occurred in late October and included the fish from the behavioral assessments. 1b. Timeline for BY 2013 experiment: growth sampling, behavioral trial and morphological comparisons. Surface and bottom fish were separated and began rearing at the OHRC in mid February 2014. Initial body and head morphometric comparisons were made in late March. The mirror stimulation trials began in early July. The final sampling for size and morphometric comparison for the 12°C treatment groups occurred in late June occurred and the 7°C groups were sampled in early August to attempt to size match groups for morphometric comparison.

Activity	Sorting Phenotypes	Growth Sampling	Morphometric Comparison	Behavioral Assessment
1a. Brood Year 2012 at FPGL				
Date	Early February	Monthly February-October	March and October	August
Months of Rearing	0	1-8	2 and 8	6
1b. Brood Year 2013 at OHRC				
Date	Mid-February	Monthly March-August	March, June and August	July
Months of Rearing	0	1-6	2, 4, 6	5

Table 2. Candidate Gamma Regression models for explaining variation in average time fish spent interacting with the mirror. The top model shows the fixes effects of phenotype, behavioral tank (Btank) and random effect of rearing tank (Rtank). Model selection procedure included Log likelihood ratio tests followed by comparing top models using second order Akaike's Information Criterion (AICc) for Gamma Regression. The K represents the number of parameters estimated for each model. The change in AIC values from the top model, ΔAIC_c are given, along with the Akaike's model weights and Log-Likelihood values.

Model Selection for Gamma Regression	K	AIC_c	ΔAIC_c	w_i	$Log_e(L)$
Time with Mirror					
$MT_{(pheno + Btank + 1|Rtank)}$	5	567.2	0.0	0.427	-277.98
$MT_{(pheno + trial + Btank + 1|Rtank)}$	6	567.7	0.6	0.319	-277.01
$MT_{(pheno + order + trial + Btank + 1|Rtank)}$	7	569.1	1.9	0.166	-276.36
$MT_{(Btank + 1|Rtank)}$	4	570.8	3.7	0.069	-281.01
$MT_{(pheno + feed + pheno*feed + order + trial + Btank + 1|Rtank)}$	9	573.8	6.7	0.015	-275.96
$MT_{(pheno + feed + length + pheno \times feed + + trial + order + Btank + 1|Rtank)}$	10	576.6	9.4	0.004	-275.85

Table 3. Candidate Negative Binomial Regression models for explaining variation in the number of SAM behaviors an individual performed. Final model selection used second order Akaike's Information Criterion (AICc). *K* represents the number of parameters estimated for each model. The change in AIC values from the top model, ΔAIC_c are given, along with the Akaike's model weights and the Log-Likelihood values.

Models	*K*	*AIC$_c$*	*ΔAIC$_c$*	*w$_i$*	*Log$_e$(L)*
MB$_{(pheno)}$	3	225.6	0.0	0.66	-219.06
MB$_{(pheno + length)}$	5	228.0	2.4	0.20	-219.04
MB$_{(1)}$	2	229.1	3.5	0.11	-224.85
MB$_{(pheno + length + trial + Btank + Rtank)}$	7	232.6	7.0	0.02	-215.83

Table 4. Average fork length in mm (± SE) between surface and bottom phenotypes in three consecutive BYs used for early morphometric comparisons (size ~ 55 mm) 2 months of rearing as separate phenotype groups (p values obtained from two-sample t-test).

Phenotype	Brood Year	Days Post-Ponding	n	Fork Length (mm)	FL comparison p value
Surface	2011		31	54 (±0.6)	
Bottom	2011	67	37	53 (±0.5)	0.059
Surface	2012		21	53 (±0.7)	
Bottom	2012	62	22	53 (±0.9)	0.863
Surface	2013		20	58 (±0.8)	
Bottom	2013	76	20	57 (±0.8)	0.400

Table 5a. Results of mixed repeated measures MANOVA modeling the effect of orientation (surface and bottom) phenotype on body shape in juvenile Chinook salmon across three BYs (2011, 2012 and 2013). Shape variables were the first 15 relative warps (principal components) from an analysis using landmark-base geometric morphometrics. 5b. Results of mixed repeated measures MANOVA modeling the effect of orientation (surface and bottom) phenotype on head shape in juvenile Chinook salmon. Shape variables were the first 8 relative warps (principal components) from an analysis using landmark-base geometric morphometrics.

Effect	Degrees of Freedom	F	P
Body Shape (15 Landmarks)			
Orientation	1/1061	0.09	0.766
Centroid size	1/138	10.16	0.002
Index variable	14/832	0.01	1.000
Orientation x index variable	14/832	2.58	0.001
Head Shape (8 Landmarks)			
Orientation	1/212	1.25	0.2644
Centroid size	7/122	0	0.8463
Index variable	1/212	0.04	1
Orientation x index variable	7/122	3.95	0.0006

Table 6a. Results of mixed repeated measures MANOVA representing the effect of orientation phenotype (surface and bottom) on the morphology of the BY 2012 fish after 8 months of rearing at the FPGL. Shape variables = first 15 relative warps (principal components) from landmark-base geometric morphometrics. 6b. Results of mixed repeated measures MANOVA showing the effect of orientation on the morphology of the BY 2013 fish after 4 months of rearing under the 12°C treatment at OHRC. Shape variables = first 14 relative warps. 6c. Results of mixed repeated measures MANOVA modeling the effect of orientation phenotype and rearing as separate vs. combined phenotype groups on the morphology of the BY 2013 fish after 4 months of rearing under the 7°C treatment at the OHRC. Shape variables = first 15 relative warps.

Effect	Degrees of Freedom	F	P
a. BY 2012 FPGL			
Orientation	1/846	0.93	0.3461
Centroid size	1/846	2.21	0.1378
Index variable	14/846	0.01	1.000
Orientation x index variable	14/846	1.86	0.0277
b. BY 2013 OHRC 12°C			
Orientation	1/537	0.34	0.5616
Centroid size	1/514	0.10	0.7490
Index variable	13/332	0.01	1.000
Orientation x index variable	13/332	1.86	0.0291
c. BY 2013 OHRC 7°C			
Orientation	1/16.2	0.27	0.6114
Treatment	1/4.92	0.06	0.8120
Centroid size	1/951	0.07	0.7973
Index variable	13/614	0.01	1.000
Orientation x index variable	14/614	2.37	0.0041
Treatment x index variable	14/614	2.52	0.0023
Orientation x Treatment x index	14/284	1.85	0.0323
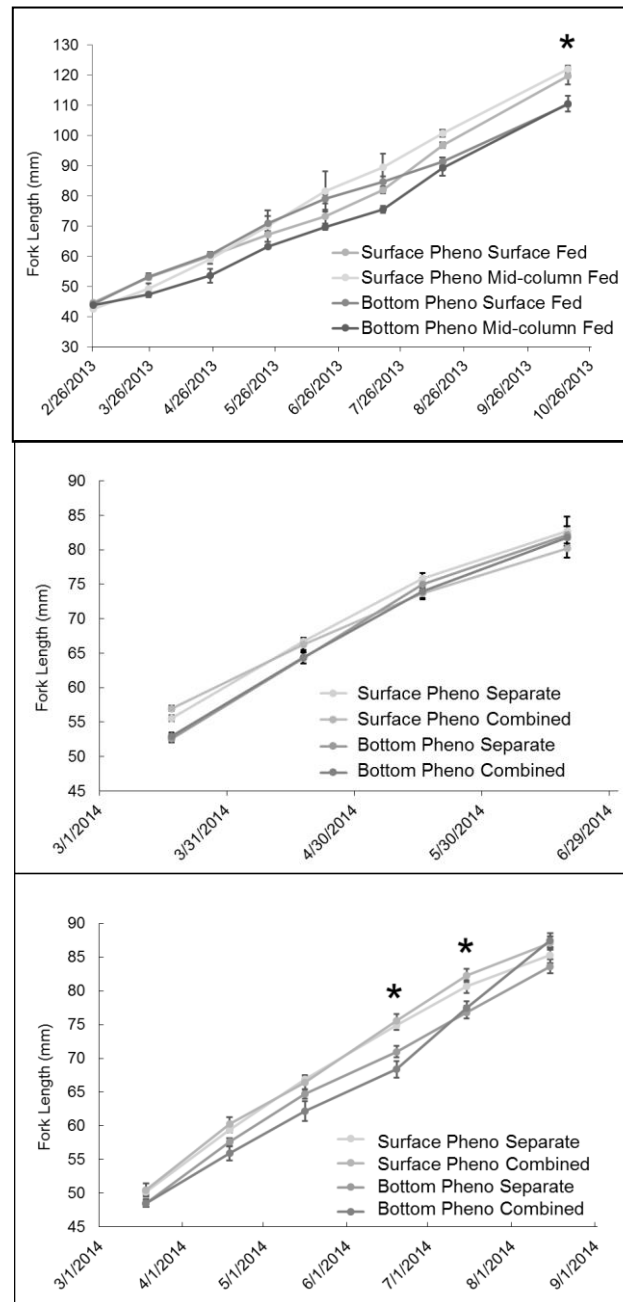
Figure 1a. Average monthly fork length between BY 2012 surface and bottom phenotype groups at the FPGL Bars represent ± SE. Letters indicate significant differences (n = 80, Welch's *t-* test, p < 0.0001). 1b. Average monthly fork length between the BY 2013 surface and bottom phenotypes reared at 12°C at the OHRC. 1c. Average monthly fork length between BY 2013 surface and bottom phenotypes reared at 7°C at the OHRC. Asterisks represent sampling dates where phenotypes were significantly different (n = 40, Welch's *t-* test, p < 0.05).
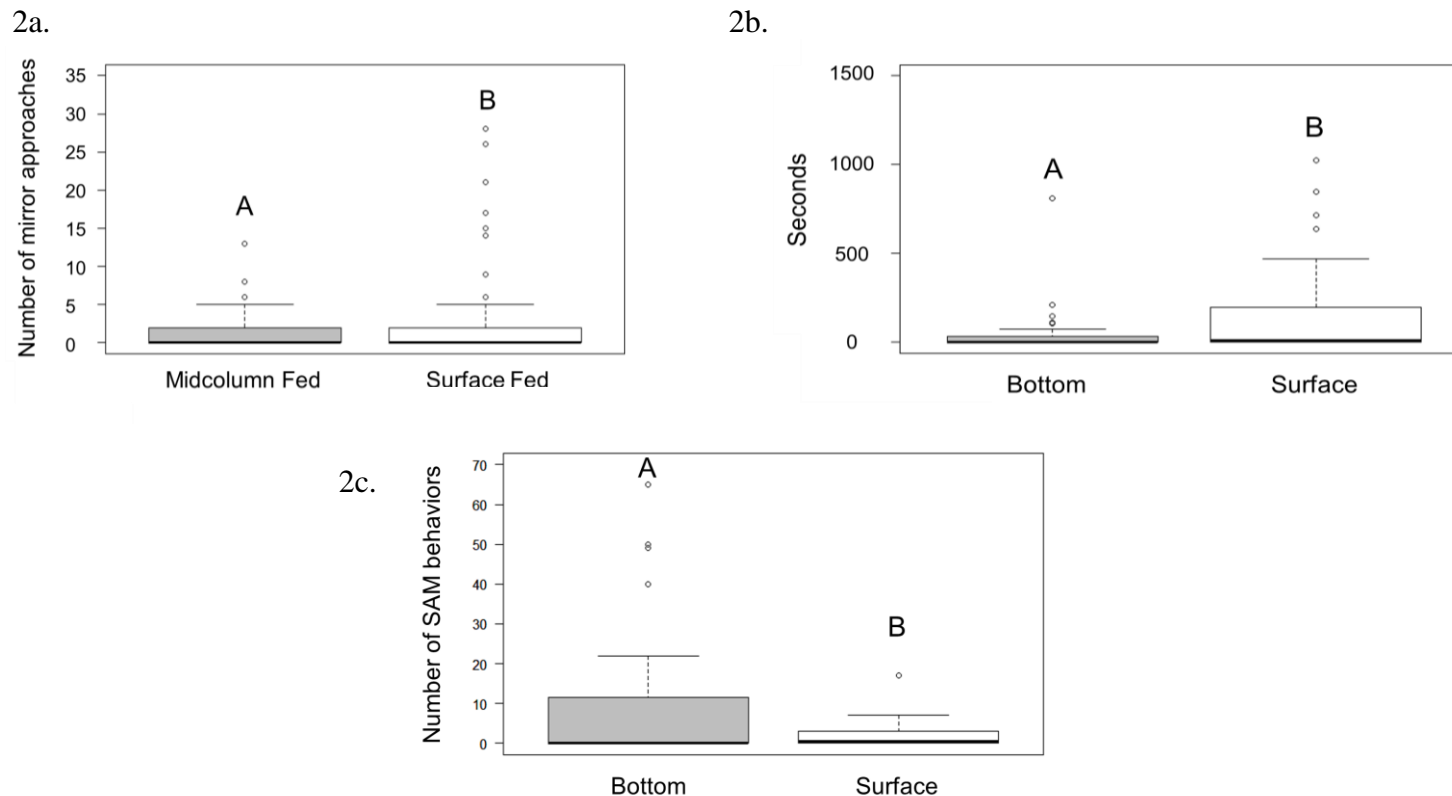
Figure 2a. Average number of mirror approaches performed by surface and mid-column fed BY 2012 individuals. Bars represent 95% Confidence Intervals. Asterisk represents significant differences (Negative Binomial Regression, $p < 0.05$, n = 60). 2b. Average time (in seconds) BY 2012 surface and bottom oriented fish spent interacting with mirror image. Different letters indicate significant differences (Gamma Regression $p < 0.05$, n= 60). 2c. Average number of distinct SAM routines performed between surface and bottom fish from BY 2013 OHRC 7°C treatments. (Negative Binomial Regression, $p = 0.0084$, n= 48).

Figure 3. (a) Fifteen landmarks used for geometric morphometric analysis of surface and bottom oriented phenotypes:1. posterior point on the maxilla; 2. Anterior most point on the snout; 3. anterior point of the orbital socket; 4. Posterior portion of the eye; 5. Pectoral fin insertion, 6. Nape of the head; 7. Pelvic fin insertion; 8.) A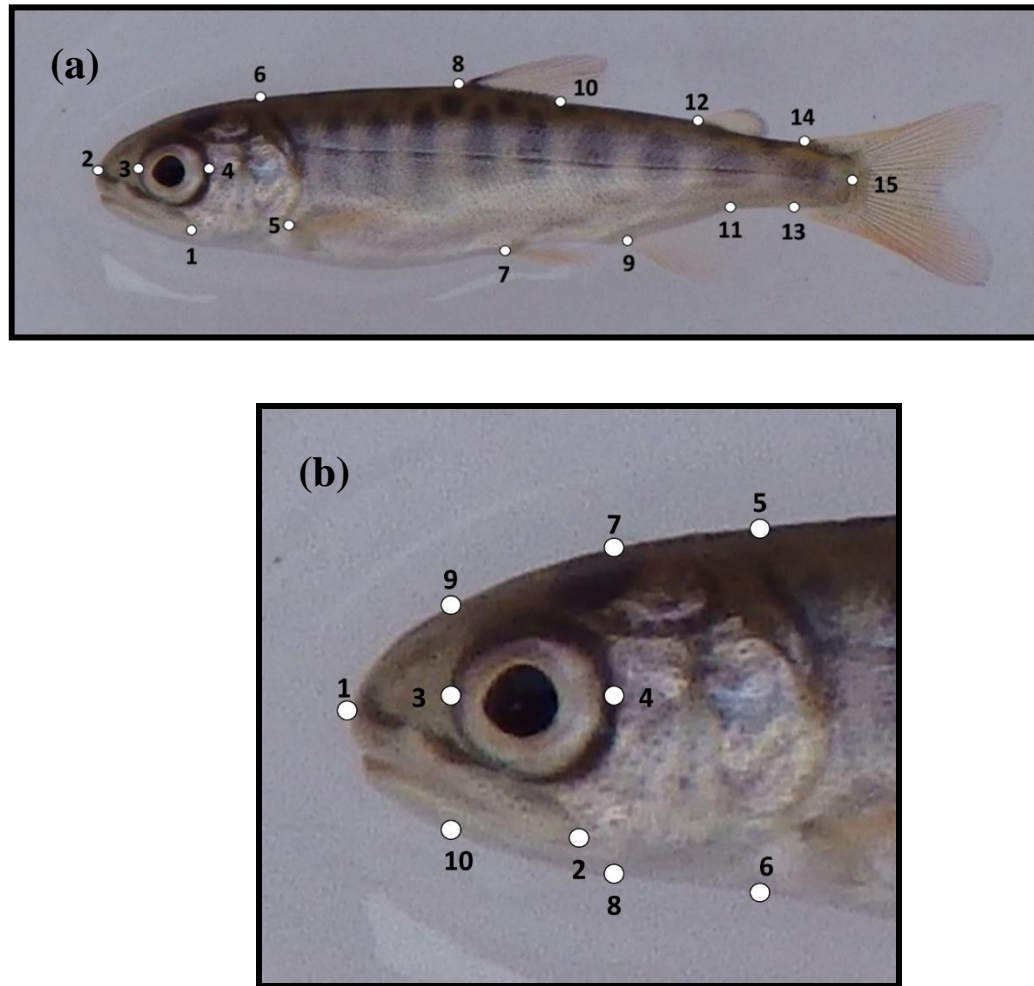nterior dorsal fin insertion; 9.) Anterior anal fin insertion; 10.) Posterior dorsal fin insertion; 11.) Posterior anal fin insertion; 12.) Anterior adipose fin insertion 13.) Ventral side of caudal fin insertion; 14.) Dorsal side of the caudal fin insertion; 15.) Posterior point of the lateral line. (b) Ten landmarks used for head morphology comparison: 1. Anterior most point on the snout; 2. posterior point on the maxilla; 3 anterior point of the orbital socket; 4. posterior point on the orbital socket; 5. Nape of the head; 6. anterior edge of the pectoral girdle; 7. Vertical extension of landmark 4 to dorsal outline; 8. Vertical extension of landmark 4 to ventral outline; 9. Vertical extension of landmark 3 to dorsal outline; and 10. Vertical extension of landmark 3 to ventral outline. Landmarks 7 – 10 were considered semi-landmarks.
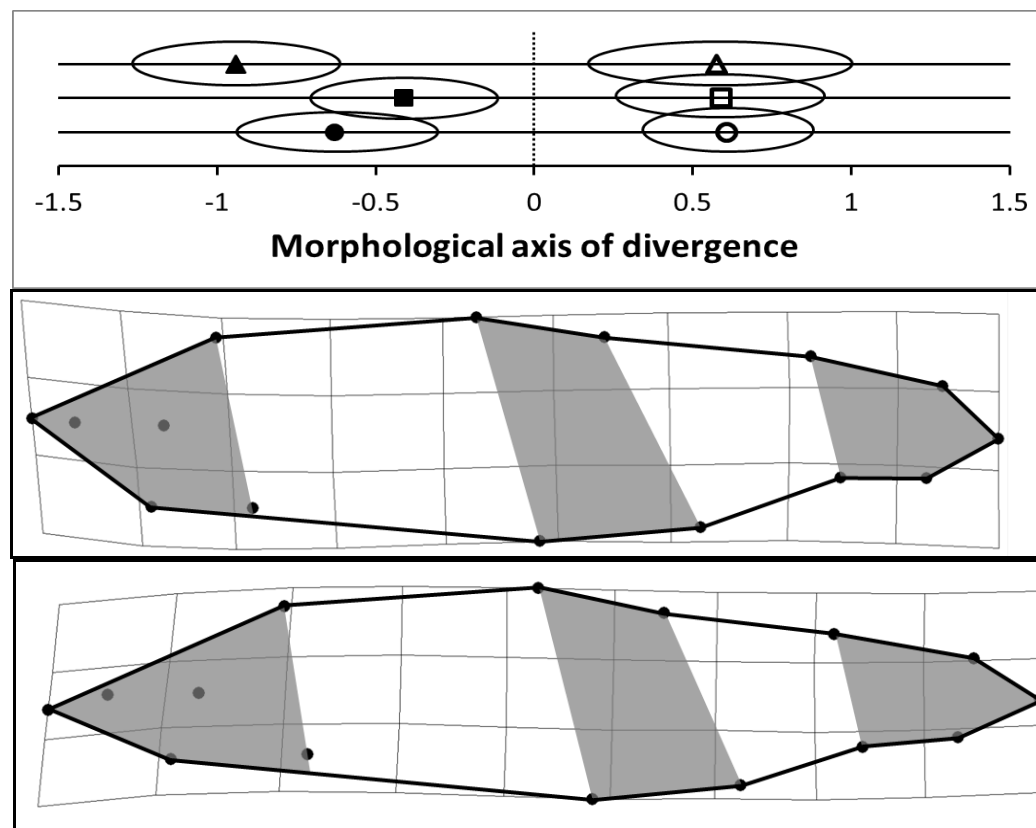
Figure 4. Morphological axis of divergence of juvenile Chinook salmon based on canonical variants derived from tank orientation (closed symbols = surface orientation; open symbols = bottom orientation) for three brood years (● = 2011; ■ = 2012; ▲ = 2013). Ellipses represent 95% confidence intervals along the axis for each brood year. Canonical correlations were conducted jointly for the brood years. Thin plate spline transformation grids represent the axis' endpoints with differences magnified three times, surface type (top) and bottom type (bottom).
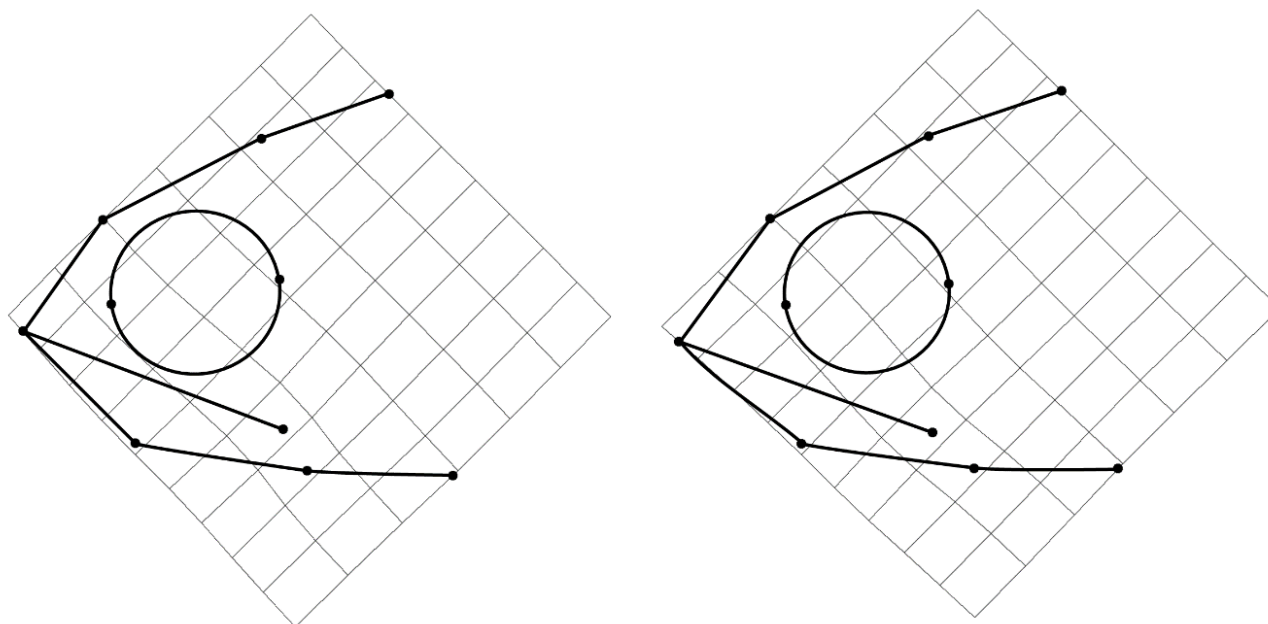
Figure 5. Head morphology of surface (left) and bottom (right) orientation phenotypes from BY 2013. Thin plate spline deformation grids represent the maximum endpoints of the observed differences between surface (left) and bottom type (right) fish.

a.
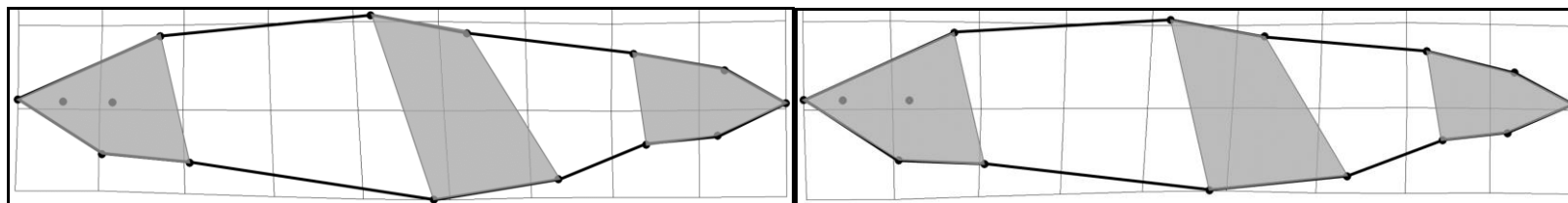
b.
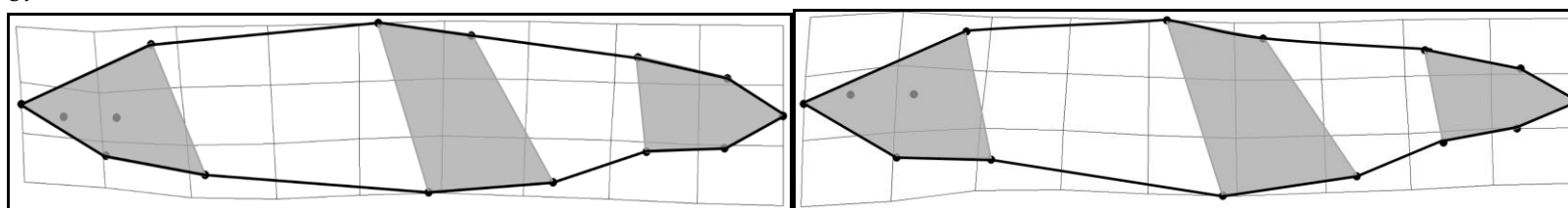
c.
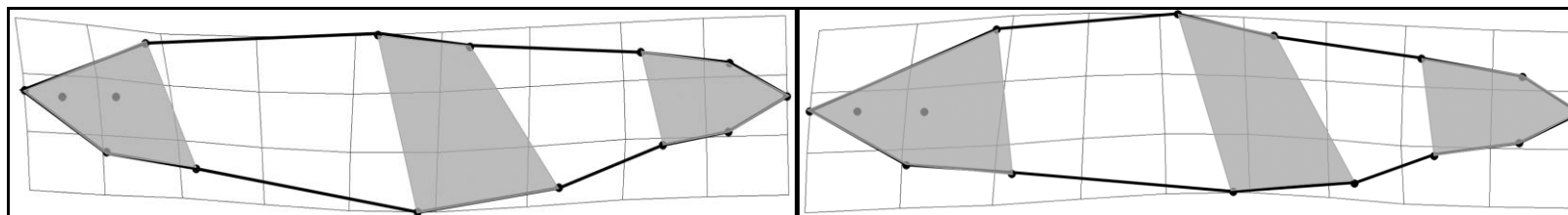
Figure 6a. Thin plate spline deformation grids representing body shape differences between surface (left) and bottom (right) phenotypes from BY 2012. Each end of the axis of morphological variation has been magnified 3 times. 6b. Deformation grids representing morphological differences between surface (left) and bottom (right) phenotypes from the OHRC BY 2013 12°C treatment. 6c. Deformation grids representing body shape differences between surface (left) and bottom phenotypes from the OHRC BY 2013 7°C treatment.

CHAPTER 3- CONCLUSION

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Understanding how and when juvenile migratory pathways are determined in life is an important goal from a fish culture, management and conservation perspective. For my purpose, I was interested in identifying early behavioral indicators that may be correlated with differences in growth and other attributes later in life. I predicted that differences in vertical orientation could be associated with other differences related to migratory timing such as growth, agonistic behavior and morphology, as these traits exhibited in the wild could lead to downstream dispersal into habitats with different growth opportunities than natal tributaries. I studied hatchery origin fish under experimental conditions in which we had already documented behavioral variation soon after emergence. In this study, I was able to link the orientation behavior expressed soon after emergence to differences in growth trajectory, agonistic behavior and body and head morphology between the two phenotypes.

Growth

After 8 months of rearing at the FPGL, the BY 2012 surface phenotype fish were on average 10% larger than the bottom phenotype, regardless of whether treatments were fed by hand or feed was injected into the mid-water column. This demonstrates there were no negative effects of the subsurface feeding on growth or condition of the surface and bottom phenotypes. Anecdotal observations verified this, as fish that were fed with the subsurface injection were seen to quickly adapt to this novel feed delivery system, even nipping at the pipette tip. Likewise, after a couple months of rearing in the surface hand fed treatment, the bottom fish began to feed closer to the surface, similar to the

surface phenotype fish. This suggests there may be some plasticity to the feeding behavior but that possibly differences in growth rates or standard metabolic rates may be responsible for maintaining the size differences between the two phenotypes.

Growth trajectories between the two phenotypes for BY 2013 experiments at the OHRC differed from previous years findings. The phenotypes reared separately or in combined groups reared at 12°C remained similar in size throughout the rearing experiment. Average size among the treatments reared at 7°C however began to diverge, reaching the maximum difference in size by the end of June, at this same time the 7°C treatments were transferred to ambient OHRC conditions (10°C). At this point the bottom phenotype groups began to catch up and by the final sampling in the middle of August the two phenotypes were no longer different in size. Interestingly the bottom fish reared alone were significantly smaller than bottom fish reared with surface fish. It is possible that the two phenotypes reared together, by occupying different portions of the water column, could forage at the surface or near the bottom where they were accustomed to. The separately reared phenotype groups, alternatively, could be competing for access to food from the portion of the water column they generally orient to.

Behavioral Assessments

The behavioral assessments of surface and bottom fish demonstrated differences in levels of agonistic behavior. Surface fish after 6 months of rearing at the FPGL spent more time interacting with their mirror image regardless of the feeding treatment. There

was however, a significant, positive effect of the surface hand feeding on the number of distinct approaches to the mirror. While agonistic response includes fight and flight responses, increased time spent interacting with their mirror image suggests increased levels of aggressiveness among surface fish. Although I did not quantify specific interactions such as nips, chases, and lateral displaying, these behaviors were observed during the trials, and individuals that spent more time with the mirror often exhibited repeated charges, nips followed by either perpendicular wig wags or extended lateral displays. In contrast, individuals which spent < 1 second with the mirror generally approached the mirror image and fled back to the refuge.

For the other behavioral metrics assessed: boldness, defined as latency to emerge from the refuge, and exploratory tendency, which was quantified by the number of 30.5 cm compartments crossings an individual completed in 30 minutes, I did not detect differences between the two phenotypes. Because all three metrics were being assessed at once, this may have influenced our interpretation of an individual's boldness and exploratory tendency. While I did not detect differences in time to emerge from the refuge, fish that emerged early did not necessarily complete more compartment crossings nor did they interact with the mirror for longer than fish that emerged later. Likewise, if fish spent significant portion of the trial interacting with their mirror image they would inherently not spend as much time swimming across compartments. This measurement for exploratory behavior did not quantify any lateral movement, which was often substantial when fish were displaying at the mirror image. Interpretation of boldness varies from study to study, and therefore I may have used an inappropriate metric for this

particular situation. One additional observation is that the aggressive response to the mirror in a novel environment may be considered a risk taking endeavor, as these fish were exposed over bare white tank bottoms and therefore potentially at greater perceived risk of predation than those that remained in the refuge.

The phenotypes' response to the mirror was somewhat different in the behavioral trials conducted with BY 2013 at the OHRC. While surface and bottom fish spent the same amount of time actively swimming, the bottom phenotype engaged in more direct contact with the mirror than surface fish. Surface fish were observed to orient to their mirror image perpendicularly for long periods of time but not make physical contact with the mirror to the extent that bottom fish did. Bottom fish also were seen to perform SAM behavior for many seconds to a minute before disengaging and returning to the gravel substrate. These repeated back and forth trips from the substrate to the mirror in fish of the bottom phenotype suggest territoriality. Comparing this to the surface fish, which were equally active, but engaged in little direct contact with the mirror, could be described more as general movement and perhaps even schooling behavior.

Previous studies with juvenile coho have shown that both agonistic response to a mirror image and depth of body may also predict dominance status and length of freshwater residency (Holtby et al. 1993). Taylor and Larkin (1986) also found that stream-type Chinook spent significantly more time engaging in SAM behavior compared to Chinook from an ocean-type population. In the BY 2013 behavioral trial the mirror was positioned closer to the substrate so that individuals did not have to venture out into an open brightly lit arena to engage with the mirror. This may also explain the extent to

which bottom fish interacted with the mirror, as in the BY 2012 behavioral assessment bottom fish spent very little time in the open arena.   In addition, for the BY 2013 OHRC rearing experiment, all fish were fed at the surface by hand that had previously demonstrated a positive effect on the number of mirror approaches in the 2013 behavioral assessment.

Despite the different trial set ups between facilities, my results suggest that surface fish may be more willing to spend time away from the gravel refuge to engage with their mirror image but when the mirror is in close proximity to gravel the bottom fish exhibit more aggressive behavior through physical contact with the mirror. For both phenotypes surface hand feeding was associated with increased mirror approaches that likely further drove the increased agonistic response of the bottom phenotype in BY 2013. Use of conventional hatchery rearing practices such as hand feeding has been associated with differences in behavior of hatchery fish compared to their wild counterparts. For example, in masu salmon (*O. masou*), Reinhardt (2001) compared orientation behavior of farmed, ocean ranched and wild stock juveniles and demonstrated both farmed and ranched fish were more surface oriented than wild juveniles, but that all fish became more surface oriented as they became conditioned to being fed pellets by hand at the surface.

A primary concern of the surface feeding method has been that surface oriented fish will be vulnerable to avian predators once released (Sosiak et al. 1979; Reinhardt 2001). Although some experiments have been conducted with Chinook to determine the benefits of employing subsurface feeders, the results have not yet demonstrated reduced

predation risk (Maynard et al. 1996b; Maynard et al. 2001b). This method for delivering

feed may drive polarization in size, foraging strategies and aggressive behavior,

especially in high density rearing conditions (Adams et al. 1998). This may drive

selection pressure for either highly competitive individuals or very non-competitive

individuals in a hatchery environment. Once released, however these behaviors may be

maladaptive and may increase competition with their wild counterparts.

Morphology

In the wild, morphological variation associated with foraging strategy and vertical

orientation in the water column has been observed primarily in lake dwelling

polymorphic species: brook trout (McLaughlin et al. 1994), arctic trout, stickleback

(Kristjansson et al. 2002) and several Coregonids (Bodaly 1979). In riverine habitats,

variation in morphology is often attributed to resident vs. anadromous forms, but also

between individuals occupying slow vs. fast moving water (Pakkasmaa and Piironen

2000; Imre et al. 2002; Langerhans et al. 2003; Keeley et al. 2007). Beeman *et al.* (1994)

found that migrating Chinook salmon smolts and anadromous rainbow trout demonstrate

deeper caudal peduncles compared to their resident counterparts.

In this laboratory study, I discovered that differences in vertical partitioning

observed in recently emerged Chinook salmon demonstrated variation in both body shape

and head morphology later in life. Across three brood years the surface phenotype

exhibited a shorter head, deeper body and caudal peduncle compared to the bottom

phenotype (see Chapter 2 Figures 3, 5a). Interestingly, the morphological attributes

observed in the surface fish was similar to fish captured at downstream mainstem site in the Willamette while the bottom phenotype more closely resembled the shape of fish rearing in the natal and upper tributaries of the McKenzie (Billman et al. 2014). The differences in head shape between the two phenotypes persisted and were detected at the end of both BY 2012 and BY 2013 rearing experiments, despite location of food delivery and rearing under different temperatures regimes (Figures 6a, 6b and 6c).

The variation in body morphology among the surface and bottom phenotypes reared at the OHRC was similar to the previous findings, in that the head shape of surface fish was generally shorter than the bottom phenotype. Unlike previous BYs 2011 and 2012, the depth of body was not greater in the surface fish at the OHRC treatments. At the time of the final morphometric comparison of the two phenotypes under the 12°C and 7°C rearing treatments, the average size was not significantly different which may account for the depth of body being similar, while head morphologies remained consistent. In feeding regime studies conducted with juvenile Chinook salmon, the depth of body in the trunk region has been shown to change in response to food availability and growth more so than in other regions of the body such as the caudal peduncle and head (Currens et al. 1989).

From a hatchery rearing perspective, these orientation phenotypes could be an artifact of captive rearing. In Atlantic salmon, similar vertical orientation behaviors and associated feeding strategies are observed under dense hatchery rearing conditions; morphological assessments were not conducted but size differences and degree of fin damage were noted (Adams et al. 1998). These aggressive surface feeding individuals

were found to be on average, larger in size and had experienced higher degrees of fin damage than the shy bottom feeders or intermediately aggressive fish that employed a sit-and-wait feeding tactic. In our study, however, fish were introduced to the stock tanks at densities considered low by conservation hatchery standards, i.e. less than 1.04 kg/m3/cm (Flagg and Nash 1999). At the time our morphological comparisons were made, the two phenotypes did not differ significantly in size (Table 2) nor exhibited evidence of fin damage from aggressive interaction (unpublished data). In my experience, even after removal of many fish from the stock tank, the remaining fish continued to maintain orientation to either the surface or the bottom of the tank for many months. Under conventional rearing practices these morphological differences and feeding strategies may be have been selected for through competition for food which is delivered at the surface.

The association between vertical orientation behaviors, body shape and head morphology is encouraging because it suggests that there may be at least two phenotypes present in hatchery origin fish that exhibit characteristics associated with different juvenile life-history tactics soon after emergence. My study however did not extend to the migration timing of these juveniles, so further research is needed to understand the role these early self-sorting behaviors may play in determining juvenile life-history tactics. Because this study is limited to an artificial environment, translation to naturally produced fish in the wild cannot be inferred; however, the shape differences observed between the surface and bottom phenotypes is consistent with morphological variation observed between wild Chinook juveniles rearing in mainstem habitats that migrate out

of the Willamette in the fall of their first year and tributary rearing, yearling smolts

(Billman et al. 2014).

Implications for Conservation and Management

The differences in growth trajectory, body and head morphology and agonistic

behavior between the two phenotypes suggest these groups may be on different migratory

trajectories, however; from these results alone I cannot infer when migration would occur

for these phenotypes in the wild. The questions of whether these early orientation

behaviors are present in the wild or the result of captive rearing, and to what extent the

behavior is genetically determined, remain to be answered. Studies have indicated that

behavioral syndromes in captive reared salmonids do exist and can be further

domesticated through selective breeding of proactive and reactive individuals (Pottinger

and Carrick 1999b), suggesting some behavioral traits are heritable and can be directly or

indirectly selected upon.

I have demonstrated for the first time that vertical self-sorting behavior occurs in

hatchery-origin Chinook juveniles and their vertical orientation is correlated with

variation in both body shape and head morphology.   This work suggests that these

phenotypes (and likely more) are present in a given rearing tank or raceway; therefore,

culture practices and management decisions that treat these fish as a homogenous group

have the potential to select for particular phenotypes. If these orientation phenotypes are

present but ignored, it could lead to loss of phenotype diversity. For example, in some

Willamette Hatchery stocks, fish exhibit a bimodal size distribution, when fish are

released as one group, some proportion may not have reached size thresholds and the physiological status to migrate out of the system which may lead to the loss of those individuals through predation (Cameron Sharpe pers. com).

There may be benefit in using this early behavior to develop targeted rearing methods for specific subyearling and yearling release groups that are better suited for fall or spring outmigration. Further research needs to be conducted in order to determine if behavioral phenotypes may be predictive of juvenile migration timing and the feasibility of isolating large numbers of surface and bottom phenotypes for developing scheduled release groups.

Our hatchery culture practices, management and conservation actions, whether through conscious or unconscious selection may alter expression of life-history tactics in imperiled wild fish. Whether these early behaviors are indicative of juvenile life-history tactics or result of generations of hatchery rearing, maintaining the diversity of phenotypes that are present is critical for both wild and hatchery stocks.

Bibliography

Adams, C.E., Huntingford, F.A., Turnbull, J.F., and Beattie, C. (1998). Alternative competitive strategies and the cost of food acquisition in juvenile Atlantic salmon (*Salmo salar*). Aquaculture 167: 17–26.

Angilletta Jr, M.J., Ashley Steel, E., Bartz, K.K., Kingsolver, J.G., Scheuerell, M.D., Beckman, B.R., and Crozier, L.G. (2008). Big dams and salmon evolution: changes in thermal regimes and their potential evolutionary consequences. Evolutionary Applications 1: 286–299.

Arendt, J.D., and Wilson, D.S. (2000). Population differences in the onset of cranial ossification in pumpkinseed (*Lepomis gibbosus*), a potential cost of rapid growth. Canadian Journal of Fisheries and Aquatic Sciences 57: 351–356.

Arendt, J., Wilson, D.S., and Stark, E. (2001). Scale strength as a cost of rapid growth in sunfish. Oikos, 93: 95–100.

Armstrong, J.D., Braithwaite, V.A., Huntingford, F.A. (1997). Spatial strategies of wild Atlantic salmon parr: Exploration and settlement in unfamiliar areas. The Journal of Animal Ecology 66: 203–211.

Beckman, B.R. (2002). Growth and the plasticity of smolting in Chinook salmon. Ph.D. Dissertation, University of Washington, Seattle, WA.

Beckman, B.R., Larsen, D.A., and Dickhoff, W.W. (2003). Life history plasticity in Chinook salmon: relation of size and growth rate to autumnal smolting. Aquaculture 222: 149–165.

Beeman, J.W., Rondorf, D.W., and Tilson, M.E. (1994). Assessing smoltification of juvenile spring Chinook salmon (*Oncorhynchus tshawytscha*) using changes in body morphology. Canadian Journal of Fisheries and Aquatic Sciences 51: 836–844.

Beer, W.N., and Anderson, J.J. (2001). Effect of spawning day and temperature on salmon emergence: interpretations of a growth model for Methow River chinook. Canadian Journal of Fisheries and Aquatic Sciences 58: 943–949.

Berejikian, B.A., Mathews, S.B., and Quinn, T.P. (1996). Effects of hatchery and wild ancestry and rearing environments on the development of agonistic behavior in steelhead trout (*Oncorhynchus mykiss*) fry. Canadian Journal of Fisheries and Aquatic Sciences 53: 2004–2014.

Billman, E.J., Whitman, L.D., Schroeder, R.K., Sharpe, C.S., Noakes, D.L.G., and Schreck, C.B., (2014). Body morphology differs in wild juvenile Chinook salmon (*Oncorhynchus tshawytscha)* that express different migratory phenotypes in the Willamette River, Oregon, U.S.A. Journal of Fish Biology 85: 1097–1110.

Blaser, R., and Gerlai, R. (2006). Behavioral phenotyping in zebrafish: comparison of three behavioral quantification methods. Behavior Research Methods 38: 456–469.

Brown, C., Burgess, F., and Braithwaite, V.A. (2007). Heritable and experiential effects on boldness in a tropical poeciliid. Behavioral Ecology and Sociobiology 62: 237–243.

Bochdansky, A.B., Grønkjær, P., Herra, T.P., and Leggett, W.C. (2005). Experimental evidence for selection against fish larvae with high metabolic rates in a food limited environment. Marine Biology 147:1413–1417.

Bodaly, RA. (1979). Morphological and ecological divergence within the lake whitefish (*Coregonus clupeaformis*) species complex in Yukon Territory. Journal of the Fisheries Research Board of Canada 36: 1214–1222.

Buchanan, R.A., Skalski, J.R., and Giorgi, A.E. (2010). Evaluating surrogacy of hatchery releases for the performance of wild yearling Chinook salmon from the Snake River Basin. North American Journal of Fisheries Management 30: 1258–1269.

Burnham, K.P., and Anderson, D.R. (2002). Model selection and multimodel inference: a practical information-theoretic approach, 2nd edition. Springer, New York.

Canty, A., and Ripley, B. (2014). boot: Bootstrap R (S-Plus) Functions. R package version1.3–11.

Chapman, B.B., Hulthén, K., Blomqvist, D.R., Hansson, L.-A., Nilsson, J.-Å., Brodersen, J., Anders Nilsson, P., Skov, C., and Brönmark, C. (2011). To boldly go: individual differences in boldness influence migratory tendency. Ecology Letters 14: 871–876.

Chittenden, C.M., Biagi, C.A., Davidsen, J.G., Davidsen, A.G., Kondo, H., McKnight, A., Pedersen, O., Raven, P.A., Rikardsen, A.H., Shrimpton, J.M., Zuehlke, B., McKinley, R.S., and Devlin, R.H. (2010). Genetic versus rearing-environment effects on phenotype: hatchery and natural rearing effects on hatchery and wild born coho salmon. PLoS ONE 5: e12261.

Coleman, K., and Wilson, D.S. (1998). Shyness and boldness in pumpkinseed sunfish: individual differences are context specific. Animal Behaviour 56: 927–936.

Conrad, J.L., and Sih, A. (2009). Behavioural type in newly emerged steelhead *Oncorhynchus mykiss* does not predict growth rate in a conventional hatchery rearing environment. Journal of Fish Biology 75: 1410–1426.

Crozier, L.G., Hendry, A.P., Lawson, P.W., Quinn, T.P., Mantua, N.J., Battin, J., Shaw, R.G., and Huey, R.B. (2008). Potential responses to climate change in organisms with complex life histories: evolution and plasticity in Pacific salmon: Evolutionary responses to climate change in salmon. Evolutionary Applications 1: 252–270.

Currens, K.P., Sharpe, C.S., Hjort, R., Schreck, C.B., and Li, H.W. (1989). Effects of different feeding regimes on the morphometrics of Chinook salmon (*Oncorhynchus tshawytscha*) and rainbow trout (*O. mykiss*). Copeia 1989: 689–695.

Cutts, C.J., Metcalfe, N.B., and Taylor, A.C. (1998). Aggression and growth depression in juvenile Atlantic salmon: the consequences of individual variation in standard metabolic rate.Journal of Fish Biology 52: 1026–1037.

Davison, A.C., and Hinkley, D.V. (1997). Bootstrap Methods and Their Applications. Cambridge University Press, Cambridge.

Farwell, M., Fuzzen, M.L.M., Bernier, N.J., and McLaughlin, R.L. (2014). Individual differences in foraging behavior and cortisol levels in recently emerged brook charr (*Salvelinus fontinalis*). Behavioral Ecology and Sociobiology 68: 781–790.

Farwell, M., and McLaughlin, R.L. (2009). Alternative foraging tactics and risk taking in brook charr (*Salvelinus fontinalis*). Behavioral Ecology 20: 913–921.

Finstad, A.G., Forseth, T., Ugedal, O., and NæSje, T.F. (2007). Metabolic rate, behavior and winter performance in juvenile Atlantic salmon. Functional Ecology 21: 905–912.

Flagg, T.A., and Nash, C.E. (1999). A conceptual framework for conservation hatchery strategies for Pacific salmonids. NOAA Technical Memo NMFS-NWFSC-38. 2725 Montlake Blvd. E. Seattle, WA 98112.

Fournier D.A., Skaug H.J., Ancheta J., Ianelli J., Magnusson A., Maunder M., Nielsen, A., and Sibert J. (2012). "AD Model Builder: using automatic differentiation for statistical inference of highly parameterized complex nonlinear models." Optimization Methods and Software 27: 233–249.

Fritts, A.L., Scott, J.L., and Pearsons, T.N. (2007). The effects of domestication on the relative vulnerability of hatchery and wild origin spring Chinook salmon (*Oncorhynchus tshawytscha*) to predation. Canadian Journal of Fisheries and Aquatic Sciences 64: 813-818.

Fulton, T. W. (1904). The rate of growth of fishes. Twenty-second Annual Report, Part III. Fisheries Board of Scotland, Edinburgh, 141–241

Gilbert, C. H. (1912). Age at maturity of the Pacific coast salmon of the genus *Oncorhynchus*. Bulletin of the Bureau of Fisheries (U. S.) 32: 1–22.

Hassell, E.M.A., Meyers, P.J., Billman, E.J., Rasmussen, J.E., and Belk, M.C. (2012). Ontogeny and sex alter the effect of predation on body shape in a livebearing fish: sexual dimorphism, parallelism, and costs of reproduction. Ecology and Evolution 2: 1738–1746.

Healey, M.C. (1991). Life history of Chinook salmon (*Oncorhynchus tshawytscha*). Pages 313–393 in Groot, C. and Margolis, L., editors. Pacific Salmon Life Histories. UBC Press, Vancouver, British Columbia.

Hoar, W.S. (1953). Control and timing of fish migration. Biological Reviews 28: 437–452.

Hoar, W.S. (1976). Smolt transformation: evolution, behavior, and physiology. Journal of the Fisheries Board of Canada 33: 1233–1252.

Holtby, L.B., Swain, D.P., and Allan, G.M. (1993). Mirror-elicited agonistic behavior and body morphology as predictors of dominance status in juvenile coho salmon (*Oncorhynchus kisutch*). Canadian Journal of Fisheries and Aquatic Sciences 50: 676–684.

Hothorn, T. Bretz, F., and Westfall, P. (2008). Simultaneous inference in general parametric models. Biometrical Journal 50: 346–363.

Huntingford, F., and Adams, C. (2005). Behavioural syndromes in farmed fish: implications for production and welfare. Behaviour 142: 1213–1228.

Imre, I., McLaughlin, R.L., and Noakes, D.L.G. (2002). Phenotypic plasticity in brook charr: changes in caudal fin induced by water flow. Journal of Fish Biology 61: 1171–1181.

Iwata, M. (1995). Downstream migratory behavior of salmonids and its relationship with cortisol and thyroid hormones: a review. Aquaculture 135: 131–139.

Jonsson, B. (1985). Life history patterns of freshwater resident and sea-run migrant brown trout in Norway. Transactions of the American Fisheries Society 114: 182–194.

Jonsson B and Jonsson, N. (1993). Partial migration: niche shift versus sexual maturation in fishes. Reviews in Fish Biology and Fisheries 3: 348–365.

Jonsson, B., Jonsson, N., Brodtkorb, E., and Ingebrigtsen, P.J. (2001). Life-history traits of brown trout vary with the size of small streams. Functional Ecology 15: 310–317.

Jonsson, B., Jonsson, N., and Hansen, L.P. (1991). Differences in life history and migratory behavior between wild and hatchery-reared Atlantic salmon in nature. Aquaculture 98: 69–78.

Keeley, E.R., Parkinson, E.A., and Taylor, E.B. (2007). The origins of ecotypic variation of rainbow trout: a test of environmental vs. genetically based differences in morphology. Journal of Evolutionary Biology 20: 725–736.

Kihslinger, R.L., Lema, S.C., and Nevitt, G.A. (2006). Environmental rearing conditions produce forebrain differences in wild Chinook salmon *Oncorhynchus*

tshawytscha. Comparative Biochemistry and Physiology-Part A: Molecular and Integrative Physiology 145: 145-151.

Konietschke, F. (2012). nparcomp: Perform multiple comparisons and compute simultaneous confidence intervals for the nonparametric relative contrast effects. R package version 2.0.

Koolhaas, J.M., Korte, S.M., De Boer, S.F., Van Der Vegt, B.J., Van Reenen, C.G., Hopster, H., De Jong, I.C., Ruis, M.A.W., and Blokhuis, H.J. (1999). Coping styles in animals: current status in behavior and stress-physiology. Neuroscience and Biobehavioral Reviews 23: 925–936.

Kristjansson, B.K., Skulason, S., and Noakes, D.L.G. (2002). Morphological segregation of Icelandic threespine stickleback (*Gasterosteus aculeatus* L). Biological Journal of the Linnean Society 76: 247–257.

Langerhans, R.B., Layman, C.A., Langerhans, A.K., and Dewitt, T.J. (2003). Habitat associated morphological divergence in two Neotropical fish species. Biological Journal of the Linnean Society 80: 689–698.

Langerhans, R.B., Layman, C.A., Mona Shokrollahi, A., and DeWitt, T.J. (2004). Predator driven phenotypic diversification in *Gambusia affinis*. Evolution 58: 2305–2318.

Larsen, D.A., Beckman, B.R., Cooper, K.A., Barrett, D., Johnston, M., Swanson, P., and Dickhoff, W.W. (2004). Assessment of high rates of precocious male maturation in a spring Chinook salmon supplementation hatchery program. Transactions of the American Fisheries Society 133: 98–120.

Larsen, D.A., Beckman, B.R., Strom, C.R., Parkins, P.J., Cooper, K.A., Fast, D.E., and Dickhoff, W.W. (2006). Growth modulation alters the incidence of early male maturation and physiological development of hatchery-reared spring Chinook salmon: a comparison with wild fish. Transactions of the American Fisheries Society 135: 1017–1032.

Leblanc, C.A., and Noakes, D.L.G. (2012). Visible Implant Elastomer (VIE) tags for marking small rainbow trout. North American Journal of Fisheries Management 32: 716–719.

Lumley, T. using Fortran code by Alan Miller (2009). leaps: regression subset selection. R package version 2.9. http://CRAN.R-project.org/package=leaps

Lundqvist, H., McKinnell, S., Fängstam, H., and Berglund, I. (1994). The effect of time, size and sex on recapture rates and yield after river releases of *Salmo salar* smolts. Aquaculture 121: 245–257.

Martin, W.R. (1949). The mechanics of environmental control of body form in fishes. Publications of the Ontario Fisheries Research Laboratory, University of Toronto Studies: Biology Series 70: 1–72.

Maynard, D.J., Crewson, M., Tezak, E.P., McAuley, W.C., Schroder, S.L., Knudsen, C., Flagg, T.A., and Mahnken, C.V.W. (1996b). The postrelease survival of Satsop River fall Chinook salmon reared in conventional and seminatural raceway habitats, 1994. Pages 78–97 in D.J. Maynard, T.A. Flagg, and C.V.W. Mahnken, editors. Development of a Natural Rearing System to Improve Supplemental Fish Quality 1991-1995. Bonneville Power Administration, Portland, Oregon.

Maynard, D.J., Hackett, G.J.L., Wastel, M., LaRae, A.L., McDowell, C., Flagg, T.A., and Mahnken, C.V.W. (2001b). Effect of automated sub-surface feeders on behavior and predator vulnerability of fall Chinook salmon. Pages 6–19 in D.J. Maynard, B.A. Berejikian, T.A. Flagg, C.V.W. Mahnken, editors. Development of a natural rearing system to improve supplemental fish quality 1996-1998. Bonneville Power Administration, Portland, Oregon.

McLaughlin, R.L., Ferguson, M.M., and Noakes, D.L.G. (1999). Adaptive peaks and alternative foraging tactics in brook charr: Evidence of short-term divergent selection for sitting-and waiting and actively searching. Behavioral Ecology and Sociobiology 45: 386–395.

McLaughlin, R.L., Grant, J.W.A., and Kramer, D.L. (1994). Foraging movements in relation to morphology, water-column use, and diet for recently emerged brook trout (*Salvelinus fontinalis*) in still-water pools. Canadian Journal of Fisheries and Aquatic Sciences 51: 268–279.

Metcalfe, N.B. (1998). The interaction between behavior and physiology in determining life history patterns in Atlantic salmon (*Salmo salar*). Canadian Journal of Fisheries and Aquatic Sciences 55: 93–103.

Metcalfe, N.B., Huntingford, F.A., and Thorpe, J.E. (1986). Seasonal changes in feeding motivation of juvenile Atlantic salmon (*Salmo salar*). Canadian Journal of Zoology 64: 2439–2446.

Metcalfe, N.B., Taylor, A.C., and Thorpe, J.E. (1995). Metabolic rate, social status and life history strategies in Atlantic salmon. Animal Behaviour 49: 431–436.

Metcalfe, N.B., and Thorpe, J.E. (1992). Early predictors of life-history events: the link between first feeding date, dominance and seaward migration in Atlantic salmon, *Salmo salar* L. Journal of Fish Biology 41: 93–99.

Millidine, K.J., Armstrong, J.D., and Metcalfe, N.B. (2009). Juvenile salmon with high standard metabolic rates have higher energy costs but can process meals faster. Proceedings of the Royal Society B: Biological Sciences 276: 2103–2108.

Morinville, G.R., and Rasmussen, J.B. (2003). Early juvenile bioenergetic differences between anadromous and resident brook trout (*Salvelinus fontinalis*). Canadian Journal of Fisheries and Aquatic Sciences 60: 401–410.

Murray, C.B., and McPhail, J.D. (1988). Effect of incubation temperature on the development of five species of Pacific salmon (*Oncorhynchus*) embryos and alevins. Canadian Journal of Zoology 66: 266–273.

National Marine Fisheries Service (NMFS). (2008). Willamette River Basin Flood Control Project. Biological Opinion. July 11, 2008, NMFS, Northwest Region, Seattle,Washington.

O'Malley, K.G., Camara, M.D., and Banks, M.A. (2007). Candidate loci reveal genetic differentiation between temporally divergent migratory runs of Chinook salmon (*Oncorhynchus tshawytscha*). Molecular Ecology 16: 4930–4941.

Pakkasmaa, S and Piironen, J. (2000). Water velocity shapes juvenile salmonids. Evolutionary Ecology 14: 721–730.

Patiño, R., Schreck, C.B., Banks, J.L., and Zaugg, W.S. (1986). Effects of rearing conditions on the developmental physiology of smolting coho salmon. Transactions of the American Fisheries Society 115: 828–837.

Perkins, T.A., and Jager, H.I. (2011). Falling behind: delayed growth explains life-history variation in Snake River fall Chinook salmon. Transactions of the American Fisheries Society 140: 959–972.

Pottinger, T.G., and Carrick, T.R. (1999b). Modification of the plasma cortisol response to stress in rainbow trout by selective breeding. General and comparative endocrinology 116: 122-132.

Pottinger, T.G., and Carrick, T.R. (2001). Stress responsiveness affects dominant subordinate relationships in rainbow trout. Hormones and Behavior 40: 419–427.

Priede, I.G. (1985). Metabolic scope in fishes. Pages 33-64 in Tytler, P., and Calow, P., editors. Fish Energetics: New Perspectives, London: Croom Helm.

Quinn, T.P. (2005). The Behavior and Ecology of Pacific Salmon and Trout, University of Washington Press, Seattle, Washington.

Quinn, T.P., Unwin, M.J., and Kinnison, M.T. (2000). Evolution of temporal isolation in the wild: genetic divergence in timing of migration and breeding by introduced Chinook salmon populations. Evolution 54: 1372–1385.

R Core Team (2014). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.Rproject.org/.

Rasmussen, J.E., and Belk, M.C. (2012). Dispersal behavior correlates with personality of a North American fish. Current Zoology 58: 260–270.

Reimers, P.E. (1973). The length of residence of juvenile fall Chinook salmon in Sixes River, Oregon. Research reports of the fish commission of Oregon 4: 1-43.

Reinhardt, U.G. (2001). Selection for surface feeding in farmed and sea-ranched masu salmon juveniles. Transactions of the American Fisheries Society 130: 155–158.

Rodnick, K.J., St.-Hilaire, S., Battiprolu, P.K., Seiler, S.M., Kent, M.L., Powell, M.S., and Ebersole, J.L. (2008). Habitat selection influences sex distribution, morphology, tissue biochemistry, and parasite load of juvenile coho salmon in the West Fork Smith River, Oregon. Transactions of the American Fisheries Society 137: 1571–1590.

Rohlf, F.J. (2003). tpsRegr, shape regression, version 1.28. Department of Ecology and Evolution, State University of New York at Stony Brook.

Rohlf, F.J. (2010a). tpsDig, version 2.16. Department of Ecology and Evolution, State University of New York at Stony Brook.

Rohlf, F.J. (2010b). tpsRelw, relative warps, version 1.49. Department of Ecology and Evolution, State University of New York at Stony Brook.

Rohlf, F.J., and Slice, D.E. (1990). Extensions of the procrustes method for the optimal superimposition of landmarks. Systematic Biology 39: 40–59.

Rowe, D.K., Thorpe, J.E., and Shanks, A.M. (1991). Role of fat stores in the maturation of male Atlantic salmon (*Salmo salar*) parr. Canadian Journal of Fisheries and Aquatic Sciences 48: 405–413.

SAS Institute. (2008). SAS 9.2 help and documentation. Cary, NC.

Schjolden, J., Stoskhus, A., and Winberg, S. (2005). Does individual variation in stress responses and agonistic behavior reflect divergent stress coping strategies in juvenile rainbow trout? Physiological and Biochemical Zoology 78: 715–723.

Schroeder, R.K., Kenaston, K.R., and McLaughlin, L.K. (2006). Spring Chinook salmon in the Willamette and Sandy Rivers. (Annual Progress Report No. F-163-R 11/12). Oregon Department of Fish and Wildlife Salem, Oregon.

Scott, L.E., and Johnson, J.B. (2010). Does sympatry predict life history and morphological diversifications in the Mexican livebearing fish *Poeciliopsis baenschi*? Biological Journal of the Linnean Society 100: 608-618.

Shearer, K., Parkins, P., Gadberry, B., Beckman, B., and Swanson, P. (2006). Effects of growth rate/body size and a low lipid diet on the incidence of early sexual maturation in juvenile male spring Chinook salmon (*Oncorhynchus tshawytscha*). Aquaculture 252: 545–556.

Sheer, M.B., and Steel, E.A. (2006). Lost watersheds: barriers, aquatic habitat connectivity, and salmon persistence in the Willamette and Lower Columbia River Basins. Transactions of the American Fisheries Society. 135: 1654–1669.

Shrimpton, M.J., Bernier, N.J., and Iwama, G.K. (1994). Differences in measurements of smolt development between wild and hatchery-reared juvenile coho salmon

(*Onchorhynchus kisutch*) before and after saltwater exposure. Canadian Journal of Fisheries and Aquatic Sciences 51: 2170–2178.

Sih, A., Bell, A.M., Johnson, J.C., and Ziemba, R.E. (2004). Behavioral Syndromes: An Integrative Overview. The Quarterly Review of Biology 79: 241–277.

Skaug H, Fournier D, Bolker B, Magnusson A and Nielsen A (2014). Generalized Linear Mixed Models using AD Model Builder_. R package version 0.8.0.

Sloat, M.R., Reeves, G.H., and Jonsson, B. (2014). Individual condition, standard metabolic rate, and rearing temperature influence steelhead and rainbow trout (*Oncorhynchus mykiss*) life histories. Canadian Journal of Fisheries and Aquatic Sciences 71: 491–501.

Sosiak, A.J., Randall, R.G., and McKenzie, J.A. (1979). Feeding by hatchery-reared and wild Atlantic salmon (*Salmo salar*) parr in streams. Journal of Fisheries Research Board Canada 36: 1408–1412.

Stamps, J.A. (2007). Growth-mortality tradeoffs and personality traits in animals. Ecology Letters 10: 355–363.

Swain, D.P., Riddell, B.E. (1990). Variation in agonistic behavior between newly emerged juveniles from hatchery and wild populations of coho salmon, *Oncorhynchus kisutch*. Canadian Journal of Fisheries and Aquatic Sciences 47: 566–571.

Taylor, E.B., and Larkin, P.A. (1986). Current response and agonistic behavior in newly emerged fry of Chinook salmon, *Oncorhynchus tshawytscha*, from ocean-and stream-type populations. Canadian Journal of Fisheries and Aquatic Sciences 43: 565–573.

Tiffan, K.F., and Connor, W.P. (2011). Distinguishing between natural and hatchery Snake River fall Chinook salmon subyearlings in the field using body morphology. Transactions of the American Fisheries Society 140: 21–30.

Toms, C.N., Echevarria, D.J., and Jouandot, D.J. (2010). A methodological review of personality related studies in fish: focus on the shy-bold axis of behavior. International Journal of Comparative Psychology 23: 1–25.

Venables, W. N., and Ripley, B. D. (2002) Modern Applied Statistics with S. Fourth Edition. Springer, New York.

Vincent, R.E. (1960). Some influences of domestication upon three stocks of brook trout (*Salvelinus fontinalis* Mitchill). Transactions of the American Fisheries Society 89: 35–52.

Warren, C.E., and Davis, G.E. (1967). Laboratory studies on the feeding bioenergetics, and growth of fish. Pages 175–214 in Gerking, S.D., editor. The biological basis of fresh water fish production, Blackwell Scientific Publications, Oxford, UK.

Waples, R.S. et al. (2001). Characterizing diversity in salmon from the Pacific Northwest. Journal of Fish Biology 59: 1–41.

Waples, R.S., Teel, D.J., Myers, J.M., and Marshall, A.R. (2004). Life-history divergence in Chinook salmon: historic contingency and parallel evolution. Evolution 58: 386–403.

Wesner, J. S., Billman, E. J., Meier, A., and Belk, M. C. (2011). Morphological convergence during pregnancy among predator and nonpredator populations of the livebearing fish *Brachyrhaphis rhabdophora* (Teleostei: Poeciliidae). Biological Journal of the Linnean Society 104: 386–392.

Webb, PW. (1984). Body form, locomotion, and foraging in aquatic vertebrates. American Zoologist 24: 107-120.

Wessel, M.L., Smoker, W.W., and Joyce, J.E. (2006). Variation of morphology among juvenile Chinook salmon of hatchery, hybrid, and wild origin. Transactions of the American Fisheries Society 135: 333–340.

Wilson, A.D., and McLaughlin, R.L. (2007). Behavioural syndromes in brook charr, *Salvelinus fontinalis*: prey-search in the field corresponds with space use in novel laboratory situations. Animal Behaviour 74: 689–698.

Wilson, A.D., and McLaughlin, R.L. (2010). Foraging behaviour and brain morphology in recently emerged brook charr, *Salvelinus fontinalis*. Behavioral Ecology and Sociobiology 64: 1905–1914.

Wolf, M., Van Doorn, G.S., Leimar, O., and Weissing, F.J. (2007). Life-history trade-offs favour the evolution of animal personalities. Nature 447: 581–584.

Wood, E.M., Yasutake, W.T., Woodall, A.N., and Halver, J.E. (1957). The nutrition of salmonid fishes. I. chemical and histological studies of wild and domestic fish. Journal of Nutrition. American Society of Nutrition 61: 465–478.

Zabel, R.W., and Williams, J.G. (2002). Selective mortality in Chinook salmon: what is the role of human disturbance? Ecological Applications 12: 173–183.

Zimmerman, M.S., Krueger, C.C., and Eshenroder, R.L. (2006). Phenotypic diversity of lake trout in Great Slave Lake: differences in morphology, buoyancy, and habitat depth. Transactions of the American Fisheries Society 135: 1056–1067.