Domestication Issues in Alaska Chinook Salmon: Maturation Timing, Egg Size, and Fecundity of Hatchery Chinook Salmon and Wild Donor Stocks

By
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Roy Martin, former station manager at Little Port Walter field station, holds a 60-lb, 6-year-old chinook salmon from the Little Port Walter Chickamin River stock.

Scientists and fisheries managers are concerned about the potential genetic effects of hatchery salmon on native (wild) salmon populations. A great deal of recent research focuses on the impacts of hatchery salmon on wild populations. Stray fish from hatchery releases may invade wild systems, interbreeding with wild fish and competing for limited resources. In extreme cases, native fish stocks may be displaced by introduced hatchery fish.

Avoiding such outcomes is one reason that a genetic management policy for Alaska's Pacific salmon resources was established in 1985. The policy mandates using local wild salmon as donor stocks for hatchery populations, to minimize genetic differences between hatchery fish and the wild fish populations they are intended to enhance. The state of Alaska controls the transport of fish and gametes, using a detailed fish transport permitting process that integrates recommendations from geneticists, pathologists, and fisheries management specialists. This effort focuses on maintaining the health and integrity of Alaska's wild salmon populations.

Despite the best efforts, domestication of hatchery salmon stocks can lead to divergence from wild salmon populations. The hatchery environment, particularly early in salmon life history, is very different from the wild environment, and selection pressures may differ. It is quite possible that domestication could produce changes in morphological and behavioral traits important for survival. For example, specific local adaptation to incubation temperature is an important fitness characteristic that has a genetic basis. Thermal regimes used in hatcheries to incubate and rear juvenile salmon often differ from conditions that wild donor salmon stocks have adapted to, and hatchery salmon may adapt to these different thermal regimes.

Domestication could also result in changes in foraging behavior and predator avoidance. Hatchery and wild rearing environments differ greatly in the availability of food and the presence (or absence) of predators. Hatchery stocks of chinook salmon (*Oncorhynchus tshawytscha)* in Southeast Alaska are derived from native salmon stocks. In their natural habitat, the salmon commonly rear in mainland rivers and migrate as age-1 smolts weighing 4 to 6 grams. During freshwater rearing, the fish forage on a variety of prey organisms throughout the water column. When cultured in regional hatcheries until they are age-1 smolts, chinook salmon typically receive a diet of fish food pellets presented at the water surface and reach a weight of 15 to 40 grams. Large hatchery smolts have been protected from predation during the freshwater rearing stage and because of their much larger size at release, are able to avoid many of the natural predators that wild salmon are exposed to in the marine environment. Such differences can ultimately affect growth and long-term survival of fish.

The degree to which domestication causes changes related to fitness in hatchery salmon is controversial; evidence is often circumstantial and based on studies that were not designed to examine divergence

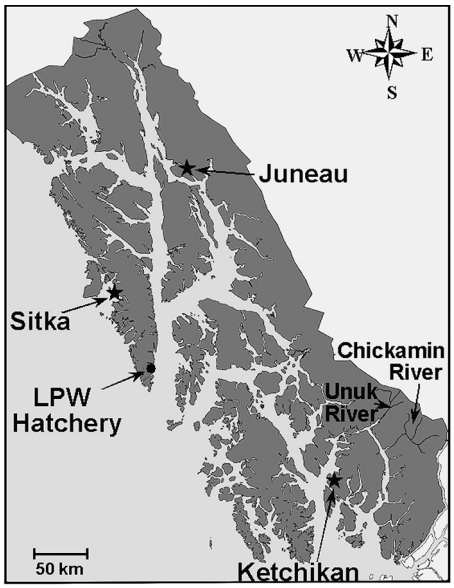


Figure 1. Locations in Southeast Alaska for the Little Port Walter research station, and the Unuk and Chickamin rivers (source of the wild chinook salmon used in this study).

from a common donor stock, or that do not separate genetic effects from the effects of environmental rearing conditions. A few studies have shown that domestication can cause genetic differences, relative to the natural donor stock, that affect behavior, growth, and survival of juvenile hatchery fish. Recent experimental evidence suggests that captive salmon may undergo rapid selection for egg size in the hatchery environment; egg size is closely related to condition during incubation and early juvenile rearing. Whether observed differences are truly genetic in nature, are the result of environmental changes, or simply represent plasticity for the characters in question remains unanswered.

A unique opportunity to compare wild salmon populations with hatchery salmon stocks exists at the Little Port Walter (LPW) Research Station in southeastern Alaska. Little Port Walter is a small bay on the Chatham Strait side of lower Baranof Island (Fig. 1) and is Alaska's oldest year-round biological research station. Operated by the Alaska Fisheries Science Center's Auke Bay Laboratory (ABL), LPW has a long history of research on Alaska fishes, invertebrates, and other marine resources. In fact, 2004 marks the 70th anniversary of federal fisheries research at Little Port Walter.

Since 1976, scientists at LPW have conducted research on chinook salmon. Early research focused on developing brood stocks and salmon enhancement technologies to support salmon ocean ranching (releasing fish from hatcheries into the wild environment) efforts in Alaska. Finfish farming (using net pens to raise fish for human consumption) is illegal in Alaska, but fisheries enhancement through ocean ranching is widespread.

Chinook salmon research and development at LPW has been primarily motivated by the need to mitigate for catch restrictions—restrictions stemming from declining stocks and subsequent conservation measures imposed by the Pacific Salmon Treaty between the United States and Canada. Production goals were established to produce chinook salmon that would contribute to fisheries and be excluded from treaty imposed quotas. In Southeast Alaska, hatchery production has succeeded in enhancing both commercial and recreational fisheries. Chinook salmon production from Southeast Alaska hatcheries exceeded 6.5 million juveniles in 2002; during the same year, the total return (catch plus hatchery returns) of adult chinooks in Southeast Alaska that originated from Alaska hatcheries was 135,000.

For the research at LPW, two distinct chinook salmon stocks have been developed through ocean ranching of tagged smolts. The Chickamin River stock resulted from a small collection of wild brood stock in 1976. The Unuk River stock was also developed in 1976, but from a larger number of individuals; the stock has periodically been infused with gametes from wild salmon. Scientists at LPW are now able to compare undisturbed wild populations of chinook salmon with fifth and sixth generation hatchery stocks that originated from the wild fish. Using the station's fish culture facilities, progeny of the various experimental groups can be cultured in a common environment, minimizing environmental bias.

In ongoing research that began in 1996, ABL scientists at LPW are examining the two hatchery brood stocks of chinook salmon and comparing them with the native donor stocks to collect evidence of divergence in important life-history characteristics. Research to date includes remote egg takes, genetic analysis, and experimental behavioral trials with juvenile salmon. Scientists have also done morphometric comparisons of hatchery and wild fish, and studies of marine survival. Two graduate students from the University of Alaska Fairbanks, Juneau Center for Fisheries and Ocean Science have conducted thesis work as part of these studies.

For our study, we compared chinook salmon progeny from the 1996 wild fish, hatchery fish, and the cross of hatchery and wild fish for each river

system. Our objective was to determine if domestication in the hatchery stocks has resulted in genetic-based differences in maturation timing, fecundity, or egg size.

Methods

Chinook salmon used for the research originated from the Chickamin and Unuk rivers, which drain into Behm Canal in Southeast Alaska (Fig. 1). Both rivers are large, cold, glacially fed systems near the U.S.-Canada border in Southeast Alaska. Chickamin River chinook salmon spawners were taken from either the Barrier Creek tributary or the South Fork of the Chickamin River within 2 km of Barrier Creek. In 1976, a hatchery population of Chickamin River chinook salmon was established at LPW using gametes from six females and eight males. Smolts from this population were tagged with stock-specific coded-wire tags; only spawners of Chickamin origin (identified by tags) were used to maintain the brood line at LPW. In 1996, fourth-generation progeny returned to LPW from the original transplant. Also during 1996, gametes from wild fish in the Chickamin River were collected with Alaska Department of Fish and Game (ADF&G) personnel; fish were from the same spawning area used in 1976. Unfertilized gametes from 5 females and 14 males were transported by helicopter to LPW on 12 August 1996 (ADF&G requirements limited collection to 5 females due to concern about adequate escapement to the system).

Unuk River chinook salmon spawners were taken from Cripple Creek, a clearwater tributary about 30 km from the river mouth. A hatchery population was established at LPW using gametes collected from wild chinook salmon in 1976-81 (n = 250). In 1998, fourth-generation progeny returned to LPW, and wild gametes were obtained from the Cripple Creek spawning area of the Unuk River. Unfertilized gametes from 10 females and 12 males were transported to LPW by helicopter on 5 August 1998. The remoteness of these rivers provides protection from development and human influence, but also creates significant logistical impediments to obtaining information and samples from the salmon populations there. Considerable logistical support from the ADF&G enabled collection of fish and samples for this study.

Reciprocal crosses were made at LPW between wild fish and hatchery fish for the Chickamin stock in 1996 and the Unuk stock in 1998, resulting in four categories of progeny for each stock: 1) hatchery female × hatchery male (HH), 2) hatchery female × wild male (HW), 3) wild female × hatchery male (WH), and 4) wild female × wild male (WW). Fertilized eggs were incubated in vertical tray incubators at LPW from August through hatching in November and December. Egg survival data were collected several times during incubation. Chilled incubation water was used during some of the incubation period to mitigate for the warmer lake-fed water at LPW. Fry were transferred from the incubators to freshwater raceways in April (3-4 months after hatching). Fish from the four treatment groups were cultured in vertical raceways for approximately 1 year, tagged with group-specific coded-wire tags, and released to the estuary at LPW in mid-May.

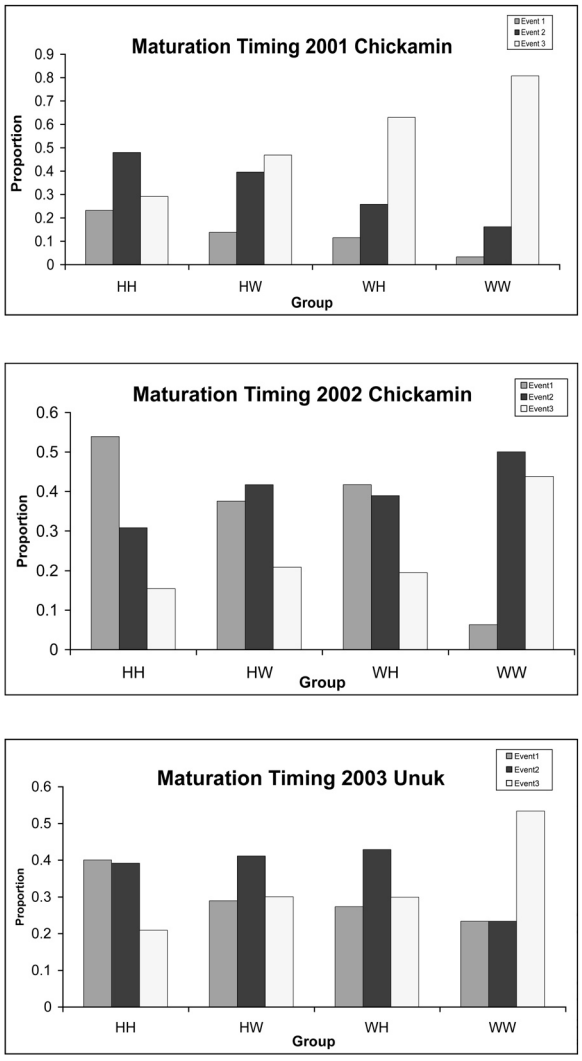


Figure 2. Maturation timing for female hatchery wild chinook salmon returning to LPW, showing proportions of females by group maturing at early, middle, and late spawning events.

Female chinook salmon in Southeast Alaska typically mature at age 5 or 6 before returning to their home stream (or hatchery) for spawning. We collected mature female salmon for the study in 2001 (Chickamin), 2002 (Chickamin), and 2003 (Unuk). Returning fish were captured in the inner bay of LPW in June, July, and August of those years and were held in ripening pens until mature; females were checked every 6-8 days for maturity. The spawning period generally extends from 1 to 21 August. During this time, mature females were spawned in three consecutive weekly spawning events (Events 1, 2, and 3). At the time of spawning, the fish were sacrificed and photographed. A unique code was assigned to each fish and a variety of tissue samples and measurements were taken. The cross type of each fish was confirmed by coded-wire tag detection and code verification. In addition, because some mixing of groups occurred prior to tagging in 1997, the identity of each Chickamin adult was confirmed by micro-satellite DNA analysis.

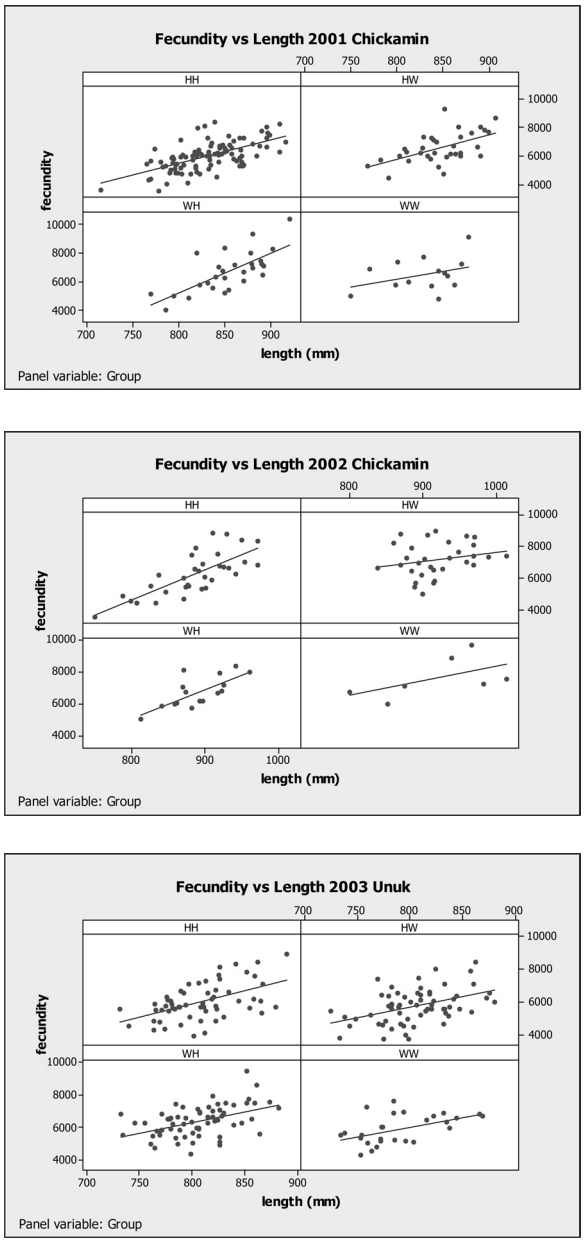


Figure 3. Relationship between fecundity and length for experimental groups of chinook salmon returning to LPW in 2001, 2002, and 2003.

Using standard hatchery procedure, female fish were bled by cutting into the caudal peduncle prior to removing the eggs from the body cavity. After bleeding, the total egg volume was removed from the fish, collected in plastic bags, and stored on ice prior to spawning. Just prior to mixing of gametes, the eggs for each female were drained of ovarian fluid in a screened bucket for 30 seconds so that the total egg production could be weighed. At this time, 50 unfertilized eggs were counted and weighed. Ten unfertilized eggs were preserved in a 10% formalin solution so they could be individually weighed later. These eggs were individually weighed to the nearest milligram after curing for approximately 6 months.

Fecundity and grouped egg size data were analyzed using the Minitab General Linear Model (GLM) procedure. Effects of cross type (HH, HW, WH, WW) were estimated for each variable. Because egg characteristics are related to female size, female length was included as a covariate in the model. An interaction term was also included in the model to test for equality of slopes among cross types. If interaction was not detected, the term was dropped from the model. All pair-wise comparisons were made among groups if the effect was significant. Tukey's multiple comparison method was used with an overall confidence level of 95%. Individual egg data from the 10-egg sample were analyzed with a nested GLM procedure to measure the variation within and among females nested within cross type. Females were treated as a random factor while cross type was treated as a fixed factor.

Results

FEMALE MATURATION TIMING

Female offspring of wild chinook salmon tended to mature later in the spawning season than hatchery fish (Fig. 2). This was evident for all years; the

lack of wild fish early in the season and hatchery fish late in the season created significant logistical problems in producing experimental crosses. Age of female maturation (either age 5 or 6) did not differ significantly for the Chickamin stock cross types.

LENGTH ADJUSTED FECUNDITY

In all cases, there was a strong positive relationship between female length and fecundity (Fig. 3). For this reason, all fecundity data were analyzed with a length covariance model to adjust raw fecundity for female egg size. In both 2001 and 2002, Chickamin WW females had the highest fecundity (adjusted for female size) and HH females had the lowest (Fig. 4). Females from the HW and WH crosses had intermediate mean fecundity. Length was a significant covariate in all fecundity analyses. The interaction term was also significant in year 2001 and 2002 Chickamin experiments, indicating differential length vs. fecundity relationships among the cross types. The GLM analysis indicated a significant effect of female parentage on fecundity in the Chickamin experiment. However, none of the four cross-type mean pair-wise comparisons were significant.

In contrast, the adjusted mean fecundities of WW and HH females were very similar in 2003 Unuk stock experiment (Fig. 4). The HW crosses had lower mean fecundity and the WH crosses had higher fecundity when compared with the HH and WW females. The length covariate was highly significant in 2003, but the interaction of length and cross type was not. When the reduced model GLM was run excluding interaction from the model, the effect of cross type was highly significant. Pair-wise comparisons indicated a difference in the mean adjusted fecundity between the HH cross type and the two HW and WH cross types.

INDIVIDUAL EGG SIZE

Analysis of the 50-egg weight samples indicated a significant relationship between female length and mean egg size in all years. Fecundity was strongly related to female length in the Chickamin females (Fig. 3), while mean egg size was not strongly related to female length (Fig. 5); adjusted mean egg weights did not vary from raw weights in any of the 3 years. The Chickamin 2001 and 2002 cross types did not differ from the 50-egg sample with respect to mean egg weight; the 2003 Unuk cross type did

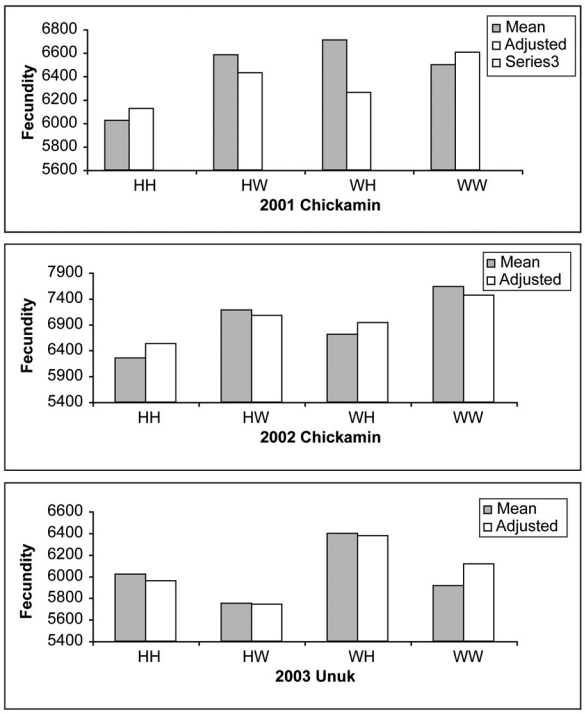


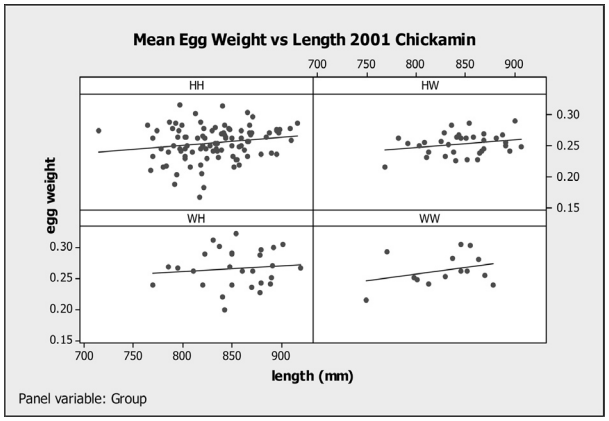
Figure 4. Raw and adjusted mean fecundity for experimental groups of chinook salmon returning to LPW in 2001, 2002, and 2003.

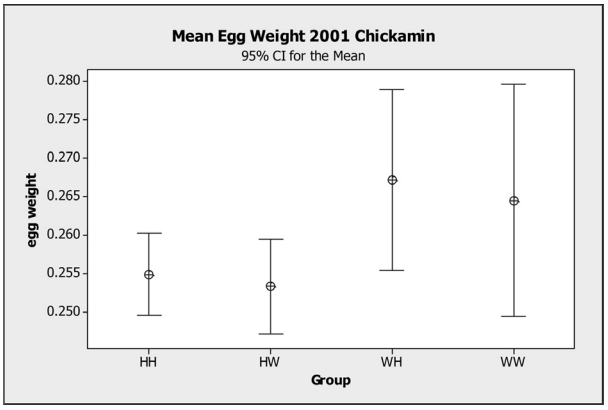
differ in mean egg weight. Tukey's pair-wise comparisons indicated that HW eggs (mean weight) were larger than HH eggs, and that HH eggs were larger than WH eggs (Fig. 6).

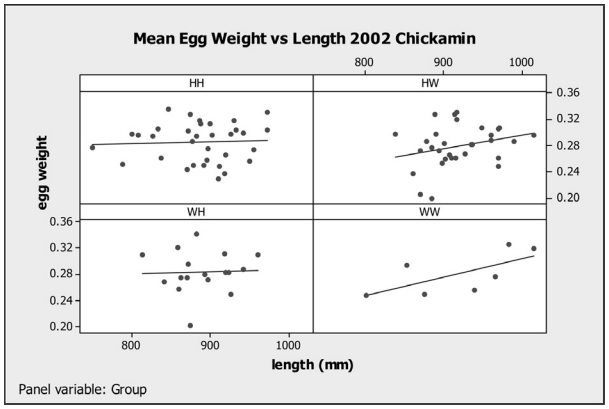
Weighing 10 eggs from each female provided a more precise measurement of average egg mass than measurements from the 50-egg samples. The 10 eggs from each female, preserved in 10% formalin at the time of spawning, were weighed to the nearest 0.001 g. Differences among females accounted for almost all of the variability explained by the nested model in all years. The Chickamin 2001 data indicated that wild fish may have larger eggs, especially considering the relatively small female length of the wild fish, but differences in egg size between cross types were not significant in either the 2001 or 2002 Chickamin data. The 2003 Unuk cross type varied in individual egg size according to the same pattern as the 50-egg weight samples (Fig. 6); mean egg weights of the two wild-hatchery crosses differed from mean egg weights of the hatchery fish.

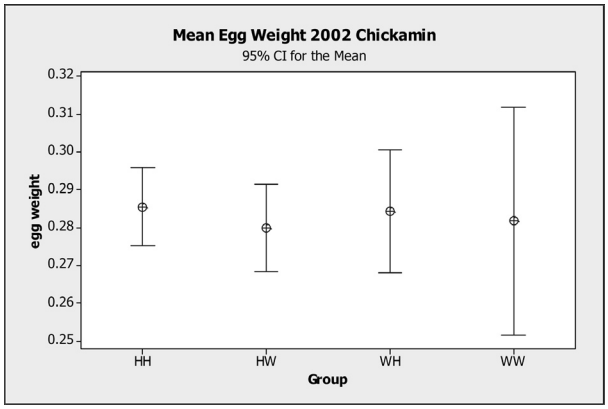
Discussion

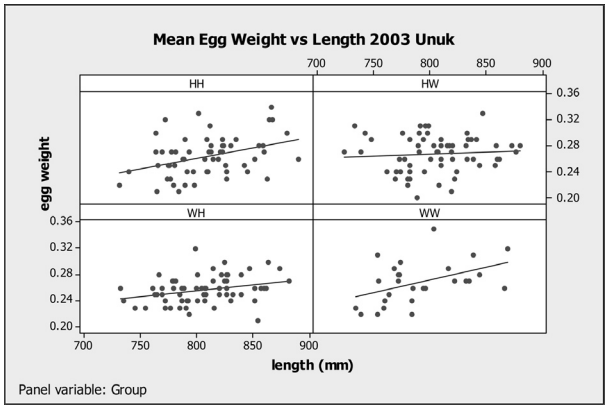
The consistent pattern of late maturing females from WW cross types, early maturing HH females, and intermediate female maturation of the wild-











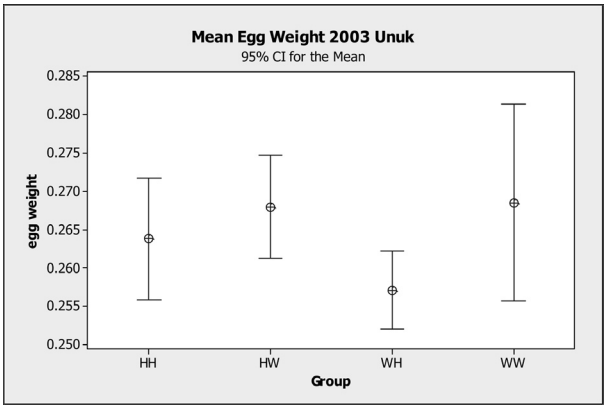


Figure 5. Relationship between mean egg size and female length for experimental groups of chinook salmon returning to LPW in 2001, 2002, and 2003.

Figure 6. Mean egg size for experimental groups of chinook returning to LPW in 2001, 2002, and 2003.

hatchery cross types is indicative of a genetic-based difference in maturation timing. This result was observed in both stocks for all brood years (2001-2003, Fig. 2). First generation differences between hatchery and wild groups reared and matured in very different environments may largely reflect environmental rather than genetic differences between the groups; the LPW environment is significantly different than wild systems experienced by donor fish with regard to juvenile incubation, rearing, and final maturation conditions. However, all experimental fish experienced essentially the same culture (hatchery) and final maturation environment, suggesting that environmental differences do not explain differences in female maturation timing.

For the parental generations in 1996 and 1998, all female and male fish used in experiments were mature at the time of the experiments, but wild fish and hatchery fish had experienced very different final maturation environments. Wild fish mi-

grate into the rivers at approximately the same time that LPW hatchery stocks return to the inner bay estuary. However, the LPW fish are captured and then held in estuarine pens with a freshwater surface lens. The freshwater temperature in August on the Unuk and Chickamin rivers is a fairly steady 9°-10°C, while the lake-fed fresh water at LPW may reach 16°-19°C during the final maturation period. The warmer fresh water at LPW is tempered to some degree by the cooler salt water, which averages 8°-12°C.

Salmon adapt to the thermal regimes of their natal streams. The hatchery environment at LPW has a very different thermal regime than the natal streams; therefore, selection pressures are changed and because of generally low mortality during early life stages, relaxed for such traits as developmental rates, emigration timing, and return timing. Other researchers have found evidence consistent with this apparent selection for earlier spawning salmon in hatchery stocks. There was no apparent difference in the proportion of females maturing at ages 5 or 6 among cross types. During the 2004 field season, we are collecting additional information from the Unuk stock and will perform a more detailed analysis of age at maturation, using all returns of males and females.

There are strong indications that wild Chickamin females have higher fecundity than female fish from the hatchery lines. The WW cross type had the highest mean fecundity in both of the Chickamin return years. The average fecundity of WW females was nearly 500 eggs higher in 2001 and almost 1,000 eggs higher in 2002 than egg numbers from the hatchery lines. Unfortunately, experimental power was compromised due to the 1997 mixing issues and lower sample sizes for the wild female salmon.

There are also indications that the relationship between female size and fecundity of the wild Chickamin fish differs from that of the hatchery fish, but again, the lack of observations of WW females did not allow a powerful test. In contrast, the 2003 Unuk stock HH and WW fecundities were quite similar; HW and WH fecundities differed from those of the hatchery lines.

For the Chickamin stock, there are indications of differences in egg size between the hatchery and wild lines, although specific pair-wise comparisons were not significant. However, the consistency and pattern of the differences gives some indication of genetic-based differences. Unuk WW and HH crosses were quite similar in egg size and the only significant differences were between the HW crosses and the hatchery line. The Unuk experiment provided more statistical power for resolution with larger numbers of observations, especially for WW and WH crosses.

It is interesting to note the differences in how the two LPW chinook stocks were founded. The Chickamin River was targeted in 1976 because the fish, especially those of the South Fork and Barrier Creek, reputedly were especially large and had low rates of precocious male maturation. While the escapements for this system may in total appear large (up to a few thousand), the escapements are composed of relatively small and somewhat distinct sub-populations. These subpopulations may consist of relatively few fish, perhaps only several dozen in some years.

The Unuk River stock was founded at LPW in 1976, but additional fish were collected over the next 5 years, bringing the total close to 250. The Unuk stock therefore has a much broader genetic base and has retained more population level genetic variability. Micro-satellite DNA analysis showed considerable loss of genetic variation for the Chickamin hatchery stock when compared with the wild line. Whether these differences resulted from a founder effect or selection is still open to question.

There are strong indications that the establishment and culture of hatchery populations of chinook salmon at LPW has produced stocks that differ from the donor stocks with respect to important life-history characteristics such as maturation timing and fecundity. These differences appear to be more pronounced in the Chickamin stock that was founded by a very small egg take in 1976. More observations of female salmon, particularly of wild females, would improve the ability of researchers to measure these differences. As this study continues, we plan to look for differences in additional life history characteristics such as age at maturation, marine survival, and behavior.