



Run-Type Composition of Juvenile Chinook Salmon in the Upper Chehalis River Basin in 2020

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Executive Summary

The status of Chinook salmon in the Chehalis River Basin (Basin) has primarily been based on compilations of harvest and escapement records produced by the Washington Department of Fish and Wildlife (WDFW) and Quinault Department of Fisheries (QDFI). A general declining trend in annual run sizes of spring Chinook and dramatic declines in the numbers of redds classified as being produced by spring Chinook raised concerns about their status.

Important findings of recent research have demonstrated genetic differences between spring and fall Chinook that provide a precise means of assessing the relative abundance of the run types. In 2020, QDFI initiated a pilot project of a three-year study to employ genetic methods to identify the genotypes of emergent Chinook fry as being homozygous fall, homozygous spring or heterozygous hybrids for the run type alleles. This study was conducted to improve understanding of the status of spring Chinook in the Chehalis Basin. This study is intended to cover at least three seasons of Chinook spawning and fry emergence, depending on annual results and limitations of staffing and funding availability.

Small inclined plane traps designed to capture emergent fry were deployed at sites downstream of spring Chinook spawning areas reported by WDFW in 2019. The traps were fabricated and deployed by West Fork Environmental (WFE) early in the 2020 winter-spring season to obtain tissue samples from up to 50 newly emergent Chinook fry per week at each site. Tissue samples were analyzed by the University of California, Davis (UC-Davis). Data collected during the first year of the pilot project were analyzed by QDFI and are reported in this information report.

Seven traps were deployed and operated in four subbasins of the Chehalis system during statistical weeks 6 through 20. Sampling was terminated when fry emergence was nearly ceased. Although traps were installed at different sites as they became fishable during the season, trapping results indicate they were operating during a vast majority of the targeted fry emergence.

A total of 6,266 Chinook fry were captured during the study and 2,243 tissue samples were genotyped. Analyses of data regarding trap efficiency, environmental conditions, and duration of trapping resulted in estimates of approximately 449 thousand fall homozygote (84.3%), 15 thousand spring homozygote (2.8%) and 69 thousand heterozygote (12.9%) emergent fry passed the trapping sites during their period of operation. The study results suggest Chehalis River Chinook currently conform more closely to a model of random mating than to a scenario of strong reproductive isolation between the run types.

These estimates do not represent total emergent fry production from the entire Chehalis Basin since sites were chosen based on the distribution of spring Chinook redds identified by WDFW. The non-trapped areas of the basin, where WDFW surveys indicated no spring Chinook redds were present, account for a majority of Chinook spawning activity.

The first year of this study demonstrated the feasibility of using fry traps to capture emergent Chinook fry during variable environmental conditions and the capacity to determine genotypes from tissue samples rapidly and accurately. Pilot project results indicated that genetic analysis could provide important information to complement data from spawning escapement surveys.

Introduction

Rapid environmental changes have led to declines in abundance of many species, including salmon (genus *Oncorhynchus*). Accelerating trends of global climate change and human population growth in Washington indicate an urgent need to take action to protect and restore aquatic habitats. The Aquatic

Species Restoration Plan (ASRP) for the Chehalis Basin, the second largest watershed in the State of Washington, has been initiated as part of the Chehalis Strategy.

Spring Chinook salmon abundance in the Basin has declined from historical levels and it is continuing to trend downward. Recent studies warn that Chinook salmon stocks along the California, Oregon and Washington coasts are vulnerable to further climate-driven declines and virtual extinction, especially small spawning populations and population segments with more specialized adaptations like the spring-summer component of adult run timing (e.g., Crozier et.al. 2019; Crozier et.al. 2021). The decline of Chehalis spring Chinook over the past 20 years has been more precipitous than the fall run type and terminal run sizes below escapement goals have resulted in closure of terminal fisheries in recent years. The low abundance and declining trend are troubling and a high priority has been placed on restoring habitats important to spring Chinook.

The available data and information regarding spring Chinook in the Basin are not sufficient for specific planning and prioritization of restoration measures. There is considerable uncertainty regarding the distribution, timing and genetic integrity of spring and fall run types during spawning. There is also little information on very early life history distributions and behaviors of emergent juveniles, and the relative abundance of progeny of spring and fall run types. Evaluations of the apparent decline of Chehalis spring Chinook are confounded by changes in spawning escapement methodologies employed by WDFW over the past several decades and by a possible shift to later timing of spawning that could be leading to increased interbreeding of spring and fall run types. Interbreeding could pose additional challenges for preserving the genetic separation of spring Chinook. Consequently, there is a need for additional information to determine the status of spring Chinook and to help understand the extent of interbreeding.

QDFi designed the project reported here to help address uncertainties regarding the status of Chehalis River spring Chinook salmon by estimating proportions of genotypes (see Background section below) of newly emerged Chinook fry produced from areas reported to have spring Chinook spawning.

West Fork Environmental (WFE) constructed and operated traps designed to capture emergent fry at seven sites downstream of all reaches reported to have spawning spring Chinook in 2019. Up to 50 tissue samples per week were collected at each site for genetic analysis by the Department of Animal Science Genetics Lab at UC-Davis. The pilot study approach consisted of the following:

- Mini-inclined plane traps were placed within four subbasins to capture newly emerged Chinook fry. The subbasins were; Skookumchuck, Newaukum, South Fork Chehalis, and upper Chehalis mainstem (upstream of the South Fork);
- The trap sites were located where landowners approved of the activity; no traps were placed unless landowner approval was granted;
- Each trap was operated for a minimum of 24-hours each week of the fry emergence period (beginning as early as mid-January and ending in mid-May) with a target of taking tissue samples from 50 Chinook fry in each week. Weekly trapping was terminated when it was evident a sufficient number of emergent fry would not be available for sampling or a maximum of 50 tissue samples had been obtained;
- All tissue samples were held at the WFE office under a chain of custody procedure until they were transferred to a QDFi representative who recorded the transfer of samples in a log book;

- Tissue samples were stored in secured storage until shipment to UC-Davis on a regular schedule with proper tracking and insurance;
- UC-Davis genetics lab used procedures outlined in Thompson et al. (2019a) for genotyping; and,
- Results of each tissue sample were reported to QDFi for analysis and interpretation.

This is an informational report conveying the results of the 2020 pilot project. Foundational information regarding relevant aspects of Chinook salmon life histories, definitions of run types and their recently discovered genetic basis, and reasons for concern about the outlook for Chehalis spring Chinook are reported in the Background section. Details about the project study design and methods are reported in the 2020 Pilot Study section. The Study Results section reports results and findings derived from the project data and the Discussion section presents context and conclusions based on the pilot project results. Details of site-specific operations and data summaries are reported in Appendix A. The systematic procedures for estimating run type production from trap catches and tissue sample genotyping are reported in Appendix B. The project database schema are shown in Appendix C and the system of statistical weeks used in this study is provided in Appendix D.

Background

Chinook Salmon Run Types in the Chehalis Basin

Chinook salmon are classified into run types based on timing of adult river entry and spawning (Quinn et al. 2016). In rivers along the Washington coast, including the Chehalis Basin, two run types are distinguished: spring-run and fall-run. Spring Chinook salmon are those that enter rivers beginning in March or April and ending sometime in mid-to-late summer; managers usually call all Chinook salmon entering the rivers prior to the end of August spring run (Lestelle et al. 2019)³. Fall Chinook salmon are fish that enter rivers between late summer and late fall, with the majority entering after the onset of freshets, usually beginning in September and peaking in mid-to-late-October, depending on the year.

Spring Chinook salmon adults return to their home rivers in a sexually immature state, usually 3 to 5 months prior to spawning. Consequently Quinn et al. (2016) referred to this run type as premature migrants. In contrast, fall Chinook enter their home river close to being sexually mature, and hence have been called mature migrants.

Spring Chinook usually begin spawning in late August in rivers on the Washington north coast but begin somewhat later in the Chehalis Basin likely due to warmer water temperatures. Spring Chinook spawning continues through September and is believed to end in early-to-mid October in Washington coastal rivers. Fall Chinook spawning is believed to begin in October and extend into early December, peaking in many areas in early November. Spring-fall hybrids have a river entry timing that is generally intermediate between the spring and fall run types (Thompson et al. 2019a).

Recent research indicates the differences in river entry and maturation patterns have a genetic basis (Prince et al. 2017; Thompson et al. 2019a) associated mostly with variation in a small region (GREB1L

³ / Historically, Chinook salmon entered the rivers on the Washington Coast almost continuously—although often in very small numbers interspersed with pulses of larger numbers—from April through the end of November. Consequently some biologists have referred to the fish that enter between April and the end of August as spring/summer Chinook—others have called them all during this period simply spring Chinook (Lestelle et al. 2019). Those fish that enter the rivers starting in September are always considered fall Chinook salmon.

gene)⁴ of the genome. In rivers where both spring and fall Chinook salmon co-exist, some amount of spatiotemporal separation in spawning is needed to maintain genetic isolation and population structure based on the run types. Geneticists believe, while some degree of interbreeding between the run types is normal under completely natural conditions, human activities have notably increased interbreeding in many locations where spring Chinook salmon still exist (Ford et al. 2020). Over time, an increasing rate of hybridization between the run types indicates genetic separation is breaking down, which increases the risk of extirpation of the spring run type (Thompson et al. 2019a; Thompson et al. 2019b; Ford et al. 2020).

A high rate of hybridization caused by interbreeding of the spring and fall run types is a major threat to the long-term viability of the spring run because it indicates they have lost spatiotemporal-specific habitats. Spring-run-specific habitat (i.e., spatiotemporal habitat that is difficult for fall Chinook to access) is hypothesized to be the major evolutionary advantage for the spring run that offsets numerous disadvantages (Quinn et al. 2016). Loss of habitat isolation and the consequent high rate of hybridization means spring Chinook are in direct competition with and will be displaced by fall Chinook due to their demographic advantages and higher fecundity (Healey 2001).

The current time series of spawning ground survey data for the upper Chehalis basin indicates spawn timing of fish classified as spring Chinook salmon has shifted later since the mid-1980s. Peak spawning of spring Chinook salmon now appears to be largely overlapped both spatially and temporally with spawning by fall Chinook salmon (Zimmerman 2017).

Genetic methods now exist to identify with certainty whether a Chinook salmon is spring or fall run type or a hybrid (heterozygote) of the two types. Because of the potential significance of hybridization, this study classified fry genotypes as being spring (SPRING) or fall (FALL) homozygotes or spring-fall heterozygotes (HET).

Status and Trends

The entire Chehalis Basin has undergone major changes since the arrival of non-Indian settlers in the mid-1800s. These changes have resulted in profound alterations to aquatic habitats used by Chinook salmon in the Basin, most significantly to habitats used by spring Chinook salmon (ASRPSC 2019).

Run size trends differ between spring and fall Chinook salmon returning to the Chehalis River system over the past 20 years (Figure 1). The trend for fall Chinook salmon is slightly downward and the population is generally considered stable. In contrast, spring Chinook exhibit a sharply declining trend. The decline is a major concern to the entities developing and implementing the ASRP. Consequently, a high priority has been placed on restoring habitats important to spring Chinook and its continued viability (ASRPST 2020). In addition, all sanctioned harvest of spring Chinook within the waters of the Basin, including Grays Harbor, was suspended for the 2019 and 2020 seasons, except for a small ceremonial harvest by the Chehalis Tribe. The percent of the total annual Chinook salmon run size returning to the Chehalis River system (excluding the Humptulips River) comprised of spring Chinook salmon since 2000 has declined from about 20% to an average of about 5% in 2018 and 2019.

Chehalis Spring Chinook Concerns

The sharp decline in spring Chinook salmon returning to the Chehalis Basin, as reflected in existing data, is a major concern to the Co-Managers, Quinault Indian Nation (QIN) and State of Washington. The cause of the decline is believed to be cumulative impacts of many factors reflected in widespread

⁴ The spring run is homozygous for the spring allele of the GREB1L gene and the fall run is homozygous for the fall allele.

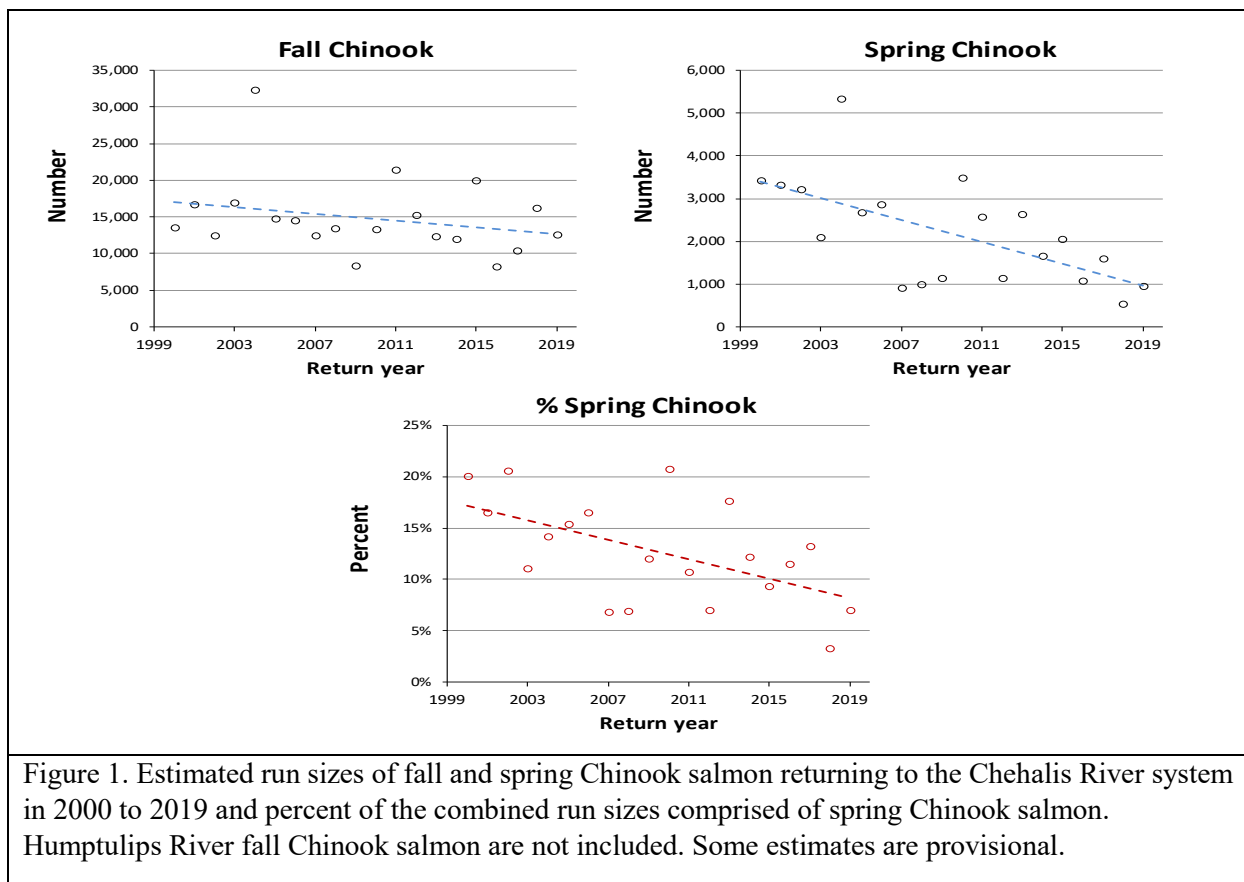


Figure 1. Estimated run sizes of fall and spring Chinook salmon returning to the Chehalis River system in 2000 to 2019 and percent of the combined run sizes comprised of spring Chinook salmon. Humptulips River fall Chinook salmon are not included. Some estimates are provisional.

habitat alterations as modelled with the Ecosystem Diagnosis and Treatment (EDT) model (McConnaha et al. 2017). Spring Chinook salmon are particularly vulnerable to unusual water temperatures and summer flow conditions because of their prolonged holding time in freshwater waiting to begin spawning (Quinn et al. 2016).

The apparent decline of Chehalis spring Chinook salmon is confounded by changes in methods used to estimate spawning escapements over the past several decades and by a shift to later spawning by these fish. The spawning survey program to estimate both spring and fall Chinook salmon abundance in the Basin evolved from about 1980 to 2000. To distinguish redds associated with either spring or fall Chinook salmon, a cutoff of October 15 was applied, a date adopted from this use in rivers on the Washington north coast (i.e., major rivers north of the Chehalis basin). This date has been used since the late 1970s in north coastal rivers. Redds dug on or before October 15 have been assumed to be from spring Chinook salmon; redds dug later are assumed to have been produced by fall Chinook salmon.

Over a period of years since 2000, as spawning survey data were being collected in the Chehalis basin, it appeared that spring Chinook salmon spawn timing was shifting later—to the point that Ashcraft et al. (2017) stated that peak spawning by this run type in the upper Chehalis River was occurring around the threshold date of October 15. Zimmerman (2017), in reviewing data back to the early 1980s, concluded there was a clear shift to later timing by fish being classified as spring Chinook salmon by WDFW surveyors. Peak spawning by fish classified as spring Chinook salmon in the 1980s occurred in mid-September but had shifted toward early-to-mid October in recent years.

This led WDFW to incorporate additional criteria to its protocols for distinguishing spring and fall Chinook salmon redds. Ashcraft et al. (2017) stated:

Due to overlap in spawn timing of spring and fall Chinook, the WDFW Region 6 District 17 protocol is to determine run-type (spring or fall) of a redd based on timing, redd condition, and phenotypic characteristics, behavior, and condition of associated live fish observed with the redd, as well as prior observations of fall Chinook activity, flow levels, and other spawning activity within the basin. Redds constructed after October 15th were all assumed to be fall Chinook, but redds constructed on or prior to October 15th were assigned either spring or fall Chinook based on the condition of redd and fish associated with the redd.

Further, Ashcraft et al. (2017) added:

The overlap in spawning location and timing of spring and fall Chinook means that field calls are necessarily subjective and additional investigation on the distinctions between these runs is being conducted as part of a separate project. This additional work should help to clarify the proportion of spring and fall Chinook spawners using information not available to field surveyors such as genetics and otoliths (microchemistry composition).

The true status of spring Chinook in the Chehalis Basin is difficult to determine from spawning escapement estimates alone. The methodology for estimating spawning escapements only attempts to distinguish between spring and fall Chinook. No provisions are incorporated to identify potential run type hybrids from interbreeding of spring and fall Chinook.

The Need for Genetic Sampling

Genetic sampling of Chinook juvenile populations is necessary to address uncertainties about the relative abundance of spring and fall run types and the extent of their interbreeding. This study was designed to collect unbiased genetic samples that represent relative abundances of the run types in portions of the Basin where the spring run type spawns.

During development of the ASRP, concerns over the status of spring Chinook salmon were heightened when it became known that the spatiotemporal overlap in spawning with fall Chinook salmon appeared to be significant (Lestelle et al. 2019). Genomic tools developed by the UC-Davis lab were employed in 2018 to identify run types of post-spawning Chinook carcasses that WDFW had collected tissues samples from over the previous 20 years. Results identified spring and fall homozygotes and heterozygote genotypes in the Chehalis River system (Thompson et al. 2019b). That study found a high proportion of carcasses that had been classified by spawning surveyors as spring Chinook in recent years were in fact fall Chinook or hybrids. Moreover, the results suggested that hybridization had increased in recent years compared to pre-2010.

Observations of later spawn timing by the spring run indicates potential for increased interbreeding with fall Chinook and hybrids. There is a need for additional information to determine the status and vulnerability of spring Chinook in the Basin and to help understand the extent of interbreeding and presence of hybrids.

The potential threat of hybridization to the maintenance of spring Chinook salmon in the Chehalis Basin appears to be substantial. Michael Miller (co-author of this report) informed the Science Review Team (SRT) for the ASRP that he considers high rates of interbreeding between spring and fall Chinook as a major threat to the viability of spring Chinook in the Basin. High levels of interbreeding indicate spring Chinook salmon have either lost spatiotemporal-specific habitats or that the numbers of spring Chinook salmon have been reduced to such low numbers that reproduction is being swamped by much more abundant fall Chinook. Consequently, spring Chinook then become in direct competition with fall Chinook for reproductive success and will eventually be displaced by fall Chinook salmon (e.g., because fall Chinook have higher fecundity [Healey 2001]).

2020 Pilot Study

Purposes and Objectives

- Gain landowner cooperation and permission to operate fry traps on stream reaches within the major spawning areas of the upper Chehalis basin where spring Chinook are believed to spawn;
- Design, build, deploy, and test mini-inclined plane traps to capture newly emerged Chinook fry at approximately seven locations in the upper Chehalis River system;
- Operate the fry traps over the period of Chinook fry emergence (approximately mid-January to mid-May) on a weekly basis to capture and sample up to 50 fry per week for length measurements and fin tissue samples at each trapping site. Based on discussions between QDFi technical staff and UC-Davis, The target tissue sample sizes of 50 randomly selected Chinook fry from each site in each week of the assessment period was established after considering budget constraints and matters pertaining to precision and accuracy . It was acknowledged that fewer fish would be caught at some sites in some weeks especially when fry emergence was beginning as well as later when emergence was ending. High water events were also expected to reduce fish capture in some weeks;
- Obtain information on the timing of Chinook fry emergence at the trapping sites;
- Estimate weekly and seasonal genotype frequencies of Chinook emergent fry moving past each site during the trapping period;
- Provide information on the relative abundances of SPRING, FALL, and HET Chinook fry. Because of increasing overlaps in the timing of spring and fall Chinook spawning, hybridization is a major concern for the future viability of spring Chinook salmon in the Basin (ASRPSRT 2020);
- Record information regarding the cost and timeliness of project elements including genotyping by the UC-Davis genetics lab; and,
- Determine whether results warrant continuation of the study in 2021.

Study Design

Acquiring non-biased tissue samples and doing genetic assays for individual Chinook spawners would be the most direct method for estimating genotype frequencies of the adult population. However, it is logistically difficult and expensive to collect sufficient tissue samples from live or dead spawners on the spawning grounds. Sampling live fish from the spawning areas, given that fish are dispersed and typically at low densities, is extremely difficult. These approaches are uncertain, expensive, and disruptive to fish preparing for or engaged in spawning. The recovery of dead spawners in sufficient numbers is also extremely difficult and uncertain. Relatively few carcasses usually are recovered in any given year and the distribution of recoveries is uneven both spatially and temporally due to variable environmental conditions and removal by predators and scavengers.

During discussions by the SRT with UC-Davis about genetic sampling of the populations, the idea of sampling juvenile migrant Chinook salmon took form. Given the difficulty of achieving adequate sample sizes and statistical resolution by collecting tissue samples from adult spawners, considerations turned to sampling juvenile offspring. Relatively large numbers of juveniles could more easily be captured and sampled. Consideration was given to sampling juvenile Chinook captured in two rotary screw traps (RSTs) operated by WDFW; one in the mainstem Chehalis River located near Oakville (downstream of the confluence with the Skookumchuck River) and one in the lower Newaukum River. While there is merit to using these traps, a sampling regime using this gear presents questions about obtaining

unbiased samples from the emigrating juvenile populations. These uncertainties arise from characteristics of alternative Chinook life-history strategies and behavioral variations in emigration and dispersal of recently emerged fry.

Natural Chinook salmon fry on the Washington coast emerge from their incubation habitats beginning in January and extending into May (QDNR 1977; QDNR 1979; SIT and WDFW 2017). Spring Chinook fry emerge during the earlier portion of this period (QDNR unpublished data; SIT and WDFW 2017). Juvenile Chinook emigration toward the ocean in coastal rivers is especially complex because of a mix of different temporal patterns and life stages involved (Healey 1991; Quinn 2018). These life stages include newly emerged fry, transitional rearing fry, pre-smolts, and smolts that are typically several months old. The downstream movement of fry is not uniform because some fish move relatively quickly, while others move slowly, stopping to rear for periods before continuing seaward. The entire period of outmigration for age-0 juveniles extends mainly from mid-January to mid-August, with pulses of juveniles still moving out in late summer and early fall (QIN unpublished).

These complex emigration patterns make it particularly challenging to interpret point source sampling results acquired at locations with a high degree of mixing from multiple upstream subpopulations. This is especially true if the objective is to characterize quantitative attributes like run type genotype frequencies in the fry population originating from areas upstream of the capture site. Use of an RST, for example, to sample juveniles at a single location on a mainstem river like the Chehalis, where juveniles of different sizes and ages (days since emergence) are mixed from the many spawning areas upstream, require all outmigration patterns and juvenile ages to be systematically sampled over the entire period of outmigration.

Systematic sampling of the entire population of juvenile Chinook arising from areas upstream of sampling sites is not possible with the RSTs deployed by WDFW. A large part of the period when most spring Chinook fry are emerging and moving downstream occurs prior to when the RSTs are deployed. The RSTs are not installed sufficiently early in the year to sample the entirety of the emigrant populations. The traps, which are large floating devices that require several people to operate, cannot be installed in the rivers until about mid-to-late March due to high river flows commonly occurring before then (Winkowski and Zimmerman 2019; West et al. 2020a and b). A reasonable expectation is that fry of the three run type genotypes will not emerge and emigrate at the same times or rates so initiating sampling late in the emergence period will cause bias in the genotype frequency estimates for fry from the areas of interest.

With these considerations in mind, we developed a study design aimed at sampling newly emerged fry at sites within the lower reaches of all of the principal spawning areas where spring Chinook are known to spawn (based on WDFW spawning surveys). Our sampling design is based on the assumption Chinook fry less than or equal to 45 mm fork length (FL) caught at our trap sites are representative of the population of fry migrants produced from spawning areas above the sites (see the *Why Target Emergent Fry* section below). A method utilizing small inclined plane traps to capture newly emergent fry was selected because it had been used previously with success in other north coastal streams and because it provided the greatest likelihood of acquiring adequately large, unbiased samples. Relevant literature and experience with fry trapping on the Queets River during the 1970's were reviewed and the information was used to design and operate the traps in this pilot project. The pilot project design was developed through a series of discussions among QDFi, WFE, UC-Davis, and WDFW.

In the 1970s, QDFi employed small inclined plane fry traps to study the outmigration and abundance of juvenile Chinook salmon in the Queets River (QDNR 1977; QDNR 1978; QDNR 1979). These traps were designed using larger inclined plane traps, or scoop traps as models (Volkhardt et al. 2007). These mini-inclined plane traps can be easily placed within a stream or river, secured in place with fence posts, and

left to fish for a number of hours before checking. The mini-inclined plane traps—simply referred to as fry traps in this report—are particularly effective at capturing newly emerged Chinook fry during their period of initial dispersal downstream. Healey (1991) described the initial emigration of Chinook fry following emergence as fry migrants, that is, fry less than about 45 mm in length that are moving immediately seaward from the spawning grounds. Chinook fry in Washington coastal rivers emerge at sizes ranging between approximately 36-42 mm in size, based on work by QDFi in the Queets River in the 1970s and observed in this study. These small traps can be deployed in relatively shallow water where they are easily accessible by operators, and placed within the stream so that widely variable flow conditions can be fished from week to week over the entirety of the fry emigration period.

Why Target Emergent Fry?

What is known and what is not known about the downstream migration of Chinook salmon juveniles? Good reviews of juvenile chinook life history are provided by Healey (1991) and Quinn (2018) and these sources were relied on to plan this project's sampling schedule and methods and to interpret project results.

Chinook salmon are described as either being ocean-type or stream-type, based on when the juveniles migrate seaward from their natal rivers. Ocean-type chinook migrate from freshwater to the estuary and ocean as sub yearlings (i.e., in the same year that they emerge as fry from the gravel) and the stream-type moves to the estuary and ocean as yearling juveniles (i.e., in the year following emergence from the gravel). A large majority of juveniles produced by both spring and fall Chinook populations along the coasts of Washington, Oregon, and California exhibit the ocean-type life history. Most, if not all, of these populations produce some yearling (stream-type) smolts—but only in very small numbers.

Quinn (2018) describes the downstream migrations of juvenile chinook as being **...especially complicated because their populations may include a mix of ocean-type fish migrating to sea as fry just after emerging from the gravel, ocean-type fish that spent several months in the river, and stream-type smolts that spent a year in the river.** (Emphasis added). For example, Quinn, citing Healey (1991), describes the outmigration patterns of juvenile chinook in the Cowichan River (near the southeast end of Vancouver Island):

“...many fry just over 40 mm left the river from mid-March through the beginning of May, with little increase in average size over that period (presumably because each individual migrated shortly after it emerged from the gravel). There was a lull in the numbers of fish migrating, followed by a pulse of larger fish, about 60 mm to 70 mm long.”

The same pattern was observed on several consecutive years in the Queets River in the late 1970s by QDFi. Healey (1991) described the initial emigration of fry following emergence as fry migrants; that is, fry less than 45 mm in length that are moving immediately after their emergence seaward from the spawning grounds. In some rivers, particularly those of relatively short length, some fry migrants move all the way to the estuaries, where a period of rearing then begins. This pattern of movement by fry to the estuary occurs in the Chehalis River (Simenstad and Eggers 1981, Campbell et al. 2017), and in Washington north coast rivers (QIN, unpublished).

Healey describes a particularly relevant characteristic of fry migrants:

Upon emergence, fry swim, or are displaced, downstream. Thomas et al. (1969) found that fall chinook fry go through a period of reduced swimming ability just before the time of complete yolk absorption, and that this coincided with the time of peak downstream migration. They hypothesized that reduced swimming ability was the cause of downstream migration.

Healey (1991) further describes the downstream movement of ocean-type juveniles as follows:

*“Once started downstream, chinook fry may continue migrating downstream to the river estuary, or may stop migrating and take up residence in the stream for a period of time ranging from a few weeks to a year or more. **What determines whether fry will hold and rear in the river, or migrate downstream to the estuary, is unknown.**”* (Emphasis added)

Healey (1991) and Quinn (2018) refer to these juvenile migrants of ocean-type chinook larger than approximately 45 mm as parr or fingerling migrants. The efficiency of the mini-incline plane traps at catching these fish, compared to the smaller fry migrants, is poor because fish larger than emergent fry size can more effectively escape the traps.

The critical thing to realize about parr migrants of ocean-type chinook is that **this is a rearing life stage** and not strictly a migration life stage. However, it is a rearing life stage in motion. In the EDT modeling, this life stage is termed as transient rearing. The fish are feeding and growing, yet continuing to move down the river toward the estuary at different speeds likely because they are in different stages of development depending on their size and their age (days) since emergence. Some have referred to this pattern of movement as “rearing on the run”, or “migration at the speed of molasses.” Both of these descriptions and Quinn’s reference to the downstream migration of juvenile chinook as “especially complicated” are appropriate.

This pattern of movement is important to recognize in interpreting the timing curves and out-migrant estimates made at the RSTs operated by WDFW. Whereas the mini-incline plane traps operated by QIN essentially target a single age group of fish (newly emerged fry), the RSTs catch a mixture of fish from newly emerged fry to older parr migrants and likely some yearling smolts. One can think of the estimates of abundance made at the RSTs as representing a diverse mixture of chinook juveniles on different life history trajectories moving from their natal sites toward the estuary at different ages, sizes, and speeds.

Chinook fry captured in this study are classified emergent fry if they are ≤ 45 mm (FL) and as rearing fry if they are > 45 mm (FL).

Chehalis River Basin

The Chehalis River Basin (Water Resource Inventory Areas (WRIAs) 22 and 23) is one of the largest river basins in Washington State, encompassing an area exceeding 2,700 mi² (Figure 2). This pilot study is focused on portions of the watershed within the Cascade Mountains and Willapa Hills ecological regions because a vast majority of observed and documented spring Chinook spawning occurs there. WDFW conducts extensive annual surveys and estimates the number of spring and fall Chinook salmon spawning in the Basin using procedures described in the *Chehalis Spring Chinook Concerns* section above.

The Cascade Mountains Ecological Region contains the southeastern part of the Basin, including the Newaukum and Skookumchuck rivers and their tributaries, Stearns and Salzer creeks, and other tributaries on the east bank of the Chehalis River near the cities of Chehalis and Centralia. This region encompasses 424 square miles and represents approximately 16% of the overall Basin.

The Willapa Hills Ecological Region contains the upper Chehalis River (upstream of the South Fork) and tributaries, including East Fork and West Fork Chehalis rivers, Elk Creek, and the South Fork Chehalis River and its tributaries. This ecological region encompasses 316 square miles and represents approximately 12% of the overall Basin. The Willapa Hills Ecological Region is believed to have been a former stronghold of spring Chinook salmon, but species occurrence has been highly variable and notably decreasing in recent years, leading to concerns about local extirpation (ASRPSC 2019).

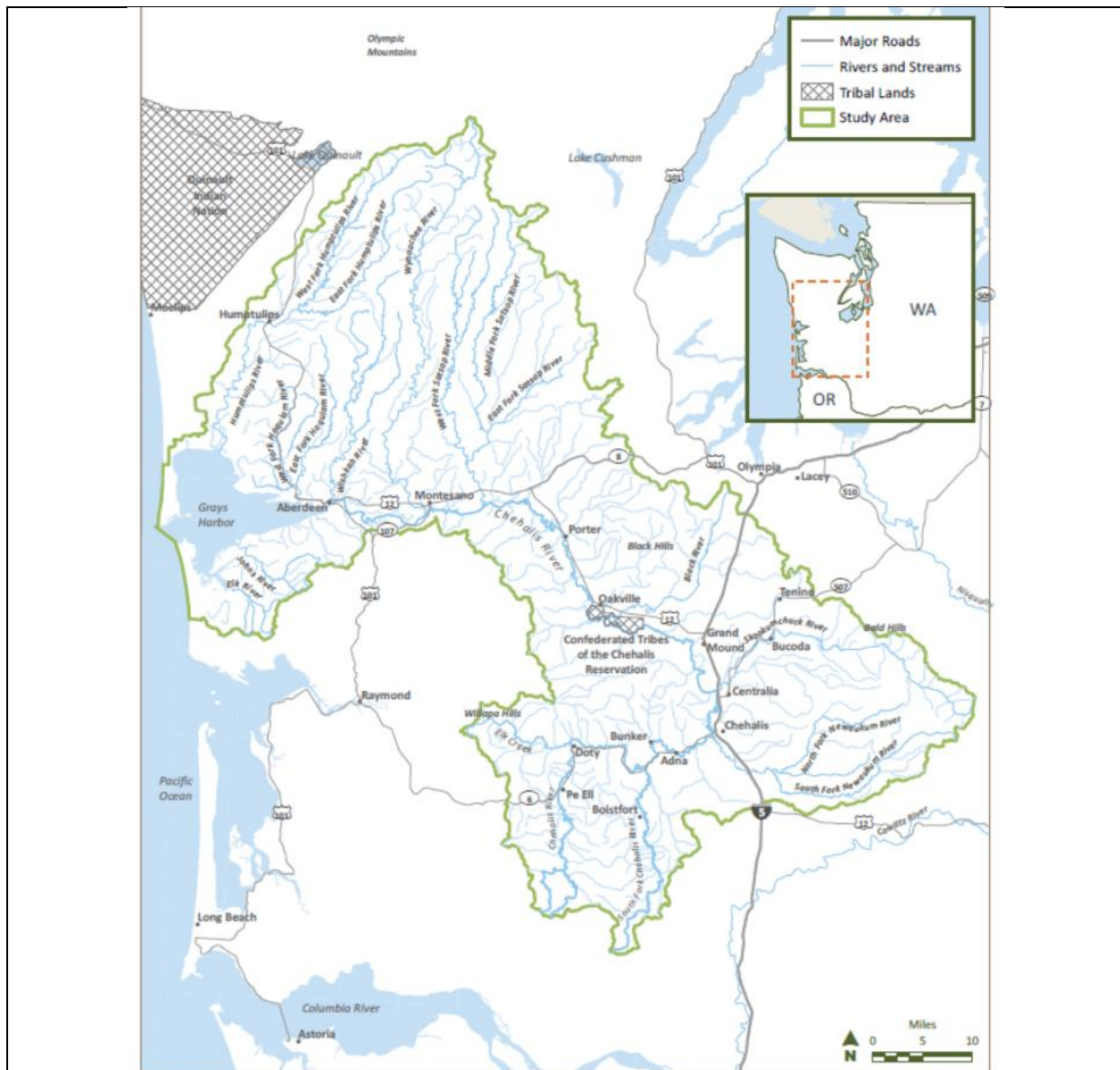


Figure 2. Map of the Chehalis River Basin. The assessment area described in this report is located entirely upstream of Centralia, including the area within the Skookumchuck River. (Source - ASRPSC 2019)

Thompson et al. (2019a) analyzed tissue samples from Chinook salmon carcasses collected by the WDFW over the previous 20 years within these subbasins as well as in areas downstream of the Skookumchuck River. They found no genetic evidence of spring Chinook spawning downstream of the Skookumchuck River.

The Newaukum and Skookumchuck rivers are believed currently to support the majority of spring run type Chinook salmon in the Basin. Fry trapping sites were located below areas reported to have spring Chinook spawning, generally in the upper third of the basin which contains the Willapa Hills and the

Cascade Mountains Ecological Regions. The Skookumchuck and Newaukum subbasins are located within the Cascade Mountains Ecological Region while the South Fork subbasin and the upper Chehalis River subbasin upstream of the South Fork are located within the Willapa Hills Ecological Region.

Trapping Sites

Sampling was directed at four subbasins within the upper Chehalis Basin: Skookumchuck subbasin, Newaukum subbasin, South Fork subbasin, and the upper Chehalis River subbasin upstream of the South Fork. Within the subbasins, trap sites were selected based on landowner permission for access and the suitability of a site for trap deployment. The sites selected were (Figure 3):

- Upper Skookumchuck River (SKU) at RM 19.0;
- Mainstem Skookumchuck River (SKO) at RM 6.2;
- Mainstem Newaukum River (MSN) at RM 4.4;
- North Fork Newaukum River (NFN) at RM 2.4;
- South Fork Newaukum River (SFN) at RM 12.8;
- South Fork Chehalis River (SFC) at RM 0.8; and,
- Upper mainstem Chehalis River (MSC) at RM 90.3.

Data Collection Methods

Fry Trap Design

The design of the fry traps employed for the 2020 pilot study were developed collaboratively by WFE and QDFI. WFE fabricated the fry traps and described the design as follows:

Inclined plane traps have been used by fisheries field practitioners for decades to capture downstream migrating salmonids. These traps share the common principle of passive entrainment of fish that are moving downstream through their framed opening. The natural flow of water through the trap deposits fish into a live box located immediately downstream of the perforated incline that sieves the water flowing over it. Loss of water through the inclined screen and perforated side panels of the trap frame means only a modest amount of water enters the live box. This reduction of water flow protects fish from turbulent conditions in the confined spaces of the live box. Fish are either unable to exit the box due to high water velocity coming over the lip of the incline screen or are disinclined behaviorally to leave the confines of the box once they are in it. Many of these trap designs incorporate pontoons for floating deployments. Our smaller inclined plane traps were fabricated without pontoons for deployment in water less than 3 feet deep and for settings where T-posts could be driven into the substrate for anchorage. However, we designed the traps so they may be easily adapted for pontoons and fished as floating traps should that become desirable.

The design itself was patterned after traps recalled from the late 1970's - at that time in use by Quinalt Indian Nation biologists on tributaries of the Queets and Clearwater Rivers. Overall trap dimensions are a 3-foot-wide by 30-inch deep frame opening, with a 5-foot-long inclined plane leading to a live box 3 feet wide, 24 inches long and 24 inches deep. The back panel and half of the side panels of the live box are constructed from the same 1/8 inch thick perforated (1/8 inch diameter holes) aluminum plate that the inclined plane is made from. The side panels of the inclined plane were covered with heavy Vexar 1/8-inch plastic mesh fabric. We made some adjustments to this trap design by connecting the inclined plane and the live box portions of the trap with a "piano" hinge. This feature permitted greater flexibility in trap deployment over variable flows and locally varying channel bed elevations. A key feature of the trap is the adjustable "flap" over which the water flows at the downstream end of the inclined plane (Figure 4, top). This flap is also attached to the trap via a piano hinge and is fully adjustable to permit variable amounts of

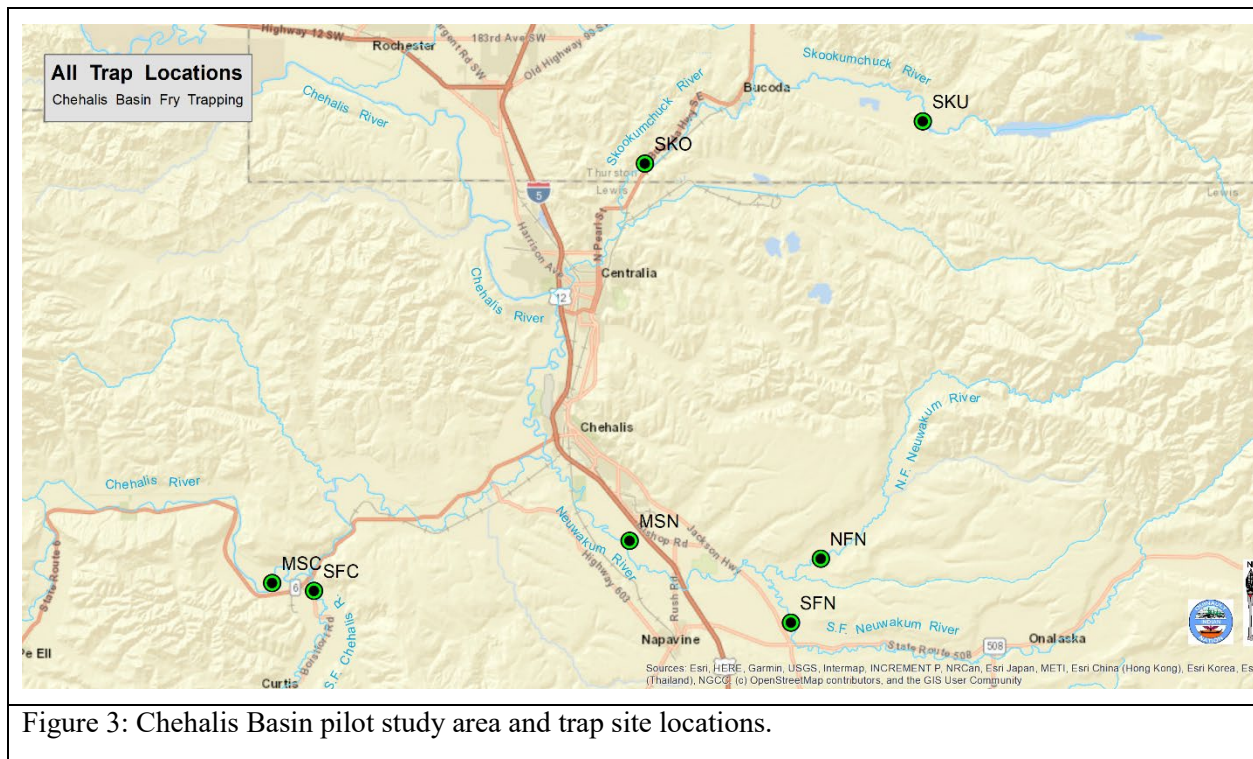


Figure 3: Chehalis Basin pilot study area and trap site locations.

water and vertical drop into the live box. It not only regulates how much water enters the live box, but it also provides protection from turbulent flow that would otherwise create unfavorable conditions for trapped fish. Adjustment is made via pegs through the perforated sides that support its position. The live box has a divider in it with space between it and the floor for fish movement between compartments and this further reduces turbulent flow while fish are being held. The trap has three adjustable supporting legs attached to the sides and one to the rear of the live box that allow flexibility in locating the trap and support the live box (Figure 4, bottom).

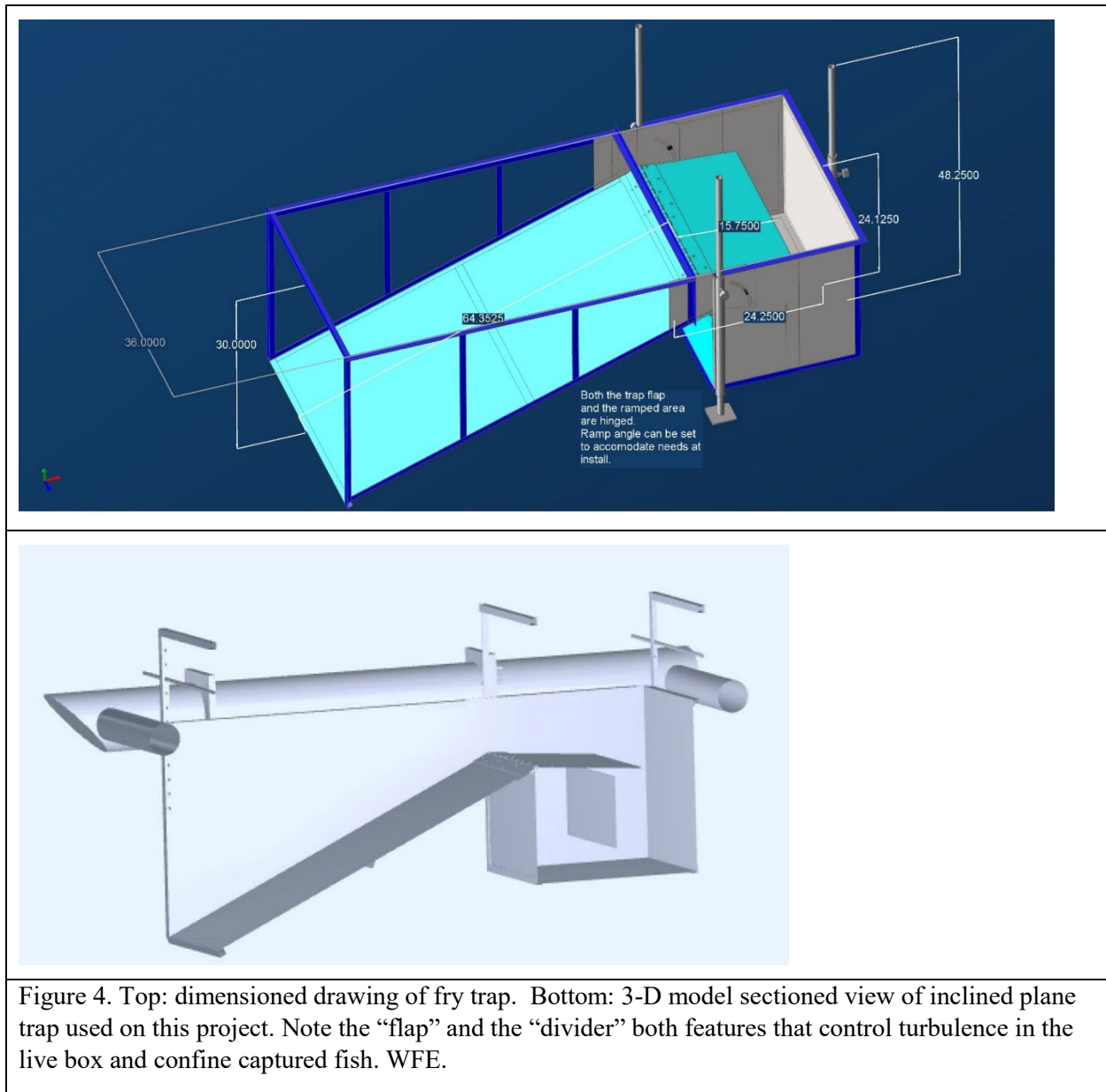
Trapping

WFE performed all the fieldwork during the 2020 pilot study, including installation and operation of the traps (Figure 5). Fry traps were deployed over most of the fry emergence period, approximately from mid-January to mid-May. Sampling was carried out systematically each week and at each site, until it was evident fry emergence was nearly ended.

The sampling plan called for initiating trapping in the second half of January 2020, depending on flow conditions. The traps were deployed for 1-3 days each week with a day consisting of an approximately 24-hr period. The number of days trapped at a site each week would be determined by when the target sample size of 50 Chinook fry was caught. Once trapping was initiated at a site, weekly samples would be collected until it was evident fry emergence was ending, as determined by the number of fish caught and fish size. Wing panels, constructed of 2" x 4" lumber covered with 1/8" Vexar mesh, were sometimes attached to the upstream ends of traps to widen the cross-section of stream being trapped, depending on flow level. The vertical dimension of the panels was 30-in, enabling them to fish the same depth as the fry traps. When deployed, the wing panels served to increase the number of fry being caught by enlarging the cross-section of the stream being trapped.

Data Collection

WFE identified and tallied fish captured at trapping sites by species and at every trap check. Photo



records were periodically taken of fish for further documentation. Environmental information on site conditions, trap configuration and placement were recorded, along with data on individual fish captured and tissue samples taken. A database was created to record information collected by WFE (see schema provided in APPENDIX C).

Chinook fry were mildly anesthetized with MS222, fork lengths measured and recorded, and a small piece of the upper lobe of the caudal fins were excised for tissue samples. The individual tissue samples were immediately folded into a piece of blotter paper and placed in a paper envelope with an identifying number on the outside (LaHood et al. 2008). When 50 tissue samples had been obtained for a trapping event (statistical week), tissue sampling was terminated, and trapping was discontinued at the site for the week. Any fish mortalities in the trap were recorded. All fish were returned to the river at the point of capture with the exception that some Chinook fry were periodically released



Figure 5: Fry trap deployments at various locations and flow conditions.

approximately 300 feet upstream for testing the feasibility of recapturing marked fish for trap efficiency trials. In these cases, the clipped caudal fin lobe served as a mark.

During each daily trapping event at each site, measurements were taken on the width of the trapping structure at its upstream end (including wing panels if used) and of the wetted stream channel width on a transect perpendicular to the flow at the upstream end of the trap. The ratio of trap structure width to total wetted channel width, i.e., the fraction of the stream channel cross-section sampled by the trap, was used as a measure of trap efficiency. The width of the trap structure was measured with a measuring tape. The width of the wetted stream channel was obtained with a range finder device appropriate for the scale of the sample sites.

Individual tissue samples collected on each sampling date were logged by WFE in logbooks, and then transferred to a QDFi biologist. Samples were stored in secured storage until shipped to the genetics lab at the Department of Animal Science UC-Davis in batches during the field season.

All tissue samples were processed using established procedures described in Thompson et al. (2019a). Genotype results for each sample were reported to QDFi for interpretation, analysis, and reporting.

Study Results

Trap Operations and Catches

The trapping operations were conducted for a total of 99 days from early February through mid-May although effort varied among sites (Table 1). Trapping duration (calendar days) and effort (total hours fished) were similar for the upper Chehalis River and Newaukum River sites but were much less at the Skookumchuck River, especially at the upper site. Traps at the Chehalis (MSC and SFC) and Newaukum (MSN, SFN and NFN) sites were fished for a range of 750 to 940 hours whereas the Skookumchuck (SKU and SKO) sites were fished for only 327 to 421 hours, less than half the time. The lesser effort at the Skookumchuck sites was due to two factors; first, Chinook catches were much greater at the Skookumchuck sites so the weekly sample objective of 50 tissue samples was achieved much sooner (often in one day) than at the other sites and, secondly, the SKU site was installed 4-6 weeks later than the other sites.

Table 1: Sampling duration, total hours fished and mean coverage (trap width/wetted width) at the seven study locations in 2020.

Location	Start Date	Start Week	End Date	End Week	Total Days	Total Hours Fished	Mean Trap Coverage
Mainstem Chehalis (MSC)	12-Feb	7	6-May	19	84	750	0.018
South Fork Chehalis (SFC)	11-Feb	7	6-May	19	85	940	0.066
Mainstem Newaukum (MSN)	20-Feb	8	6-May	19	76	823	0.061
South Fork Newaukum (SFN)	4-Feb	6	6-May	19	92	877	0.066
North Fork Newaukum (NFN)	10-Feb	7	13-May	20	93	890	0.075
Upper Skookumchuck (SKU)	18-Mar	12	6-May	19	49	327	0.051
Middle Skookumchuck (SKO)	11-Feb	7	13-May	20	92	421	0.035

The mean ratio of trap width (3-ft) to total wetted channel width, i.e., the fraction of the wetted stream channel cross-section sampled by the basic, non-extended trap unit is shown here for perspective regarding variation among sites for trap coverage. Seasonal mean coverages were similar, 5.1%-7.5%, at five of the sites but were lower at the MSC and SKO sites; 1.8% and 3.5%, respectively. The effects of wing panels on channel coverage and trap effectiveness were accounted for in estimates of fry emigration timing and estimates of genotype frequencies. The methods and results are described in Appendices A and B.

The traps captured 8,614 fish of 13 species⁵ during the pilot study (Table 2). Salmonids (genus *Oncorhynchus*) were the most common fishes and dominated the catch at all sites except SFN where lamprey were more abundant. Chinook salmon juveniles far exceeded the catch of other salmonid species at all sites, followed by coho salmon.

⁵ Species were identified to the following taxonomic levels: Chinook Salmon *Oncorhynchus tshawytscha*, Coho Salmon *O. kisutch*, Cutthroat Trout *O. clarki*, Rainbow Trout *O. mykiss*, Sculpin *Cottus sp.*, Lamprey *Lampetra sp.*, Dace *Rhinichthys sp.*, Pikeminnow *Ptychocheilus oregonensis*, Shiner *Richardsonius balteatus*, Stickleback *Gasterosteus aculeatus*, Sucker *Catostomus macrocheilus*, Rock Bass *Ambloplites rupestris* and Largemouth Bass *Micropterus salmoides*.

Table 2: Total catches by species at all seven study locations in 2020.

Location	Chinook Salmon	Coho Salmon	Cutthroat Trout	Rainbow Trout	Sculpin	Lamprey	Dace	Pikeminnow	Shiner	Stickleback	Sucker	Rock Bass	Largemouth Bass
Mainstem Chehalis	419	7	1	1	13	28	25	8	342	-	-	3	-
South Fork Chehalis	124	19	-	-	5	184	1	33	21	1	-	37	-
Mainstem Newaukum	128	20	-	1	80	44	2	10	5	-	-	23	2
South Fork Newaukum	611	49	1	3	80	120	61	21	14	1	-	-	-
North Fork Newaukum	307	137	1	-	34	130	34	5	6	9	1	-	-
Upper Skookumchuck	2,202	510	-	1	27	22	1	-	-	-	-	-	-
Middle Sookumchuck	2,475	100	-	-	19	13	19	-	5	6	1	1	-
Total All Sites	6,266	842	3	6	258	541	143	77	393	17	2	64	2

Environmental Conditions: Stream Temperatures

Water temperatures were measured and recorded to the nearest tenth of a degree centigrade (°C) at the beginning and end of each trapping event at all sites. Based on these temperature measurements, there were no significant differences among sites ($F_{6, 422} df = 0.71$; $p = 0.642$) but there was a significant increase in temperatures over the sampling weeks ($F_{1, 422} df = 303.19$; $p < 0.0001$). There was also a significant interaction between sites and weeks because the sites warmed at different rates ($F_{6, 422} df = 2.31$; $p = 0.033$; Figure 6). This differential rate of warming was not considered in the interpretation of trap catches and estimates of genotype frequencies.

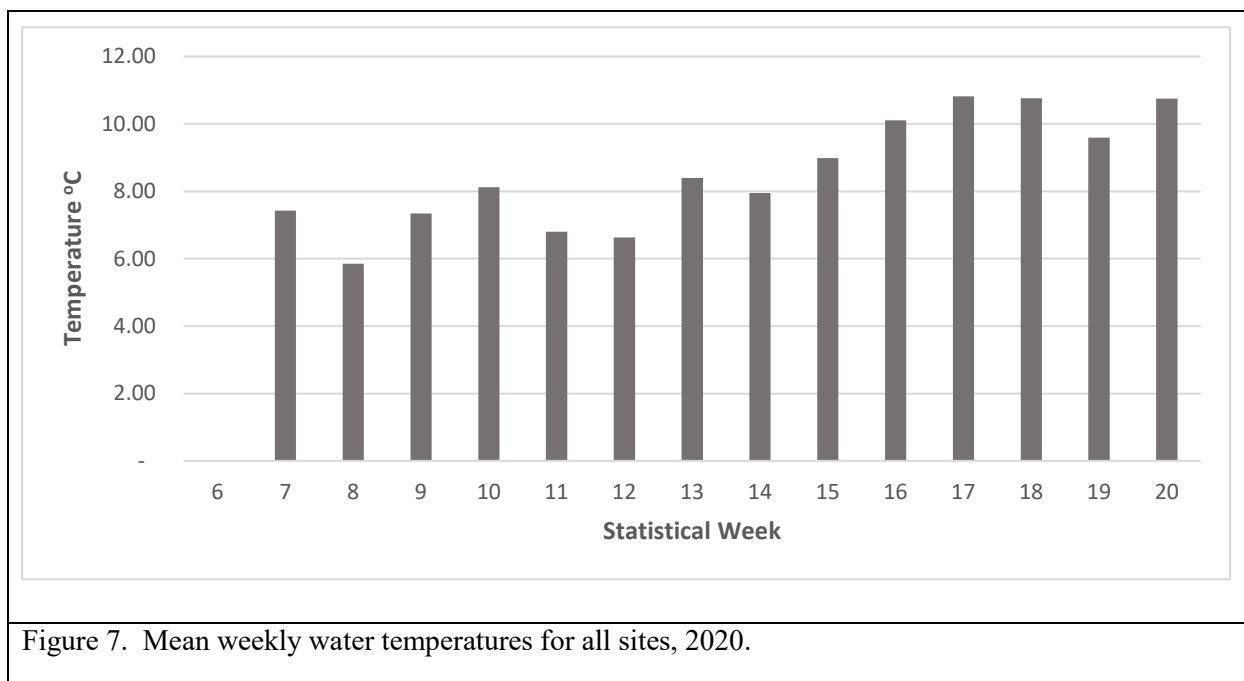
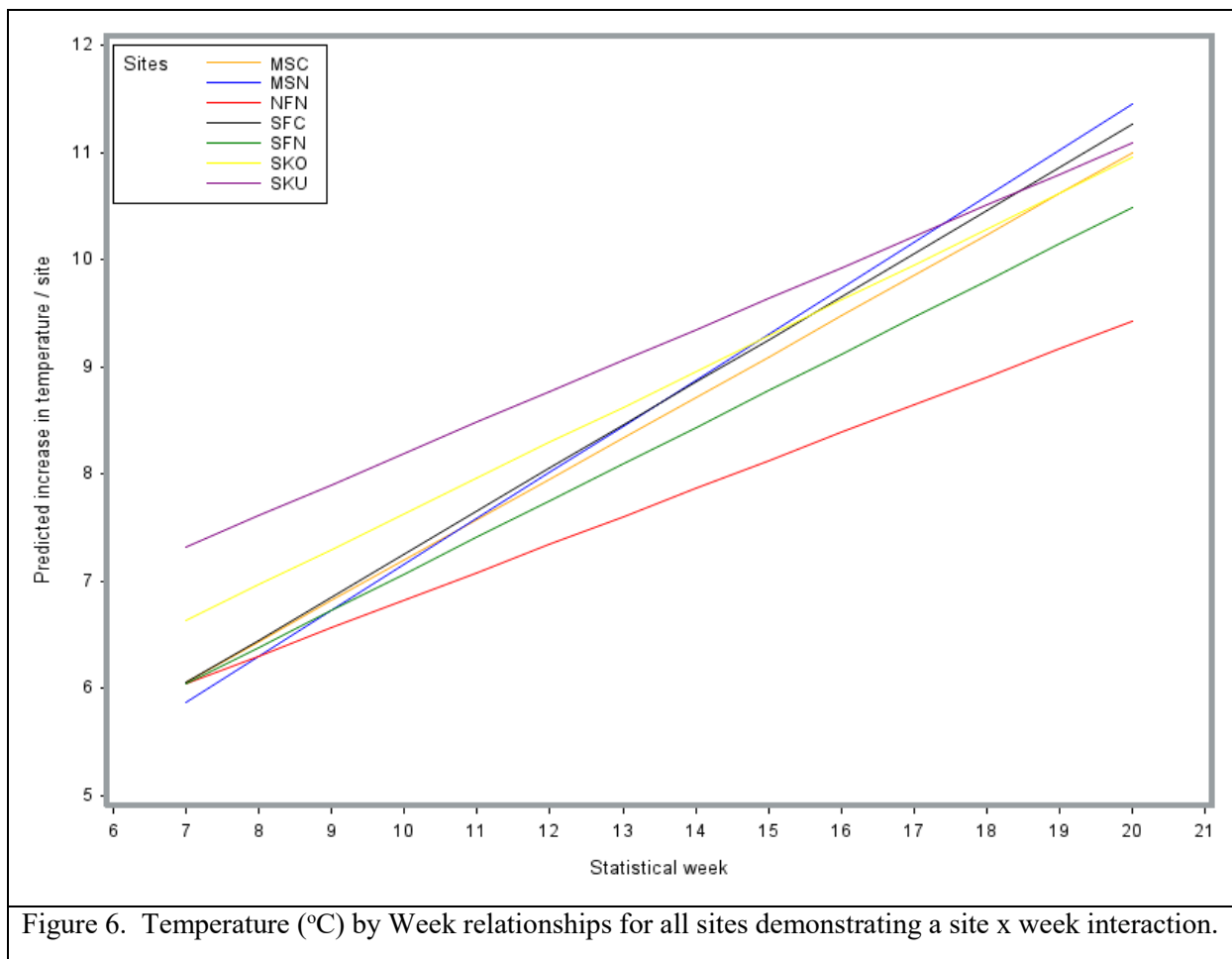
Stream temperatures ranged from 3.5 °C to 12.5 °C and trended from cool in February at the beginning of sampling to warm in May at the end of sampling. Weekly mean temperatures varied around approximately 7 °C during Weeks 7-12, gradually increased to 10.5 °C during Weeks 13-16 and leveled there for the duration of the project (Figure 7; See Appendix D for statistical week dates).

Environmental Conditions: Stream Flows

Daily stream discharge data are available for all of the streams where trap sites were located. Continuously recording gauging stations are operated by USGS at the following locations:

- Skookumchuck River (RM 6.4) - USGS station 12026400
- Newaukum River (RM 4.2) - USGS station 12025000
- North Fork Newaukum River (RM 7.9) - USGS station 12024400
- South Fork Newaukum River (RM 23.1 – miles from Chehalis R.) - USGS station 12024000
- South Fork Chehalis River (RM 16.5) - USGS station 12020800
- Upper Chehalis River (RM 101.8) - USGS station 12020000

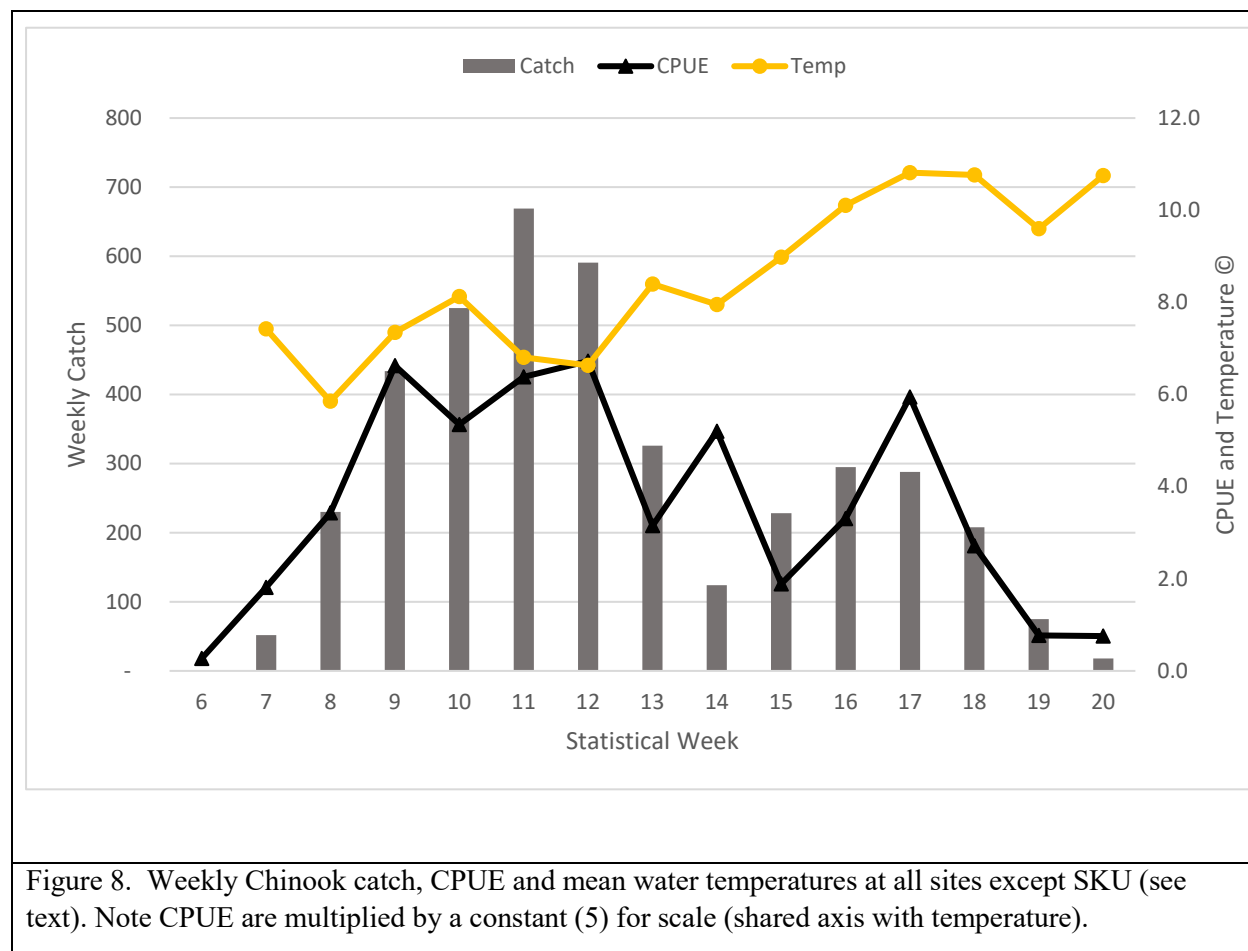
Flow measurements for each site are provided along with catch and effort data in Appendix A.



Chinook Fry Abundance and Timing

Fry Trapping results by individual sites are provided in Appendix A.

The mean timing patterns of Chinook catch and CPUE for all sites except SKU are shown in Figure 8. Data

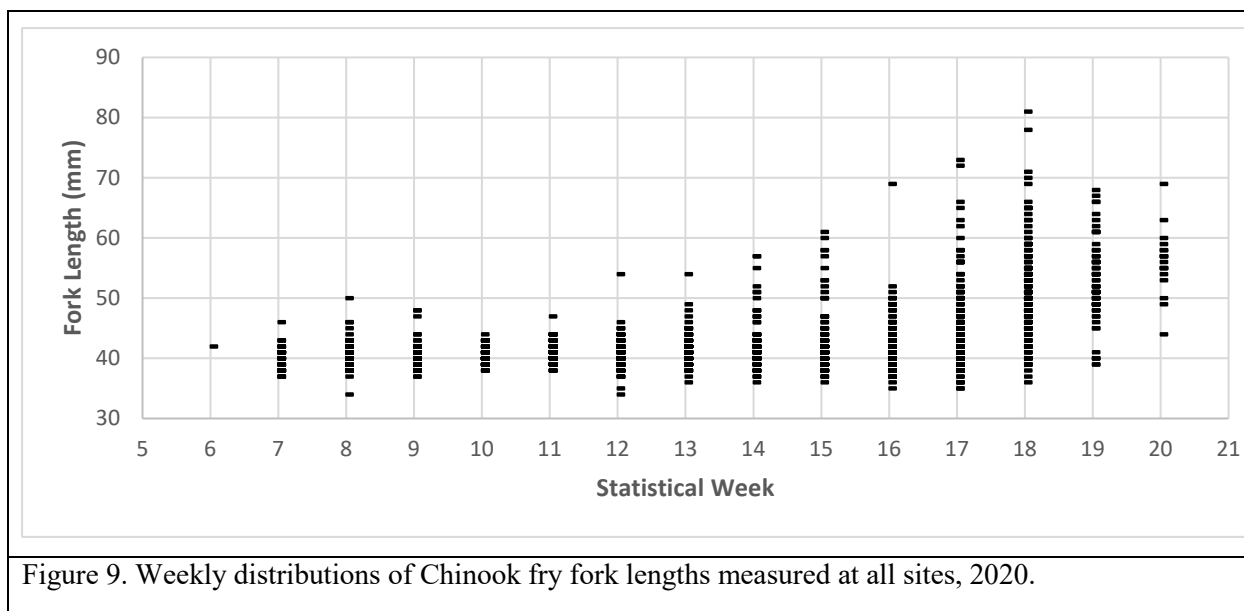


for the SKU site were excluded because trapping there did not start until Statistical Week 12, several weeks later than the other sites, and its catches were large enough to skew timing patterns from the other sites. Chinook fry abundance was relatively low in early February (Weeks 6 and 7), increased gradually to peak abundance in mid-to-late March (Weeks 11 and 12) and then declined to relatively low abundance in early May (Weeks 19 and 20). The data also show a bimodal, secondary peak abundance in Weeks 16 and 17 but it is unclear whether this pattern is due to actual fry abundance and behavior or to stream flow effects. All of the sites experienced an increased streamflow event during Week 14.

Water temperatures remained relatively low through mid-March (Weeks 7-12) and then increased progressively through April (Weeks 13-18; Figure 8). The shift toward increasing stream temperatures in Week 13 corresponds to a decline in Chinook catches that persisted for the rest of the study period. It is not known if these observed concurrent patterns of Chinook abundance and stream temperatures are related.

Chinook Fry Lengths

Fork lengths were measured and recorded to the nearest millimeter for 2,261 Chinook fry from all sites during the 2020 sampling season. The measured fry were selected without bias and represent length frequency distributions of the total numbers captured each week. The lengths ranged from 34 mm to 81 mm and the frequency distributions varied among weeks ($F_{1, 2242} \text{ df} = 776.78$; $p < 0.0001$; Figure 9). Mean lengths fluctuated around 40 mm during Weeks 7-12, gradually increased to 44 mm by Week 17 and then increased more steeply to 56 mm by Week 20 (Figure 10).⁶



The measured lengths varied among sites ($F_{6, 2237} \text{ df} = 17.18$; $p < 0.0001$) and there was a significant Week x Site interaction effect ($F_{6, 2230} \text{ df} = 35.2$; $p < 0.0001$). Mean lengths increased through the sampling season at all sites but they did not increase at the same rate. The fork length data for each site are summarized in Appendix A.

The weekly length frequency distributions and trends in mean lengths should be interpreted within the context of life-history-behavioral models applied to this study (see section *Why Target Emergent Fry?* above). The model and criteria adopted for this report emphasize the presence of two distinct groups of Chinook fry captured by the traps. The first group (Emergent Fry) is composed of newly emerged individuals, ≤ 45 mm FL, which were being carried downstream passively by streamflow. This group would soon establish themselves at temporary rearing locations downstream of the trap sites. The second group (Rearing Fry) is composed of fry > 45 mm FL that had emerged earlier at sites further upstream, established themselves at temporary rearing habitats and then initiated gradual but active downstream movement.

⁶ / Fry with protruding yolk sacs that measured as large as 39 mm were captured periodically. It is reasonable to assume that newly emerged fry ranged in sizes up to 42 or 43 mm. This is consistent with observations made by QIN biologists in the Queets River studies in the 1970s who observed that fry as large as 42 mm showed incomplete closure of the abdominal body wall associated with absorbed yolk sac (indicating recent emergence) (observations by Larry Lestelle).

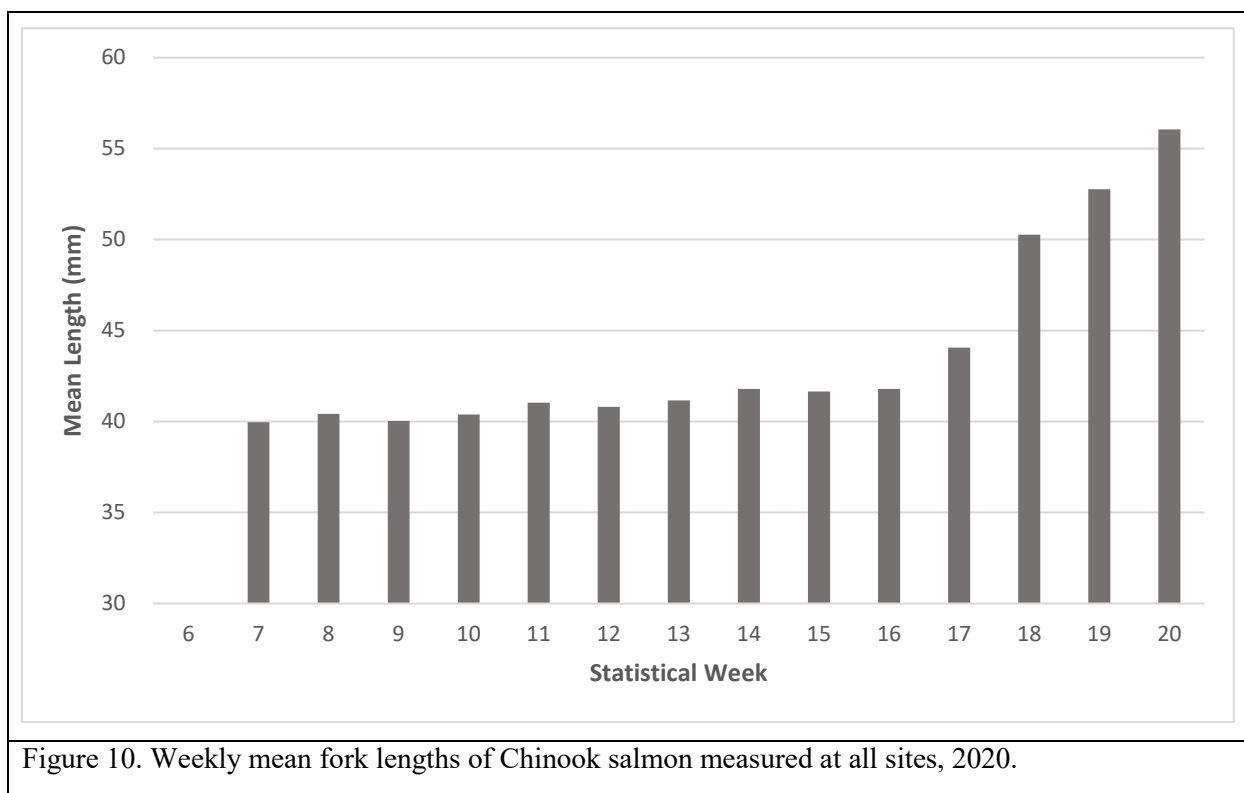


Figure 10. Weekly mean fork lengths of Chinook salmon measured at all sites, 2020.

This partitioning of weekly fry catches by length provides better clarity for interpreting trends in catches and length frequencies. Chinook captured at the trap locations were dominated by Emergent Fry during Weeks 7-16 and then transitioned to nearly all Rearing Fry by Week 20 (Figure 11). The apparent low abundance of all Chinook fry and reduced frequencies of Emergent Fry after Week 17 suggests emergence at the spawning locations targeted during site selection was nearly complete by the end of sampling and the timing and attribute data gathered through the sampling season are representative of total production from those spawning locations.

Lengths of Emergent and Rearing Fry were analyzed separately with linear models ($\text{Length} \sim \text{Site} + \text{Week} + \text{Site} \times \text{Week}$) to determine whether there were any significant differences among sites or over time (weeks). The results show, for both Emergent and Rearing fry, there were significant Site and Week effects and there was significant Week-by-Site interaction for Rearing Fry (Table 3).

The weekly catches and samples taken at each study site were not drawn from stationary populations. Rather, the weekly catches and samples were taken from transient populations with varying attributes. The trend of increasing length over time does not represent growth of individuals in a single population but represents shifting mixes of emergent and rearing fish at varying stages of development and growth.

Tissue Samples

Tissue samples were collected from captured Chinook fry in each week at each site for genetic processing at UC-Davis. The sampling protocol was to take a tissue sample and measure all Chinook fry captured up to 50 per site per week. This resulted in variation of sampling fractions among trapping sites ranging from 100% to 16.1% (Table 4). Sampling fractions were similar and large for the Chehalis and Newaukum sites but considerably smaller at the Skookumchuck sites due to the larger catches at the Skookumchuck sites and the 50-fish target for weekly tissue sample sizes.

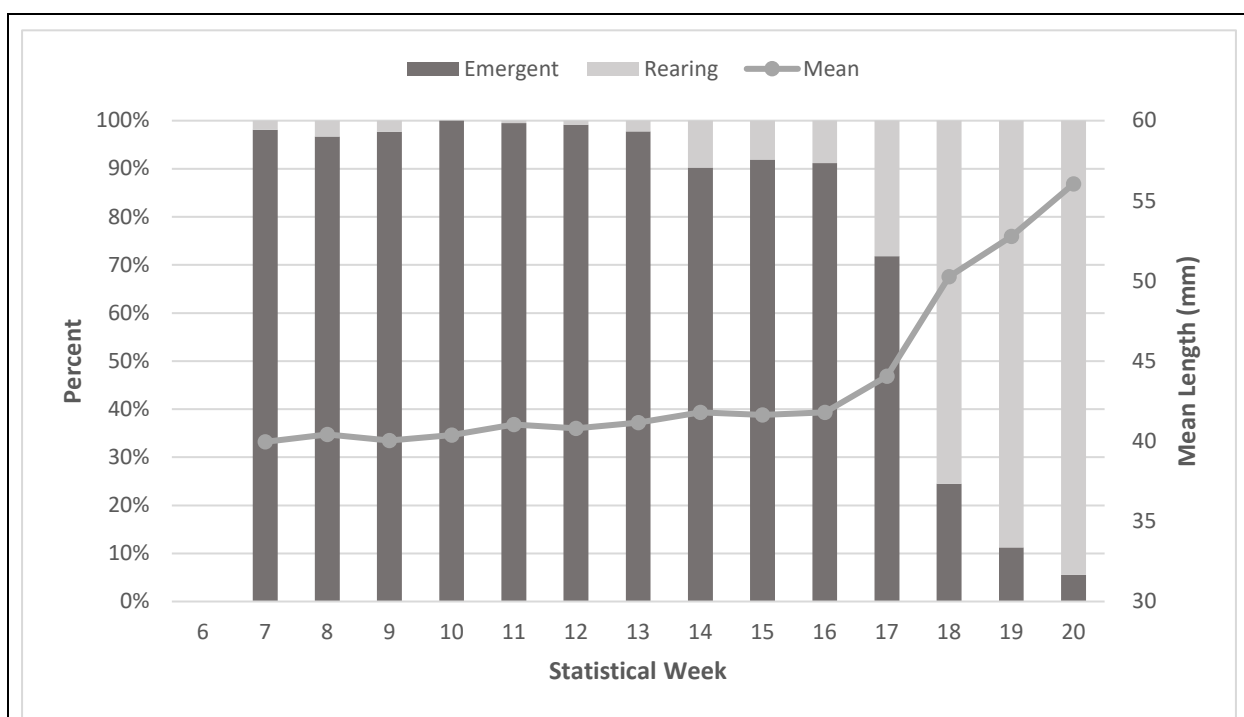


Figure 11. Weekly proportions of emergent and rearing fry and weekly mean lengths of Chinook fry captured at all sites.

Table 3: Statistical results for analysis of site and time (weeks) effects on Chinook fork lengths during the 2020 sampling season.

Effect	Emergent Fry			Rearing Fry		
	Degrees of Freedom	F Statistic	p value	Degrees of Freedom	F Statistic	p value
Week	1, 1860	11.25	<0.0001	1, 356	37.25	<0.0001
Site	6, 1860	4.78	<0.0001	6, 356	3.69	<0.01
Week x Site	6, 1860	1.82	=0.09	6, 356	3.3	<0.01

Emergent Fry Genotype Data

The acquisition and processing of tissue samples for genotype identification were successful. Two thousand two hundred sixty one tissue samples were collected through the season and 2,243 were processed and classified to genotype. Three run type genotypes of Chinook fry were identified: Homozygous for the fall allele (FALL), homozygous for the spring allele (SPRING) and heterozygous fall-spring hybrids (HET). All three genotypes were present at all sites except NFN where only FALL and HET individuals were detected. The FALL genotype was the most abundant (78.6%) in samples from all sites, ranging from 50.8% at MSN to 97.8% at NFN (Table 5). The HET genotype was second most common

Table 4: The total Chinook fry catch and tissue samples processed at each site, 2020.

Location	Total Catch	Tissue Samples	Sample Fraction
MSC	419	324	0.773
SFC	124	124	1.000
MSN	128	128	1.000
SFN	611	447	0.732
NFN	307	233	0.759
SKU	2,202	355	0.161
SKO	2,475	650	0.263
TOTAL	6,266	2,261	0.361

(17.2%) ranging from 40.6% at MSN to 2.2% at NFN. The SPRING genotype was the least abundant (4.2%) and ranged from nil at NFN to 9.5% at SFN.

There was substantial variation among the sites for genotype frequencies. The genotype frequencies at each site were compared with frequencies at each of the other sites and more than half (57.1%) of the site-to-site comparisons were significant. Frequency variation among sites was most prevalent for the FALL genotype (71.4%) followed by HET (64.3%) and SPRING (35.7%) (See Appendix B for details of the methods and comparisons).

Table 5: Genotype frequencies of total Chinook fry tissue samples collected in 2020.

Location	FALL	HET	SPRING
MSC	0.643	0.304	0.053
SFC	0.782	0.185	0.032
MSN	0.508	0.406	0.086
SFN	0.701	0.204	0.095
NFN	0.978	0.022	0.000
SKU	0.958	0.037	0.006
SKO	0.808	0.164	0.030
TOTAL	0.786	0.172	0.042

One thousand eight hundred and eighty nine of the samples processed were Emergent Fry and 1,871 of them were identified to genotype. All three genotypes were found at all sites except NFN where only FALL and HET individuals were detected (Table 6). The FALL genotype was the most abundant (78.6%) in samples from all sites, ranging from 50.9% at MSN to 97.7% at NFN. The HET genotype was the second most common (17.6%) ranging from 2.3% at NFN to 40.2% at MSN and the SPRING genotype was the least abundant (3.8%) ranging from nil at NFN to 8.9% at MSN. Genotype frequencies in the Emergent Fry were generally the same as for total fry sampled shown in Table 5. There was substantial variation of genotype frequencies among sites for the Emergent Fry samples although less than for all fry samples. Variation among sites was again most prevalent for the FALL genotype (64.3%) followed by HET (60.7%) and SPRING (14.3%) (See Appendix B for details).

Table 6: Genotype frequencies of Chinook Emergent Fry tissue samples collected in 2020.

Location	FALL	HET	SPRING
MSC	0.650	0.297	0.052
SFC	0.750	0.202	0.048
MSN	0.509	0.402	0.089
SFN	0.748	0.193	0.059
NFN	0.977	0.023	-
SKU	0.956	0.036	0.007
SKO	0.807	0.163	0.030
TOTAL	0.786	0.176	0.038

The pilot study demonstrated the feasibility of estimating run-type genotype proportions of Chinook salmon fry production from portions of the upper Chehalis Basin. Catch and effort data along with length measurements and genotype identification for Chinook fry captured at each of the trap sites were compiled and processed through analysis sequences to estimate the total number of emergent fry, by genotype, emigrating

past the trap sites during the sampling season. Details of the procedures used can be found in Appendices A and B.

The estimated number of Emergent Fry emigrating past each trap site each week is presented in Table 7. The largest populations of Emergent Fry were encountered at the Skookumchuck River sites and the smallest was at SFC. SPRING was the least abundant genotype at all sites ranging from none at NFN to 5,420 at SKO. FALL was the most abundant genotype at all sites except MSN where HET was more abundant. A very important finding based on these estimates is the abundance of HET individuals relative to SPRING. HET were more than four times more abundant than SPRING individuals were. The weekly estimates also show a tendency for SPRING fry to appear mostly early in the season, FALL fry to appear later and HET fry to be somewhat intermediate but more similar to SPRING (Table 7 and Figure 12).

Table 7: Estimated weekly Emergent Fry production by genotype by site.

Statistical Week	MSC			SFC			MSN			SFN			NFN			SKU			SKO		
	FALL	HET	SPRING	FALL	HET	SPRING	FALL	HET	SPRING	FALL	HET	SPRING	FALL	HET	SPRING	FALL	HET	SPRING	FALL	HET	SPRING
6												138									
7	586	1,173	-	-	-	-				16	16	31	-	-	-				4,438	2,034	1,109
8	910	273	-	-	-	-	150	600	150	116	136	136	-	-	-				9,171	5,774	1,019
9	1,055	767	-	-	18	-	74	221	74	588	190	35	-	-	-				22,730	10,332	689
10	1,777	957	137	41	-	-	237	79	40	776	325	100	-	-	-				13,400	11,339	1,031
11	4,118	1,611	448	101	62	-	191	222	48	278	145	44	-	-	-				25,492	4,974	622
12	3,360	6,365	4,067	79	32	16	62	43	10	699	166	37	-	-	-	111,707	4,965	4,965	28,438	605	605
13	3,912	4,157	367	22	9	4	60	26	7	509	246	16	-	-	-	65,236	1,331	-	9,814	1,115	223
14	1,896	862	-	70	-	-	57	86	-	244	92	-	158	-	-	49,291	4,286	-	27,809	1,209	-
15	1,385	-	-	95	-	-	11	33	-	3,160	70	-	592	-	-	2,414	-	-	1,454	37	-
16	12,997	366	-	108	-	-	34	-	-	924	41	-	1,233	-	-	767	40	-	4,902	245	123
17	1,063	-	-	43	-	-	28	-	-	506	17	-	1,111	72	-	379	13	-	18,685	1,099	-
18	54	-	-	6	-	-	72	-	-	14	-	-	231	14	-	123	-	-	4,381	-	-
19	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	570	-	-
20	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	284	-	-
Totals	33,113	16,531	5,018	565	120	20	976	1,311	327	7,830	1,444	537	3,326	86	-	229,918	10,636	4,965	171,569	38,764	5,420

The estimated numbers by genotype of Emergent Fry passing the trapping sites during the trapping period were approximately 447 thousand FALL (84.0%), 69 thousand HET (12.9%) and 16 thousand SPRING (3.1%; Table 8). These estimates do not represent total Emergent Fry production for the

Table 8: Genotype frequencies of Chinook Emergent Fry populations originating upstream of the trap sites.

GENOTYPE			
Location	FALL	HET	SPRING
MSC	0.606	0.302	0.092
SFC	0.801	0.171	0.028
MSN	0.373	0.502	0.125
SFN	0.798	0.147	0.055
NFN	0.975	0.025	-
SKU	0.937	0.043	0.020
SKO	0.795	0.180	0.025
ALL SITES	0.840	0.129	0.031

Chehalis Basin since areas where WDFW surveys indicated no spring Chinook redds were not trapped and some emergent fry were likely missed prior to commencement and after cessation of trapping.

Timing of Emergent Fry by Genotype

The trapping sites included in the 2020 pilot project collected data from virtually all areas where WDFW reported redds classified as produced by spring Chinook in 2019 and, with the exception of the Skookumchuck sites, the study captured samples representing a majority of emergent fry. The SKU trap was not operational until statistical week 12 and the large number of Chinook captured in the first sampling week suggests substantial numbers of Emergent Fry were likely missed; this conclusion is reinforced by

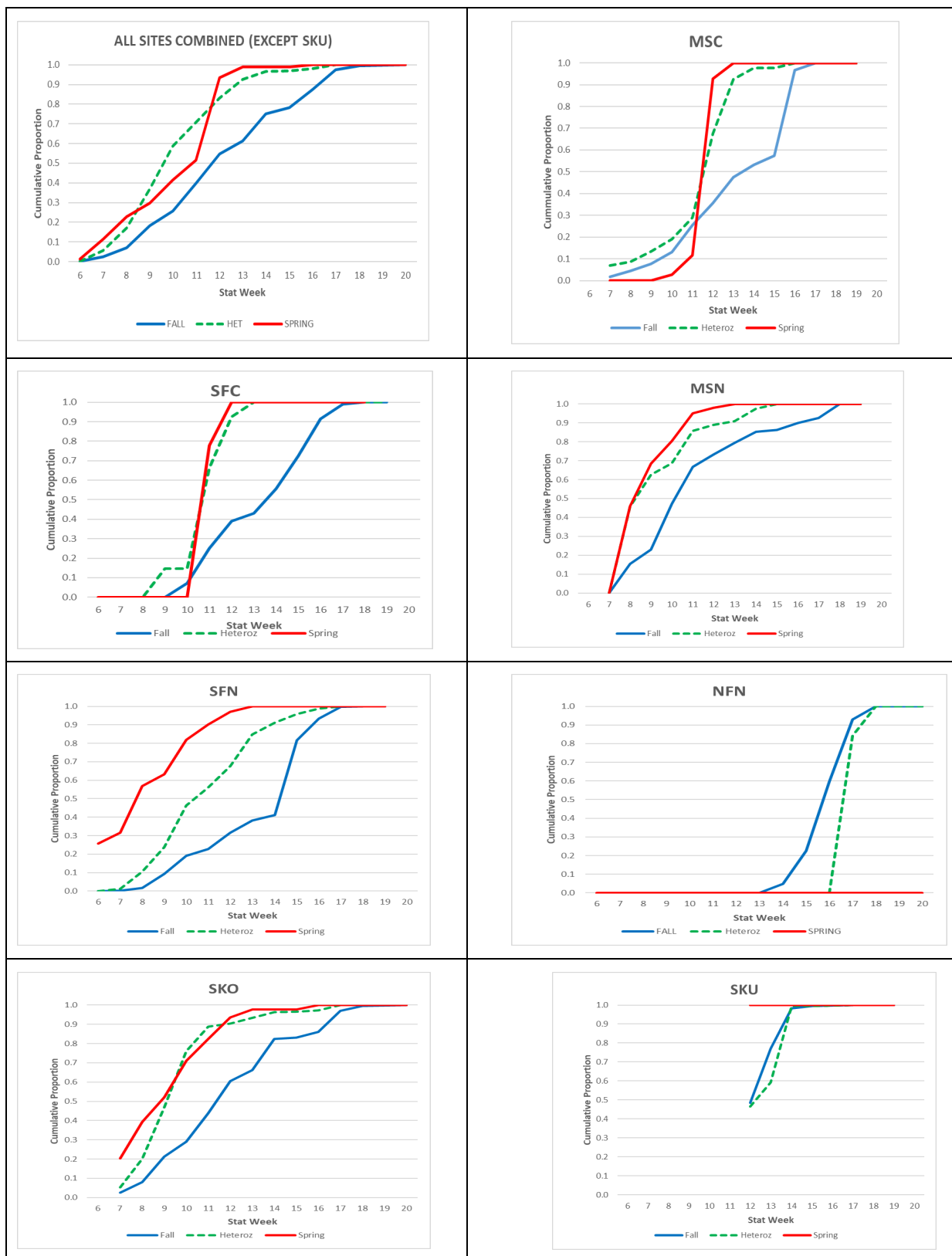


Figure 12. Relative timing of Emergent Fry by genotype based on summed estimates of weekly populations at all sites (excluding SKU) and for individual trapping sites.

the pattern of fry capture at the SKO site. Additionally, catch data for the MSC, MSN, and SKO sites suggest some small numbers of Emergent Fry likely passed those sites prior to the start of trapping. A combined effect of missing early emigration prior to initial trapping is likely underestimation of the proportion of SPRING genotype in Emergent Fry.

Figure 13 shows the weekly genotype frequencies of Emergent Fry estimated to have passed the fry traps over the duration of the pilot study. Considerable variation among sites for emergence timing is revealed although FALL emergence is usually later than SPRING and HET.

Inbreeding Analysis

To investigate levels of interbreeding between run-types, we first used the estimated Emergent Fry genotype frequencies to calculate frequencies of the spring and fall alleles. We then used these allele frequencies to calculate expected genotype frequencies under Hardy-Weinberg Equilibrium (HWE), a simple model that predicts the relationship between allele and genotype frequency using a set of basic assumptions such as random mating and no differential fitness among genotypes. Lastly, we calculated a statistic called F_{IS} (often referred to as the inbreeding coefficient) to compare the observed and HWE expected genotype frequencies.

F_{IS} values can range from -1 to 1. Identical observed and HWE expected genotype frequencies produce an F_{IS} of 0, indicating completely random mating among genotypes in the parental generation. An F_{IS} near -1 occurs when there is a strong deficit of heterozygotes in the observed genotype frequencies to HWE expectations, indicating strong reproductive isolation in the parental generation. Lastly, an F_{IS} near 1 occurs when there is an overabundance of heterozygotes in the observed genotype frequencies relative to HWE expectations, indicating strong preferential mating between individuals of different genotypes (e.g., FALL individuals preferentially mate with SPRING individuals as opposed to mating with other FALL individuals). The overall F_{IS} (i.e., from all sites combined) was -0.25, with the individual site values ranging from 0.07 to -0.46 (Table 9).

An overall F_{IS} of -0.25 means there was only a 25% reduction in the frequency of heterozygotes compared to HWE expectations. Thus, although there is some degree of non-random mating, these data indicate that Chehalis Chinook currently conform more closely to a model of random mating than to a scenario of strong reproductive isolation between the run-types (i.e., where an F_{IS} much closer to -1 would be expected). Interestingly, this pattern of interbreeding is consistent with the patterns of emergence timing where there is substantial overlap among all three genotypes even though spring Chinook fry emerge earlier on average. A general spawning scenario that is consistent with both the interbreeding and emergence time data is that there is currently little temporal difference in the start of spawning among the three genotypes (i.e., spring, heterozygote, and fall), but fall Chinook spawning continues after spring and heterozygote spawning has ended. This is indicated by the continued emergence of fall Chinook fry after spring and heterozygote fry emergence has ceased (see above).

Spawning Adult Genotype Frequencies

Direct estimates of genotype frequencies for the 2019 adult spawning population cannot be made because systematic sampling to acquire tissue samples for genetic analysis was not conducted. We estimated an overall spring-run allele frequency of approximately 9% in the emergent fry (see above; Table 9). Assuming this estimate is reasonably accurate and there was no selection (i.e., changes in allele frequency) between the parent and offspring generations, adult spawners that produced these fry would have had similar allele frequencies. However, it is not possible to estimate directly the parental



Figure 13. Genotype composition of Emergent Fry by stat week and trapping site. The total estimated Emergent Fry population of all genotypes is indicated in parentheses.

Table 9: Comparisons of observed and Hardy-Weinberg expected genotype frequencies and estimates of inbreeding coefficients (F_{IS}) for the study area.

Sites	Genotype frequencies (observed)			Spring Allele frequency (observed)	Genotype frequencies (HWE expected)			F_{IS}
	Fall	Het	Spring		Fall	Het	Spring	
MSC	0.606	0.302	0.092	0.243	0.573	0.368	0.059	-0.179
SFC	0.801	0.171	0.028	0.114	0.786	0.201	0.013	-0.150
MSN	0.373	0.502	0.125	0.376	0.389	0.469	0.141	0.070
SFN	0.798	0.147	0.055	0.129	0.760	0.224	0.017	-0.344
NFN	0.975	0.025	-	0.013	0.975	0.025	0.000	0.013
SKU	0.937	0.043	0.020	0.042	0.919	0.080	0.002	-0.459
SKO	0.795	0.180	0.025	0.115	0.783	0.204	0.013	-0.116
All sites	0.840	0.129	0.031	0.096	0.818	0.173	0.009	-0.253

genotype frequencies because the same emergent fry frequencies could be produced from distinctly different adult populations. For example, populations of spawning adults with genotype frequencies of 91% FALL, 0% HET, and 9% SPRING or with 82% FALL, 18% HET, and 0% SPRING could produce the same genotype frequencies in emergent fry. However, the highest possible adult spawner SPRING frequency would be a scenario with no adult HETs (i.e., the scenario with 91% FALL and 9% SPRING), and even this scenario has a much lower spring-run frequency than the WDFW estimate of approximately 14% based on spawning ground surveys in areas upstream of the trapping sites.

Although the parental genotype frequencies cannot be directly estimated from fry frequencies, it is useful to compare the fry genotype data from this study with the adult carcass genotype data reported in Thompson et al. 2019b. Thompson et al. analyzed 115 adult carcasses that were collected in the Chehalis Basin within and upstream of the Skookumchuck River in 2014-2016. They found 5 SPRING (4.3%), 20 HET (17.4%), and 90 FALL (78.3%) individuals. The fact that they observed many more HETs than SPRING carcasses suggests an adult spawning scenario with few HET relative to SPRING individuals is unlikely. Thus, the true frequency of SPRING individuals in the 2019 spawning population (which produced the fry collected in this study) was likely substantially less than 9%.

Since the carcasses analyzed by Thompson et al. were not collected in a systematic way, the extent to which their genotype frequencies were representative of the true frequencies from those years is unclear. For example, if the carcasses were collected in a way that favored FALL individuals, the true SPRING frequency might be higher than 4.3%. However, our observation that the emergent fry from this study, which were collected in a systematic way, contain SPRING and HET genotype frequencies that are even less than Thompson et al. values suggest the carcasses analyzed in their study were not strongly biased against SPRING individuals. This provides further evidence that the true frequency of SPRING individuals in the Chehalis Basin is much lower than WDFW estimates based on redd surveys.

Regardless of the exact genotype frequencies in the spawning adults that produced the fry collected in this study, the adult return from these emergent fry (primarily in 2023) is expected to have a genotypic composition similar to the emergent fry. In other words, the adult return to our study area in 2023 is

expected to be composed of approximately 85% FALL and only 3% SPRING individuals, with the remainder being HETs.

Findings and Conclusions

Following are the central results of this pilot study:

- The objectives of the pilot study were successfully achieved and results warrant continuation of the emergent fry study. The ability to trap, sample, and analyze newly emerged Chinook fry from each of the sites over the entirety of the emergence period was sufficient to estimate proportions of genotypes in emergent Chinook fry below areas where WDFW spawning escapement surveys identified spring Chinook redds. Continuation of the fry trapping study is warranted and should be supported for at least two additional years to obtain more information on emergence timing, variability in impacts of environmental conditions, genotype frequencies, and a clearer representation of the status of spring Chinook in the Chehalis Basin.
- The inclined-plane trap design developed for this study was efficient, durable and achieved the intended purpose. The trap captured 8,614 fish of 13 species and the trapping crew was able to process the catches with minimal fish mortality (.025 for all species; .019 for Chinook). The catches included 6,266 Chinook fry.
- A decision to partition the sampled fry population into Emergent Fry and Rearing Fry was based on review of pertinent literature and QIN experiences studying Chinook juveniles in the Queets River system. The criterion selected was to classify fry ≤ 45 mm FL as Emergent Fry and fry > 45 mm FL as Rearing Fry. This partitioning was supported by the study results (see length frequency presentations and analyses in Appendix A).
- Fry captured at the trap locations were dominated by Emergent Fry during Weeks 7-16 (Feb. 9 – April 18) and then transitioned to nearly all Rearing Fry by Week 20 (May 10-16).
- The pilot study catches suggest some Chinook fry emergence likely occurred in January, prior to the 2020 trap installation in early February. The results also suggest some emergence was still occurring after cessation of trapping in mid-May. However, the results also indicate trapping operations covered a large majority of fry emergence and emigration. Peak Chinook fry trap catches occurred early-March through mid-April.
- A large set of tissue samples (2,261) was taken from the Chinook fry and processed at UC-Davis. All three run-type genotypes, FALL, HET, and SPRING were identified in the samples and were found across all the trap sites except NFN where only FALL and HET were detected. Of the 2,261 tissue samples taken, 1,889 were Emergent Fry.
- For all sites combined, estimates of Emergent Fry passing the trapping sites during the trapping period by genotype were approximately 447 thousand FALL (84.0%), 69 thousand HET (12.9%), and 16 thousand SPRING (3.1%). These estimates do not represent total emergent fry produced by the Chehalis Basin since areas where WDFW surveys indicated no spring Chinook redds were not trapped and some emergent fry were likely missed prior to commencement and after cessation of trapping. Estimation of emergent fry from the entire Chehalis Basin would require a substantially expanded study involving trapping of all stream reaches downstream from reported Chinook redd locations.
- There was substantial variation among sites for genotype frequencies of Emergent Fry. Nearly 90% of genotype specific site-to-site comparisons were significantly different.

- There was considerable variation among sites for emergence timing of the three genotypes. However, FALL emergence was usually later than SPRING and HET.
- Estimated fall and spring allele frequencies based on the observed Emergent Fry genotype frequencies allowed comparisons of observed and expected genotype frequencies based on Hardy-Weinberg Equilibrium. An inbreeding coefficient (F_{IS}) of -0.25 was calculated from the comparisons. These results suggest Chehalis Chinook currently conform more closely to a model of random mating than to a scenario of strong reproductive isolation between the run-types. The patterns of emergence timing and substantial overlap among the genotypes supports this interpretation.
- The performance and outcomes of this pilot study were generally positive and met our expectations regarding the scope and quality of data and information generated. This pilot study provided opportunities to learn more about aspects of logistics, operations and equipment designs as applied to the Chehalis River environment and allowed adaptations for application to the second year of trapping (2021). Based on results of the 2020 pilot, we were able to eliminate one trapping site (SKU) and start trapping operations two weeks earlier in 2021 (Statistical Week 4). In addition, we set up a test for a trap design modification to improve trap effectiveness and limit the impacts of drifting materials from clogging entry into the sample box. We added a trash drum to one of the traps to test its effectiveness for reducing effects of clogging by debris drifting into the site.
- Recommendations for future studies
 - The feasibility of collecting tissue from spawners to determine the genetic composition of the fish producing Chinook redds should be investigated. Such data would inform interpretation of fry genotype estimates and provide valuable insight into timing of spawning, survivals and reproductive fitness. Genetic parentage analysis of spawners and emergent fry might also be used to relate emergent fry to specific redds.
 - Additionally, consideration should be given to installing water temperature recorders near trapping sites to monitor thermal accumulations during the Chinook incubation period and provide measures of the influence of temperature on incubation times from egg deposition to fry emergence.

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Run-Type Composition of Juvenile Chinook Salmon in the Upper Chehalis River Basin in 2020

APPENDIX A: Site-Specific Trapping Results

Quinault Indian Nation Department of Fisheries

July 2021

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Introduction

Appendix A presents a descriptive summary of data and information recorded at each trapping location (site) during the 2020 sampling season. Variation and trends in water temperatures and streamflows are presented along with trapping effort and Chinook fry catches. The catch and effort data are summarized to show relative timing of emergence, abundance and emigration.

Chinook fry captured in this study were partitioned into two life history, behavioral categories based on size. Fry less than or equal to 45 mm fork length (FL) are termed Emergent Fry and those greater than 45 mm FL are termed Rearing Fry (See the main report, *Study Design* Section for rationale). Weekly length measurements are summarized and relative capture timing of Emergent Fry and Rearing Fry are compared for each site.

Representative tissue samples were collected from Chinook fry captured at each site on each day of operation. The samples were processed and classified to run-type genotype. Three genotype classifications are used in this study; homozygous for the spring allele (SPRING), homozygous for the fall allele (FALL) and heterozygote fall-spring hybrids (HET; See the main report, *The Need for Genetic Sampling* Section for background). These data provide estimated weekly abundances and relative timing of the three genotypes, as derived by computational steps described in Appendix B. The estimated weekly abundances and relative emergence timing of the three genotypes are presented for each site.

The genotype frequencies of Emergent Fry are also compared to proportions of Chinook redds classified as fall or spring during WDFW surveys done in 2019 proximate to each site.

Trap Operations, Data Summaries and Results

Mainstem Chehalis (MSC)

Trapping Period

The Mainstem Chehalis River trap (MSC) was operated in weekly episodes from February 12 (Week 7) through May 6 (Week 19) of the 2020 sampling season (Figure A-1). The trap was fished for 750 hours during the season.

Streamflow and Temperature Conditions

Weekly mean streamflow at MSC gradually decreased from 1,950 cfs in Week 6 to 249 cfs by Week 13. The only remarkable flow event during the sampling season occurred in Week 14 when the weekly mean increased to 1,118 cfs and the flow effects persisted through Week 15. These increased flows may have hindered trap effectiveness but, since it still caught Chinook fry on those days, the magnitude of effect is not clear. Flows returned to lower levels and remained there during Weeks 16-20.

Weekly mean stream temperatures at the site ranged from 6.1°C to 11.5°C. The stream temperatures varied around 7.5°C early in the season (Weeks 7-14) and then increased during Weeks 15-17 to near 11°C.

Chinook Fry Catches

Four hundred nineteen Chinook fry were captured at MSC of which 324 were processed for length and tissue samples. Chinook fry were captured in the first week of operation so some emergence and transport past the site may have occurred before sampling was initiated. However, based on numbers and trends in catch over the initial sampling weeks, any missed production was likely a small proportion

of the total Emergent Fry population that drifted through the site. Chinook fry were captured in low numbers early in the season and then increased to bi-modal peaks in Weeks 12 and 16. Interpretations for the apparent bi-modal abundance are uncertain because potential effects of relatively high stream flows in Weeks 14 and 15 on Chinook fry transport and capture are unknown.

Length Frequencies and Life History Stages

The fork lengths of Chinook fry captured at MSC ranged from 34 mm to 60 mm and averaged 41.4 mm during the sampling season (Figure A-2). The distribution of measured lengths varied among weeks. Mean lengths increased gradually from 39.6 mm to 41.5 mm over Weeks 8-13 (Figure A-3), increased to 42.8 mm in Week 14 and then varied around 43 mm for the remainder of the sampling period. A partial explanation for the increased mean lengths after Week 13 is an increased entry of Rearing Fry into the trap catches.

Chinook catches at MSC were predominantly Emergent Fry (95.9%) throughout the sampling season (Figure A-1). Rearing Fry contributed to the catch in small numbers during Weeks 12-17 but were never more than 20% of the weekly catch. The lengths of Emergent Fry increased over weeks through the season (Figure A-4) from 39.6 mm mean length in Week 8 to 41.2 mm in Week 17 ($F_{1, 306} df=18.159$; $p<0.0001$). Although statistically significant, the small increase in length through the season may not be important. The original measurements were to the nearest millimeter so a change in mean length of 1.6 mm over nine weeks is not provoking and likely explanations are not obvious. The lengths of Rearing Fry at MSC did not vary over weeks through the season ($F_{1, 14} df=0.633$; $p=0.439$) and their overall mean length was 50.5 mm. The test for length change over time for Rearing Fry was limited by a small sample size of only 16 individuals.

Emergent Fry Abundance and Emergence Timing

The abundance and genotype frequencies of Emergent Fry moving downstream past the MSC site were estimated from the sampling data (see Appendix B for estimating procedures). An estimated 54,663 Emergent Fry drifted through the MSC site during the trapping period made up of 60.6% FALL, 30.2% HET, and 9.2% SPRING individuals (Figure A-5). Fall and HET were captured earlier than SPRING individuals were but the overall timing was much later for the FALL genotype than for HET and SPRING.

Comparison of Adult Redd and Fry Genotype Frequencies

Chinook redd counts in spawning locations upstream of MSC were classified to run types by WDFW and are compared with Emergent Fry genotype frequencies at MSC in Table A-1. Redds classified as spring run type made up 15.6% of the total redds upstream of MSC while only 6.2% of the Emergent Fry population were SPRING. Although there is considerable uncertainty in this comparison (See the main report *Spawning Adult Genotype Frequencies* Section for explanation) it certainly suggests the actual abundance of individuals homozygous for the spring allele is much less than the redd survey results imply.

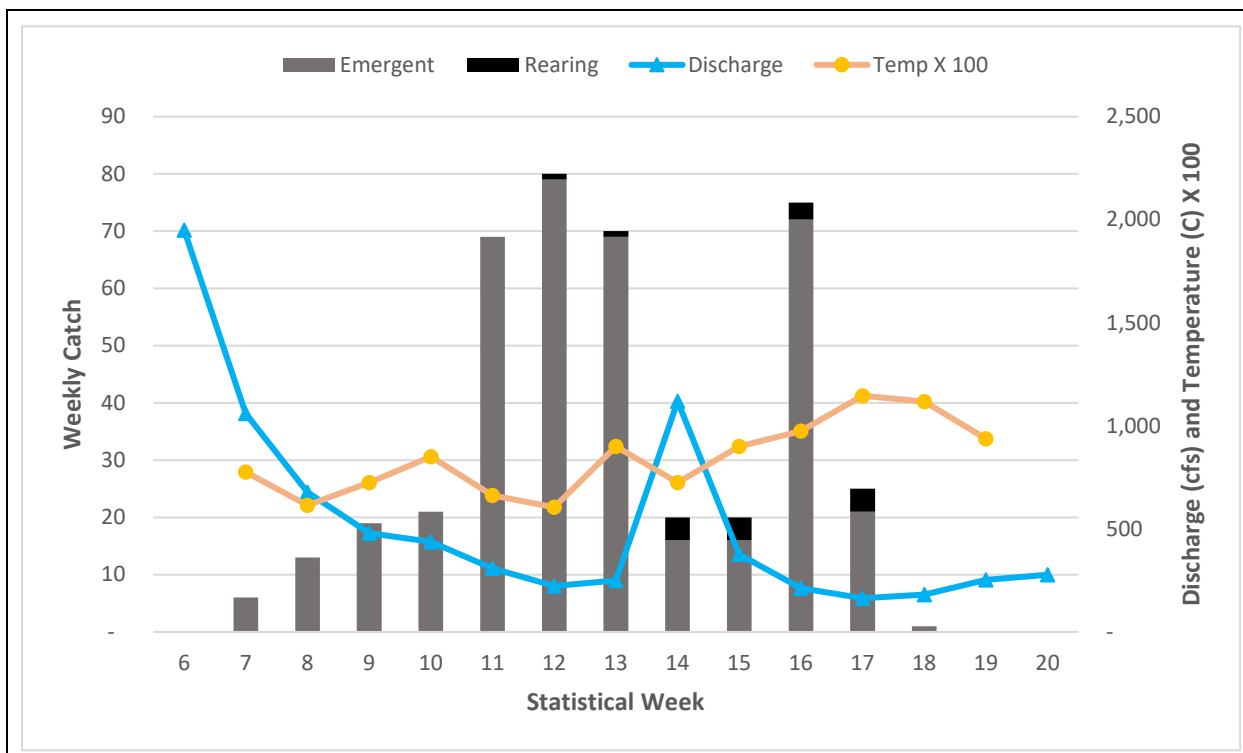


Figure A-1: Weekly Chinook catches, mean stream discharge and mean stream temperature at the MSC site, 2020. The weekly catches are partitioned into Emergent Fry and Rearing Fry based on lengths. Note the mean temperatures are multiplied by a constant (100) for scale (shared axis with discharge).

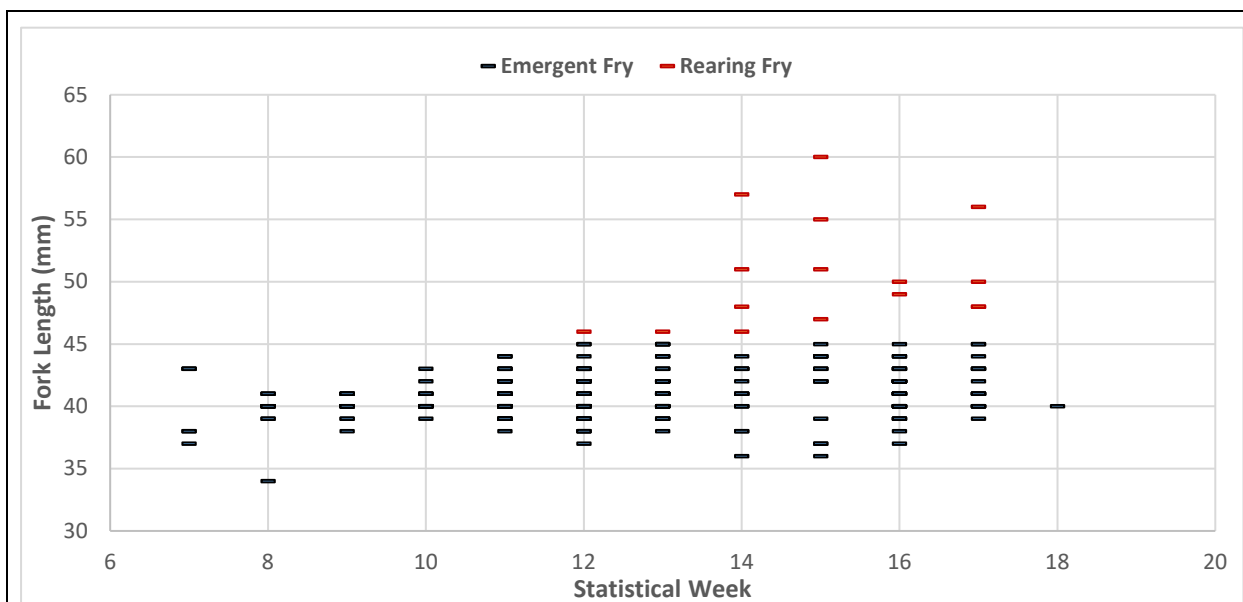


Figure A-2: Weekly fork lengths of Chinook fry measured at the MSC site, 2020.

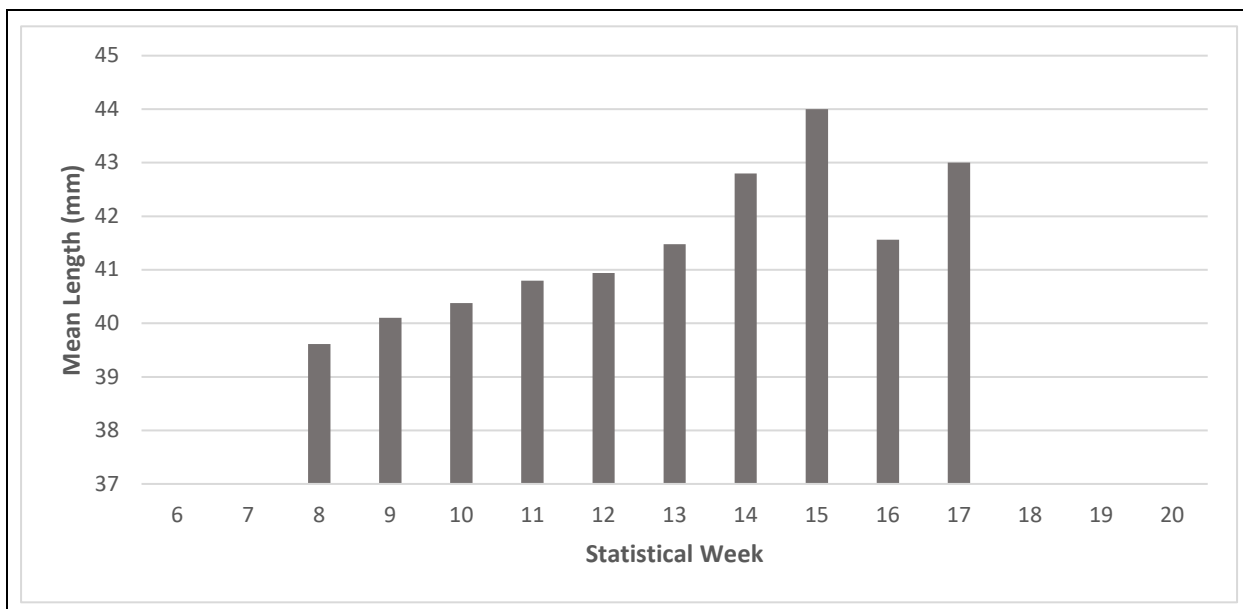


Figure A-3: Weekly mean fork lengths of Chinook fry measured at the MSC site, 2020. Note Weeks 7 and 18 are omitted due to small sample sizes.

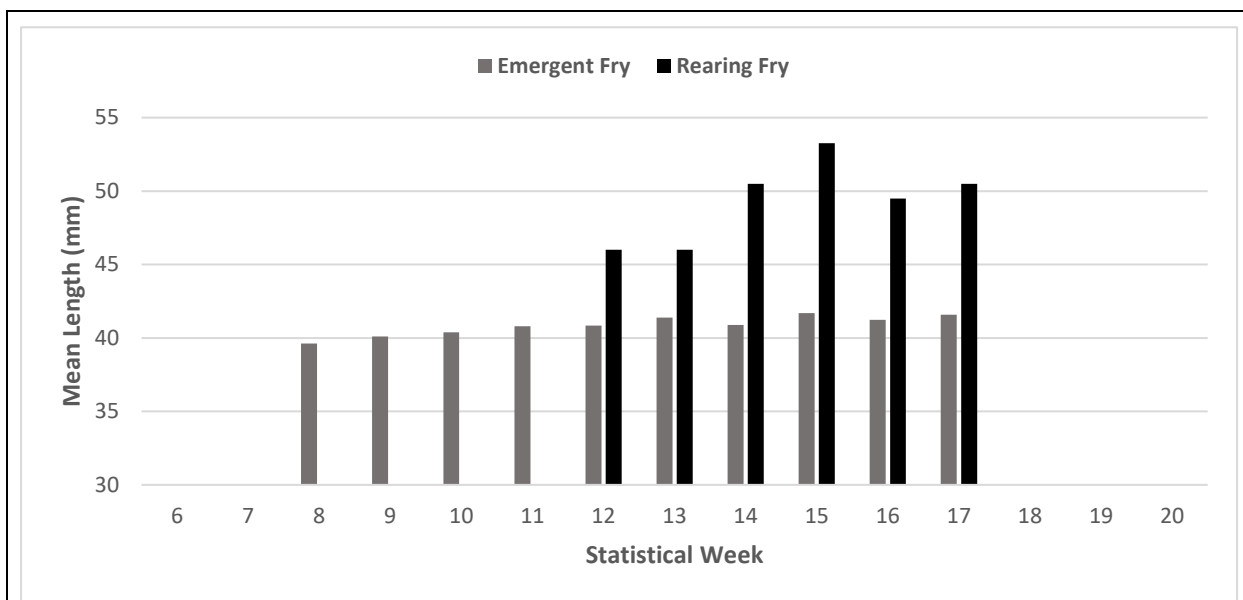


Figure A-4: Weekly mean fork lengths of Emergent Fry and Rearing Fry at MSC, 2020. Means for Emergent Fry in Weeks 7 and 18 are omitted due to small sample sizes. All weekly means of Rearing Fry are included regardless of sample size.

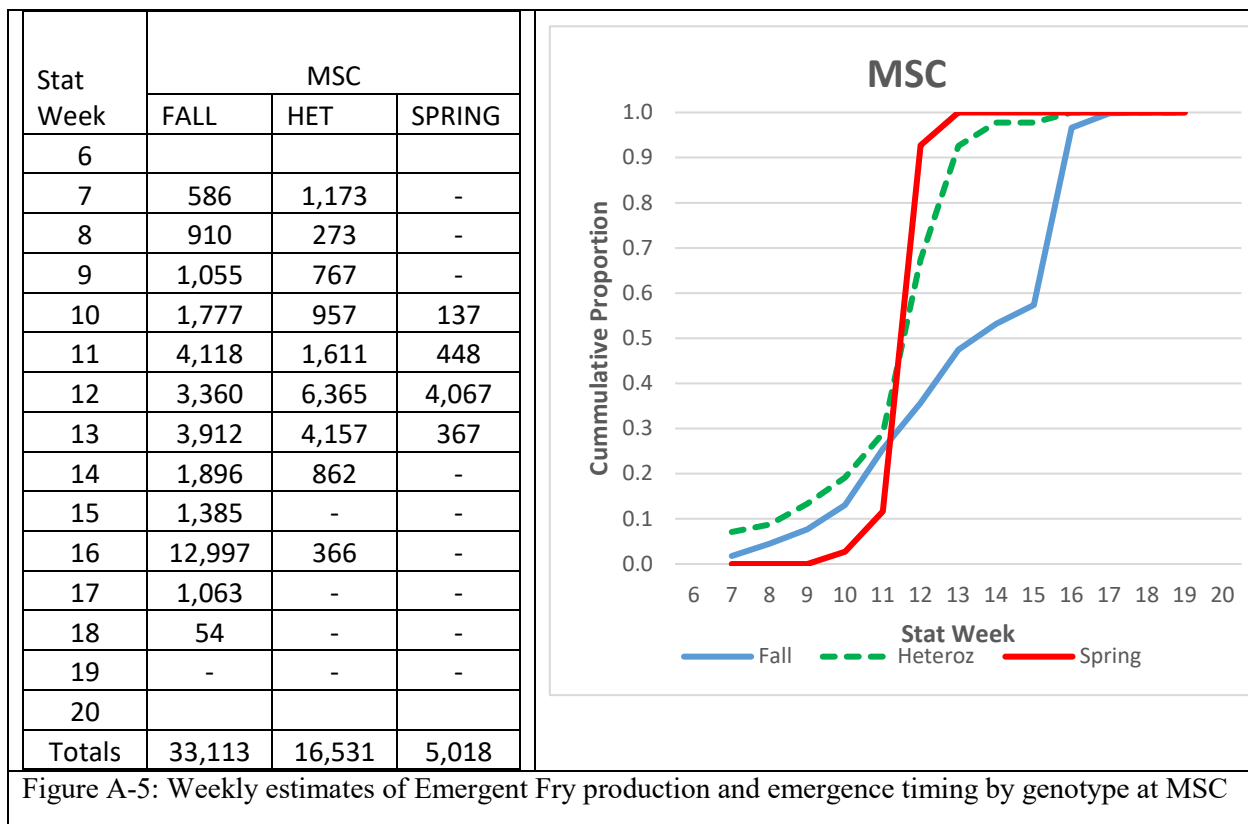


Table A-1: Chinook redd counts by run type and Emergent Fry genotype frequencies for MSC.									
2019 Run Type Classed Redds				Emergent Fry Genotypes					
Fall		Spring		FALL		HET		SPRING	
483	84.4%	89	15.6%	33,113	60.6%	16,531	30.2%	5,018	9.2%

South Fork Chehalis (SFC)

Trapping Period

The South Fork Chehalis River trap (SFC) was operated in weekly episodes from February 11 (Week 7) through May 6 (Week 19) of the 2020 sampling season (Figure A-6). The trap was fished for 940 hours during the season.

Streamflow and Temperature Conditions

Weekly mean streamflow at SFC gradually decreased from 429 cfs in Week 6 to 58 cfs by Week 13. The flows increased sharply in Week 14 and partial flow effects of the event extended into Week 15. The increased flows may have hindered trap effectiveness but, since it still caught Chinook fry on those days, the effect of flow variability on fry abundance and behavior could not be determined. Flows returned to lower levels by Week 16 and remained there for the rest of the sampling season.

Weekly mean stream temperatures at SFC ranged from 6.4° C to 11.3° C. The stream temperatures varied around 7.3° C early in the season (Weeks 7-14) and increased to near 11° C during Weeks 16-19.

Chinook Fry Catches

One hundred twenty-four Chinook fry were captured at SFC and they were all processed for length measurements and tissue samples. No Chinook fry were captured in the first two weeks of operation (Weeks 7 and 8) and then they were present in low abundance in Weeks 9 and 10. Based on these catches and trends over the initial four weeks of operation, it is likely this trap was in place early enough to capture fry representing the earliest emergence from spawning areas upstream and proximate to the site. Chinook catches increased rapidly in Week 11 and they remained relatively high in Week 12. Catches dropped to a moderate level in Week 13 and remained there through Week 17. The largest weekly catch of the season was in Week 18 and then catch dropped to nil in Week 19, the last week of operation.

Length Frequencies and Life History Stages

Chinook fry captured at SFC ranged from 39 mm to 70 mm FL and averaged 46.2 mm for the entire sampling season (Figure A-7). The distribution of measured lengths varied among weeks. Mean lengths varied moderately around 42 mm during Weeks 11-16 and then increased to 48.2 mm in Week 17 and 55.9mm in Week 18 (Figure A-8). A partial explanation for the increased mean lengths in Weeks 17 and 18 is the shift in abundance from mostly Emergent Fry to mostly Rearing Fry in the catches.

Chinook fry catches were mostly Emergent Fry (67.7%) and their numbers dominated catches during Weeks 9-16 (Figure A-6). Rearing Fry contributed to the catch in small numbers during Weeks 13 and 14 and then dominated catches in Weeks 17 and 18 (86.0%). The measured lengths of Emergent Fry did not change through the season ($F_{1, 82} \text{ df}=1.292$; $p= 0.259$) and their overall mean length was 42.1mm (Figure A-9). Lengths of Rearing Fry increased over weeks ($F_{1, 38} \text{ df}=6.910$; $p= 0.012$) from 47.5mm mean length in Week 13 to 56.2mm in Week 18.

Emergent Fry Abundance and Emergence Timing

The abundance and genotype frequencies of Emergent Fry moving downstream past the SFC site were estimated from the sampling data (see Appendix B for estimating procedures). An estimated 705 Emergent Fry drifted past SFC during the trapping period (Figure A-10) made up of 80.1% FALL, 17.0% HET and 2.8% SPRING individuals. FALL and HET individuals were captured earlier than SPRING but the overall timing for the FALL genotype was later than for HET and SPRING. The project data suggests Chinook production was small at this site for all genotypes.

Comparison of Adult Redd and Fry Genotype Frequencies

Chinook redd counts in spawning locations upstream of SFC were classified to run types by WDFW and are compared with Emergent Fry genotype frequencies at SFC in Table A-2. Redds classified as spring run type made up 16.2% of the total redds upstream of SFC while only 2.8% of the Emergent Fry population were SPRING. Although there is considerable uncertainty in this comparison (See the main report *Spawning Adult Genotype Frequencies* Section for explanation) it certainly suggests the actual abundance of individuals homozygous for the spring allele is much less than the redd survey results imply.

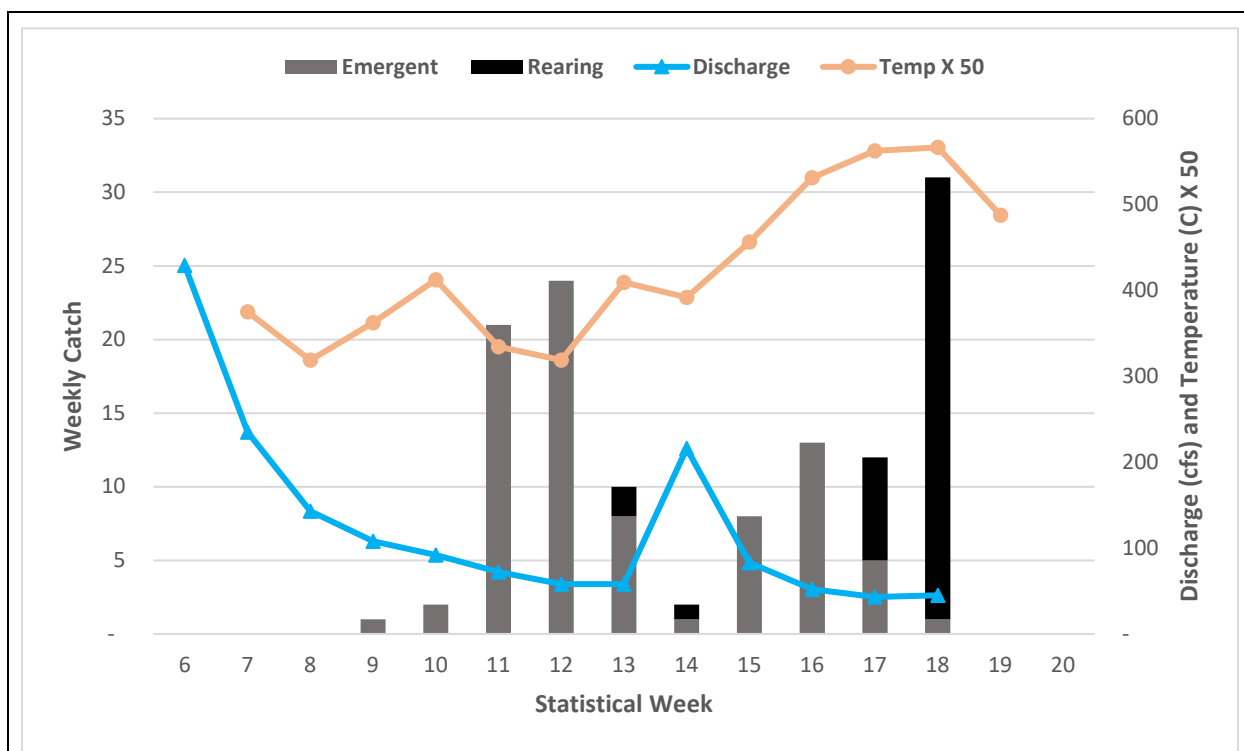


Figure A-6: Weekly Chinook catches, mean stream discharge and mean stream temperatures at the SFC site, 2020. The weekly catches are partitioned into Emergent Fry and Rearing Fry based on lengths. Note the mean temperatures are multiplied by a constant (50) for scale (shared axis with discharge).

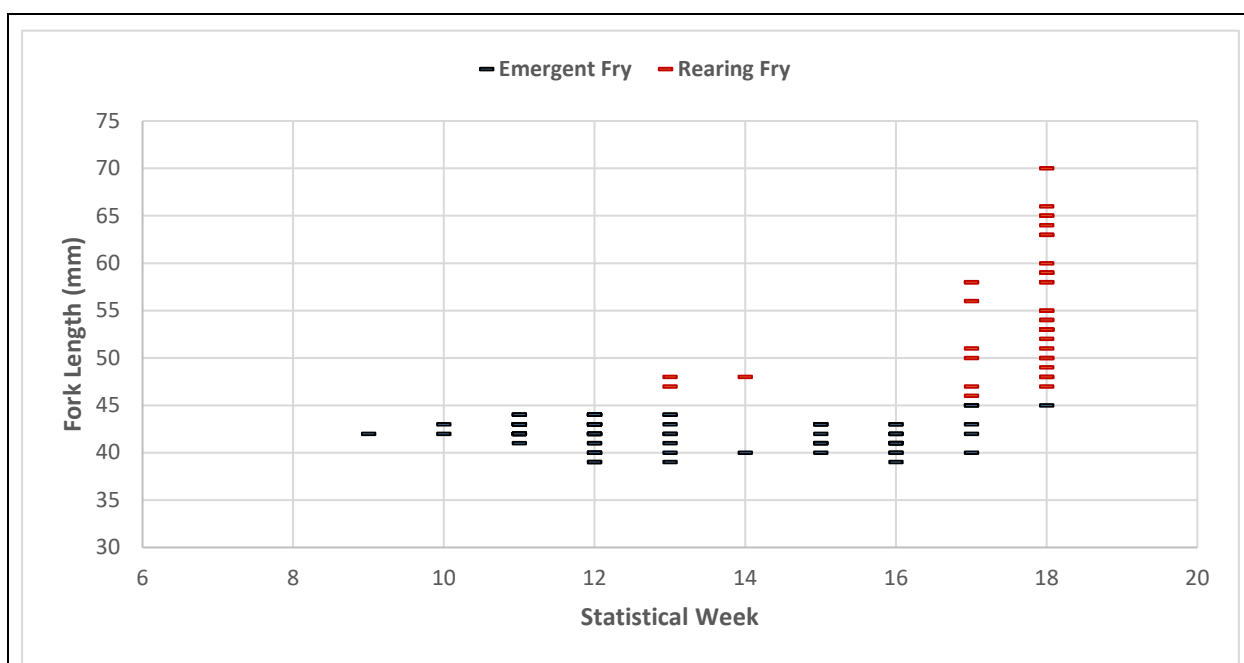
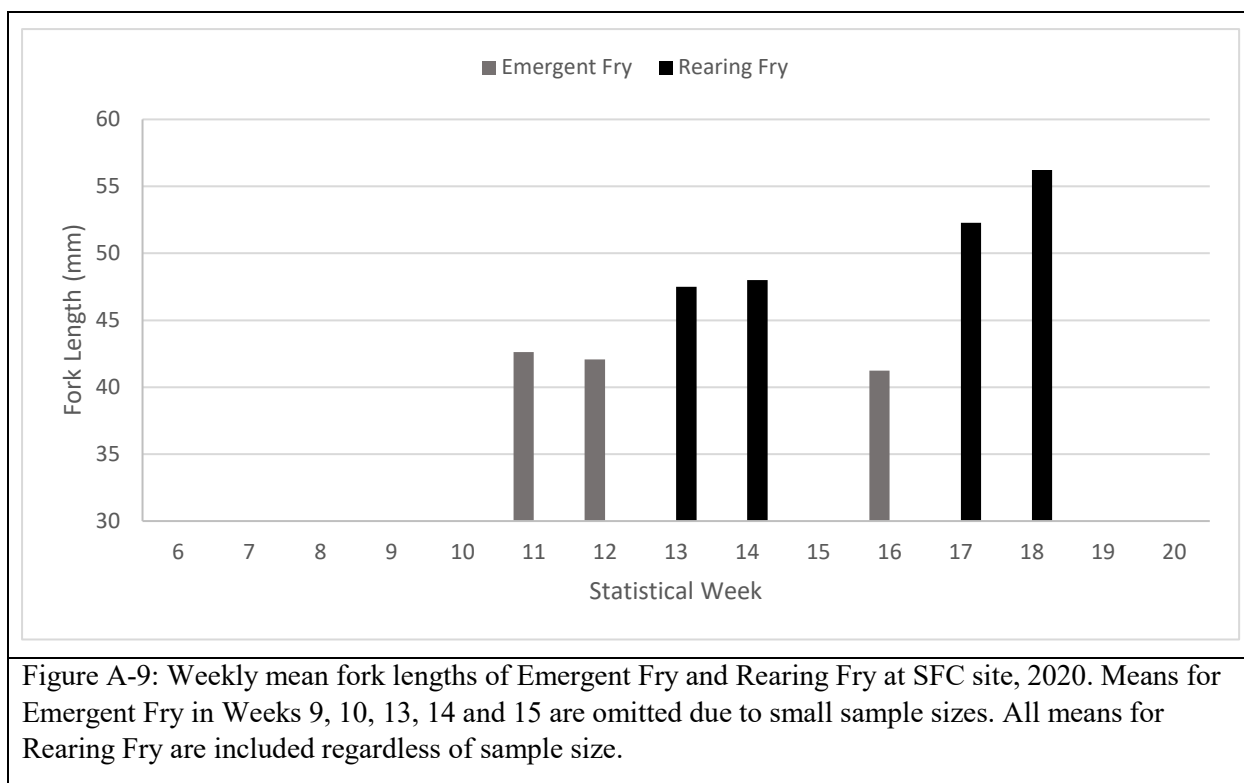
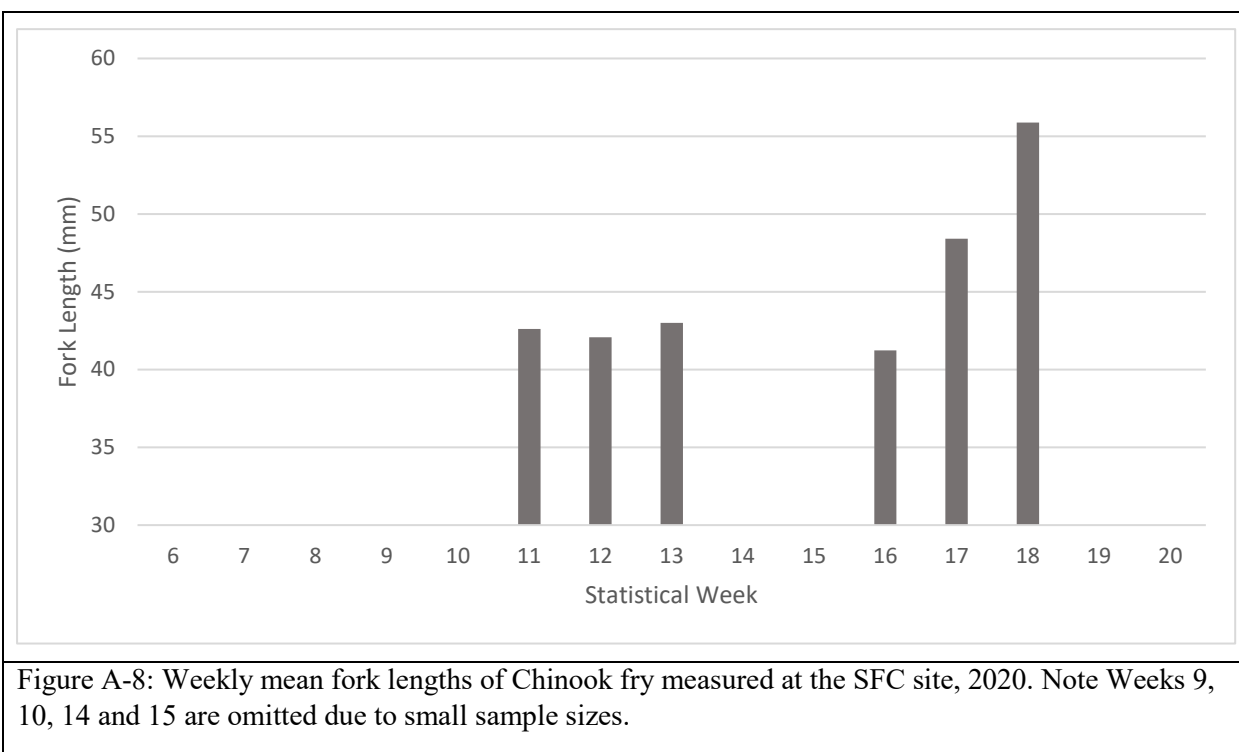


Figure A-7: Weekly fork lengths of Chinook fry measured at the SFC site, 2020.



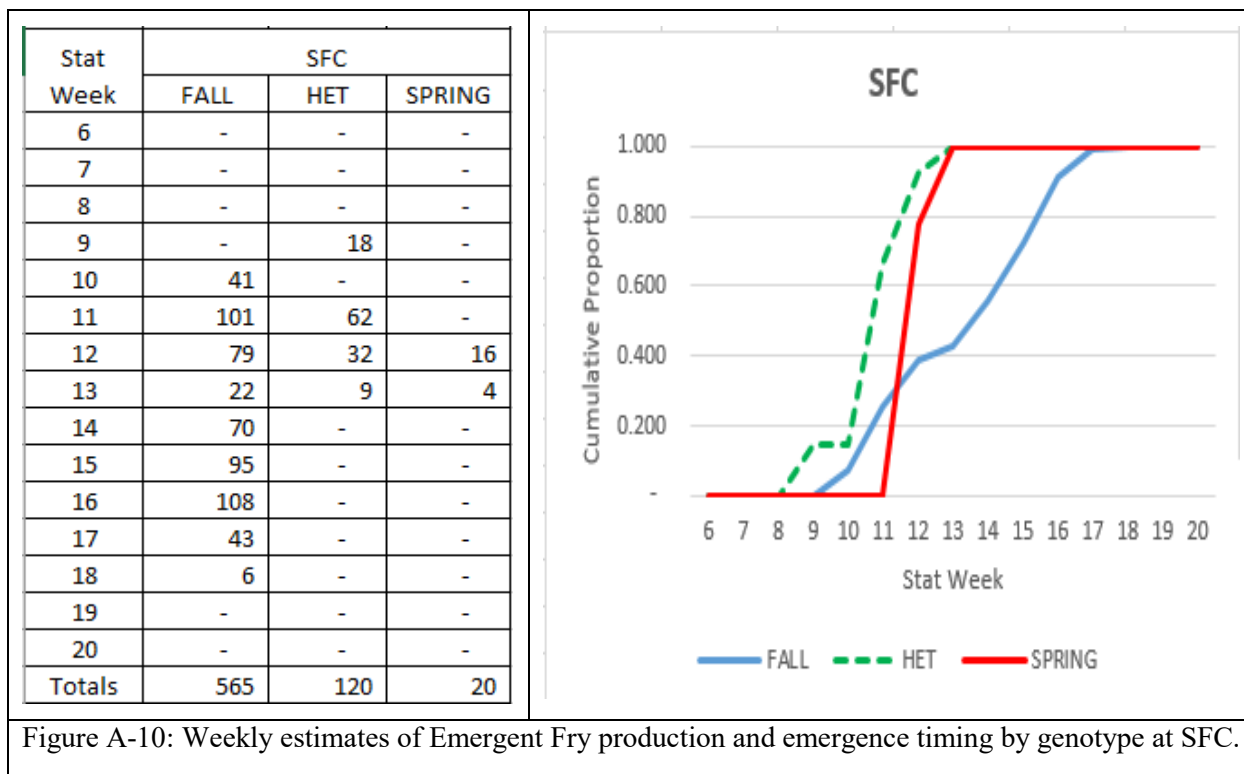


Table A-2: Chinook redd counts by run type and Emergent Fry genotype frequencies for SFC.									
2019 Run Type Classed Redds				Emergent Fry Genotypes					
Fall		Spring		FALL		HET		SPRING	
57	83.8%	11	16.2%	565	80.1%	120	17.0%	20	2.8%

Mainstem Newaukum (MSN)

Trapping Period

The Mainstem Newaukum River trap (MSN) was operated in weekly episodes from February 20 (Week 8) through May 6 (Week 19) of the 2020 sampling season (Figure A-11). The trap was fished for 823 hours during the season.

Streamflow and Temperature Conditions

Weekly mean streamflow for the site decreased rapidly from 2,849 cfs in Week 6 to 496 cfs in Week 9 and then decreased gradually to 295 cfs by Week 13. The flows increased abruptly in Week 14 and the flow effects extended into Week 15. The increased flows may have hindered trap effectiveness but, since it still caught Chinook fry on those days, the magnitude of effect is not clear. Flows returned to low levels in Week 16 and remained there for the rest of the sampling season.

Weekly mean stream temperatures at MSN ranged from 5.3°C to 11.4°C. Stream temperatures increased from 5.3°C in Week 8 to 8.0°C in Week 9, varied around 7.7°C during Weeks 10-14, increased to 10.5°C by Week 16 and then varied around 10.7°C for the remainder of the season.

Chinook Fry Catches

One hundred twenty-eight Chinook fry were captured at MSN and they were all processed for length measurements and tissue samples. Chinook fry were captured in the first week of operation so some emergence and transport past the site may have occurred before sampling was initiated. The relatively moderate catch in Week 8 and the ascending trend in catches through Weeks 11 and 12, suggest any missed production prior to Week 8 was likely only a small proportion of the total Emergent Fry population that drifted through the site. Chinook fry were captured in moderate abundance the first three weeks of operation (Weeks 8-10) and then increased rapidly to peak abundances in Weeks 11 and 12. Catches returned to moderate levels during Weeks 13-18 and no Chinook were captured in Week 19, the final week of operation. Effects of the relatively high streamflow in Weeks 14 and 15 on Chinook fry transport are unknown.

Length Frequencies and Life History Stages

Chinook fry captured at MSN ranged from 36 mm to 69 mm fork length and averaged 42.3 mm for the entire sampling season (Figure A-12). The distribution of measured lengths varied among weeks. Mean lengths varied moderately around 41.0 mm during Weeks 8-13 and then increased to greater than 44 mm for Weeks 14-18 (Figure A-13). A partial explanation for the increased mean lengths after Week 13 is the shift in abundance from mostly Emergent Fry to an increased entry of Rearing Fry during Weeks 14-18.

Chinook catches at MSN were mostly Emergent Fry (87.5%) and their numbers dominated catches through the early and middle sampling season (Weeks 8-15; Figure A-11). Rearing Fry contributed to the catch in small numbers during Weeks 12 and 14 and were a larger component in Weeks 16-18. No Chinook fry were captured in Week 19, the final week of operation. The measured lengths of Emergent Fry did not change through the season ($F_{1, 110} df=1.271$; $p=0.262$) and their overall mean was 41.1 mm (Figure A-14). Lengths of Rearing Fry did not vary over weeks ($F_{1, 14} df=0.013$; $p=0.91$) and their overall mean length was 50.4 mm. The test for length change over time for Rearing Fry was limited by a small sample size of only 16 total individuals.

Emergent Fry Abundance and Emergence Timing

The abundance and genotype frequencies of Emergent Fry moving downstream past the MSN site were estimated from the sampling data (see Appendix B for estimating procedures). An estimated 2,614 Emergent Fry drifted past MSN during the trapping period (Figure A-15) made up of 37.3% FALL, 50.2% HET and 12.5% SPRING individuals. All three genotypes were present in the initial samples in Week 8 but the overall timing was earlier for SPRING and HET and more protracted and later for FALL.

Comparison of Adult Redd and Fry Genotype Frequencies

Chinook redd counts classified to run types by WDFW in spawning locations upstream of MSN are compared with Emergent Fry genotype frequencies at MSN in Table A-3. Redds classified as spring run type made up 17.4% of the total redds upstream of MSN while 12.5% of the Emergent Fry population was SPRING. Although there is considerable uncertainty in this comparison (See the main report *Spawning Adult Genotype Frequencies* Section for explanation) it suggests the actual abundance of individuals homozygous for the spring allele is less than the redd survey results imply.

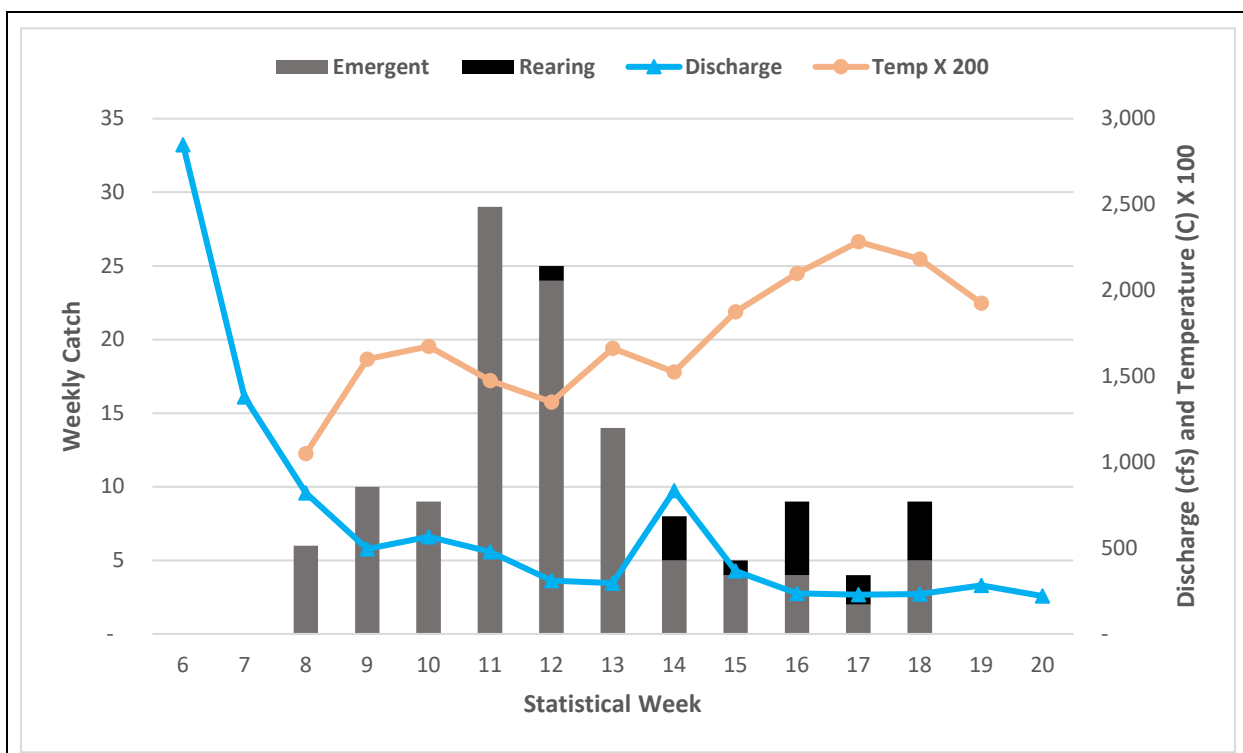


Figure A-11: Weekly Chinook catches, mean stream discharge and mean stream temperatures at the MSN site, 2020. The weekly catches are partitioned into Emergent Fry and Rearing Fry based on lengths. Note the mean temperatures are multiplied by a constant (200) for scale (shared axis with discharge).

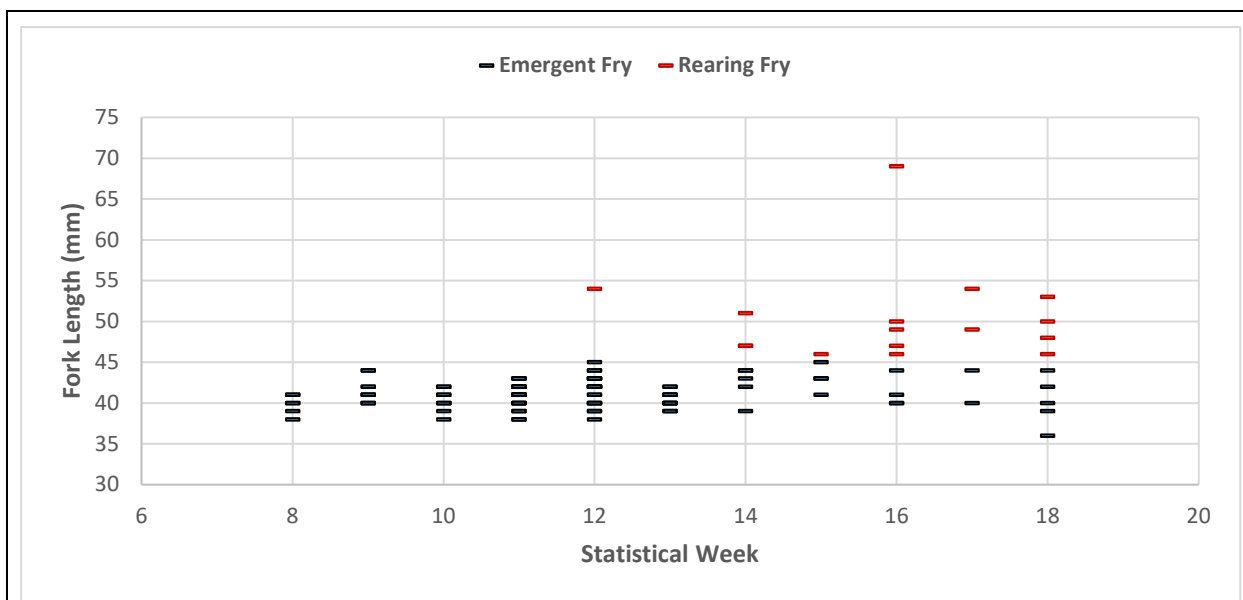


Figure A-12: Weekly fork lengths of Chinook fry measured at the MSN site, 2020.

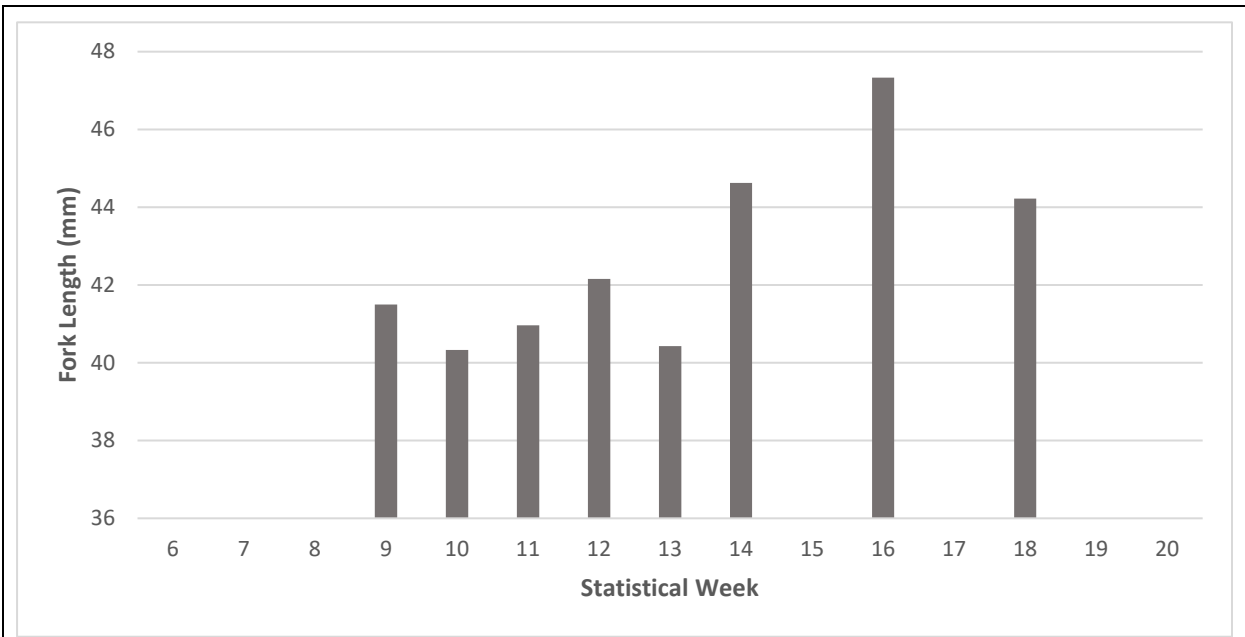


Figure A-13: Weekly mean fork lengths of Chinook fry measured at the MSN site, 2020. Note Weeks 8, 15 and 17 are omitted due to small sample sizes.

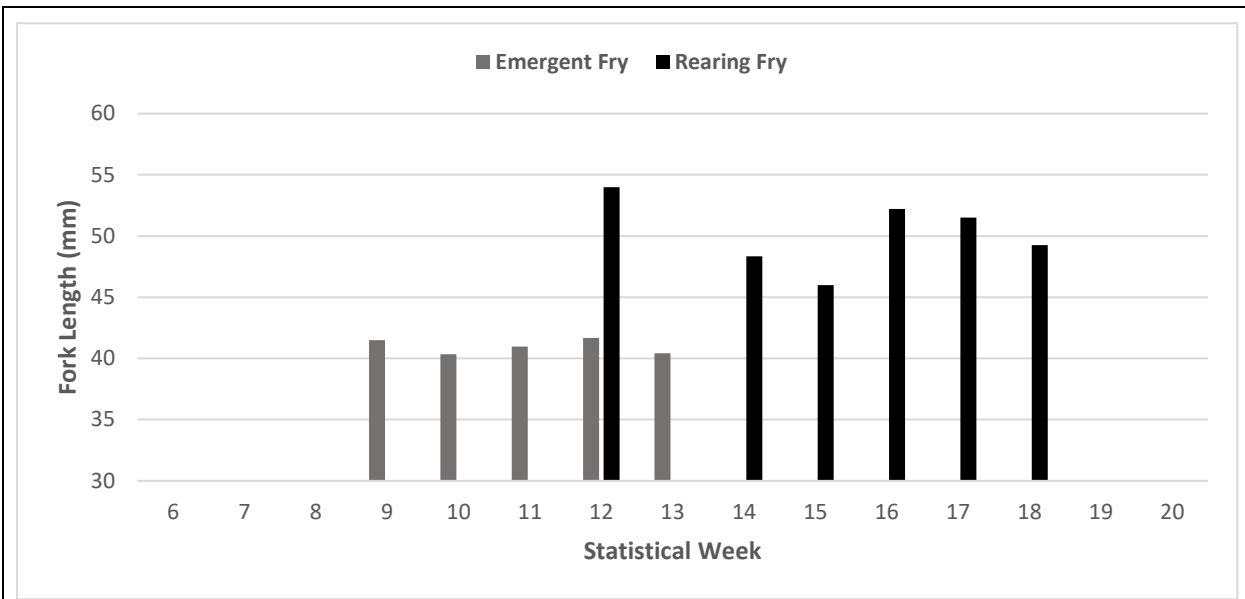


Figure A-14: Weekly mean fork lengths of Emergent Fry and Rearing Fry at MSN, 2020. Means for Emergent Fry in Weeks 8 and 14-18 are omitted due to small sample sizes. All means for Rearing Fry are included regardless of sample size.

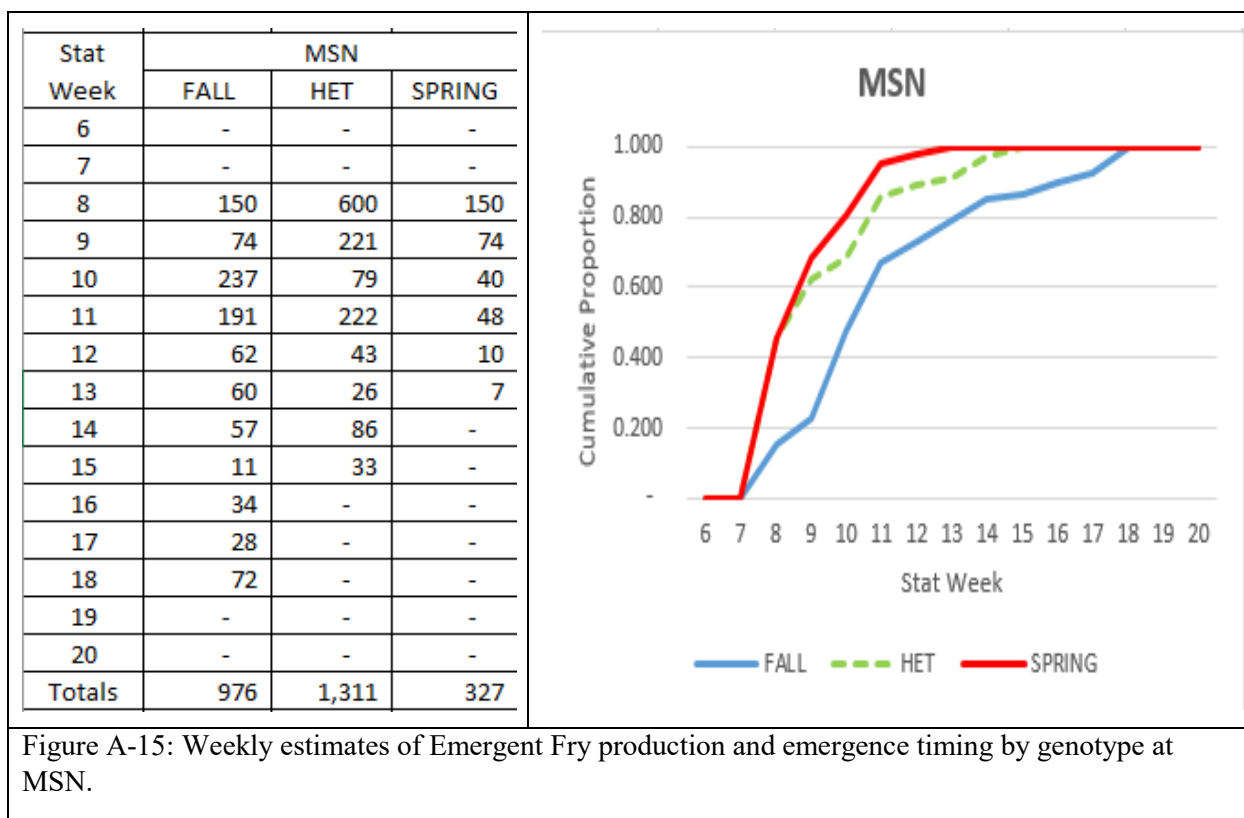


Table A-3: Chinook redd counts by run type and Emergent Fry genotype frequencies for MSN.									
2019 Run Type Classed Redds				Emergent Fry Genotypes					
Fall		Spring		FALL		HET		SPRING	
304	82.6%	64	17.4%	976	37.3%	1,311	50.2%	327	12.5%

South Fork Newaukum (SFN)

Trapping Period

The South Fork Newaukum River trap (SFN) was operated in weekly episodes from February 4 (Week 6) through May 6 (Week 19) of the 2020 sampling season (Figure A-16). The trap was fished for 877 hours during the season.

Streamflow and Temperature Conditions

Weekly mean streamflow for the site decreased rapidly from 1,379 cfs in Week 6 to 336 cfs in Week 8 and then decreased gradually to 112 cfs by Week 13. The flows increased abruptly to 340 cfs in Week 14 and the higher flow effects extended into Week 15. The increased flows may have hindered trap effectiveness but, since the trap still caught Chinook fry on those days, the magnitude of effect is not clear. Flows returned to low levels in Week 16 and remained there for the rest of the season.

Weekly mean stream temperatures at SFN ranged from 5.5° C to 10.3° C. Stream temperatures varied around 6.7° C during Weeks 7-12, increased to 10.1° C by Week 16 and then varied around 10.0° C for the remainder of the season.

Chinook Fry Catches

Six hundred eleven Chinook fry were captured at SFN of which 447 were processed for length measurements and tissue samples. Chinook fry were captured in the first week of operation so some emergence and transport past the site may have occurred before sampling was initiated. However, the small catch (1) in Week 6 and the trend of increasing catches through Week 13 suggest the missed early production was likely a very small proportion of the total Emergent Fry population that drifted through the site. Chinook fry were captured in low abundance the first two weeks of operation (Weeks 6-7) and then increased to peak abundance in Week 13. Catches gradually declined to low abundance by Week 18 and no Chinook were captured in Week 19, the final week of operation. Effects of the relatively high streamflow in Weeks 14 and 15 on Chinook fry transport are unknown.

Length Frequencies and Life History Stages

Chinook fry captured at SFN ranged from 36 mm to 78 mm fork length and averaged 41.9 mm for the entire season (Figure A-17). The distribution of measured lengths varied among weeks. Mean lengths varied moderately around 41.2 mm during Weeks 8-16 and then increased to a high of 55.2 mm in Week 18 (Figure A-18). A partial explanation for the increased mean lengths in Weeks 17 and 18 is the shift to a greater proportion of Rearing Fry in the weekly catches.

Chinook catches at SFN were mostly Emergent Fry (90.0%) and their numbers dominated the catch until late in the season (Weeks 17 and 18; Figure A-16). Rearing Fry contributed to the catch in small numbers during most of the season but were most abundant in Weeks 17 and 18. No Chinook fry were captured in Week 19, the final week of operation. The measured lengths of Emergent Fry increased over time ($F_{1, 396} = 37.584$; $p < 0.0001$; Figure A-19). Although statistically significant, the small increase in length through the season may not be important. The original measurements were to the nearest millimeter so a change in mean length of 1.6 mm over eight weeks is not provoking and probable explanations are not obvious. Lengths of Rearing Fry also increased over weeks ($F_{1, 47} = 12.216$; $p = 0.001$).

Emergent Fry Abundance and Emergence Timing

The abundance and genotype frequencies of Emergent Fry moving downstream past the SFN site were estimated from the sampling data (see Appendix B for estimating procedures). An estimated 9,811 Emergent Fry drifted through the SFN site during the trapping period made up of 79.8% FALL, 14.7% HET and 5.5% SPRING individuals (Figure A-20). The first SPRING fry in the 2020 pilot project was captured at SFN. The timing pattern of emergence indicates that SPRING fry emerged first, followed by HET and finally by FALL fry.

Comparison of Adult Redd and Fry Genotype Frequencies

Chinook redd counts in spawning locations upstream of SFN were classified to run types by WDFW and are compared with Emergent Fry genotype frequencies at SFN in Table A-4. Redds classified as spring run type made up 20.8% of the total redds upstream of SFN while only 5.5% of the Emergent Fry population were SPRING. Although there is considerable uncertainty in this comparison (See the main report *Spawning Adult Genotype Frequencies* Section for explanation) it certainly suggests the actual abundance of individuals homozygous for the spring allele is much less than the redd survey results imply.

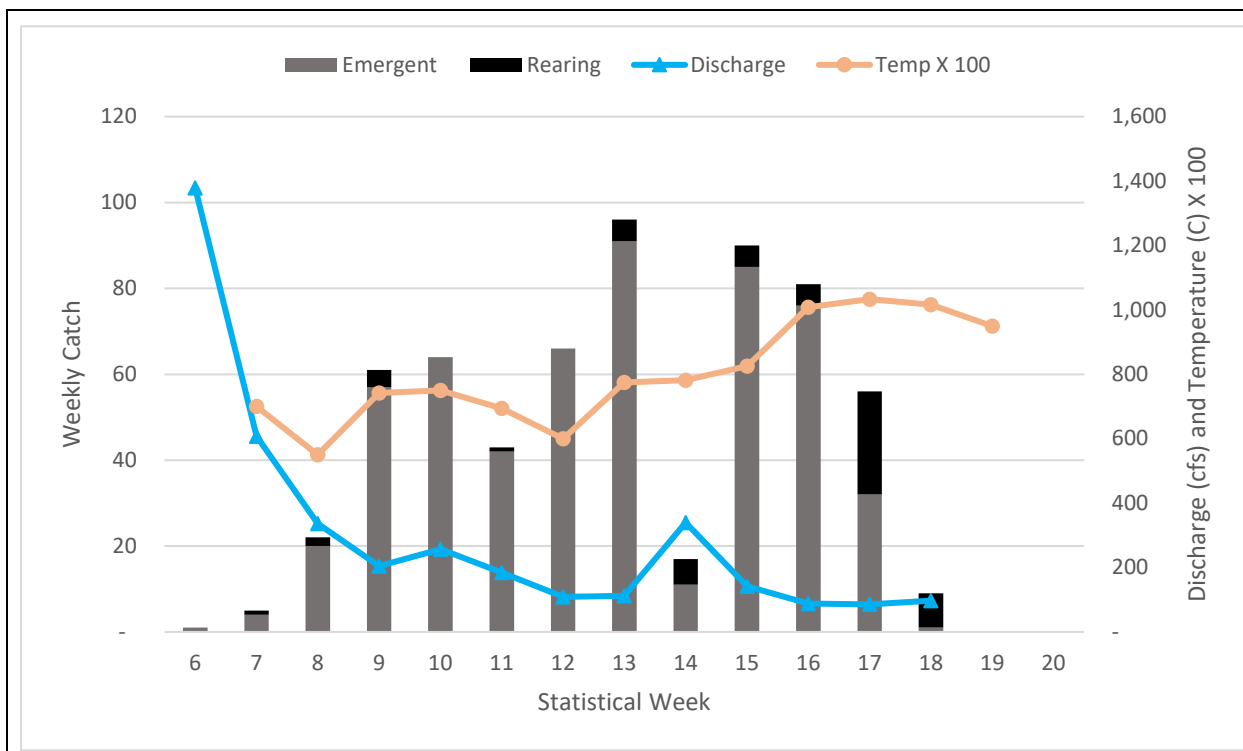


Figure A-16: Weekly Chinook catch, mean stream discharge and mean stream temperatures at the SFN site, 2020. The weekly catches are partitioned into Emergent Fry and Rearing Fry based on lengths. Note the mean temperatures are multiplied by a constant (100) for scale (shared axis with discharge)

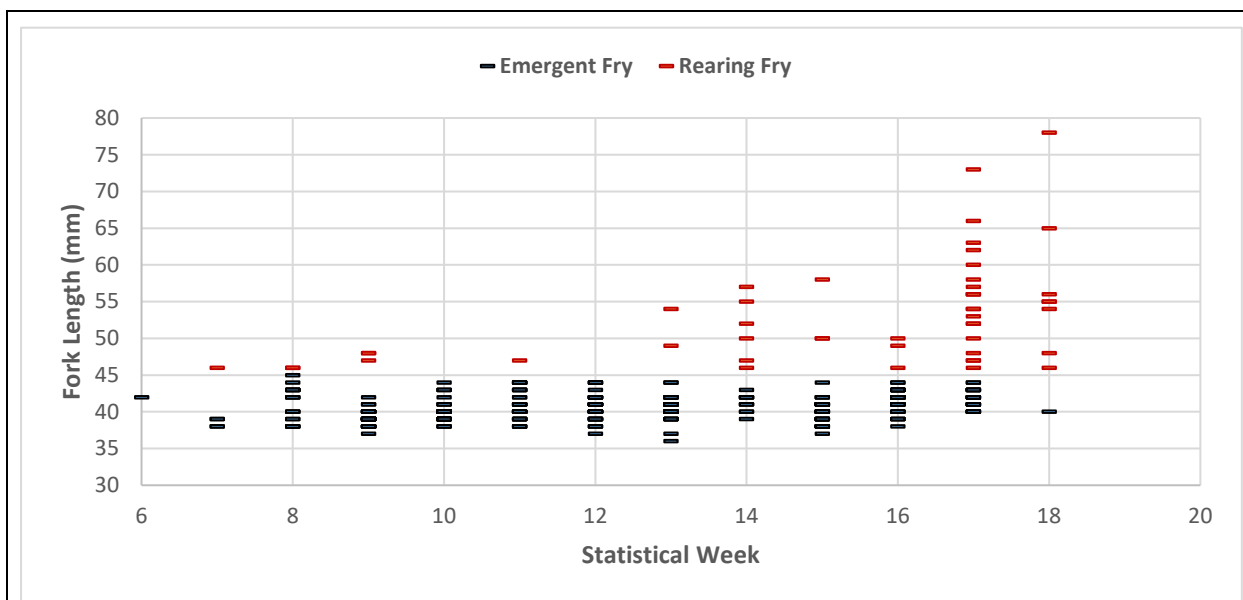


Figure A-17: Weekly fork lengths of Chinook fry measured at the SFN site, 2020.

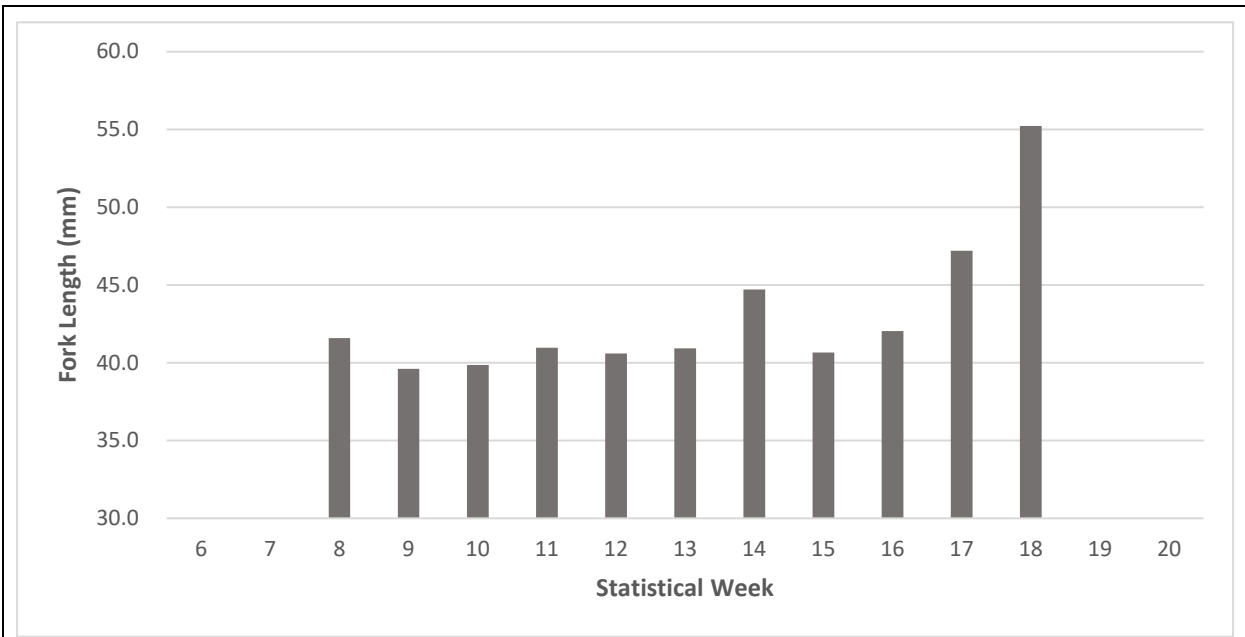


Figure A-18: Weekly mean fork lengths of Chinook fry measured at the SFN site, 2020. Note Weeks 6 and 7 are omitted due to small sample sizes.

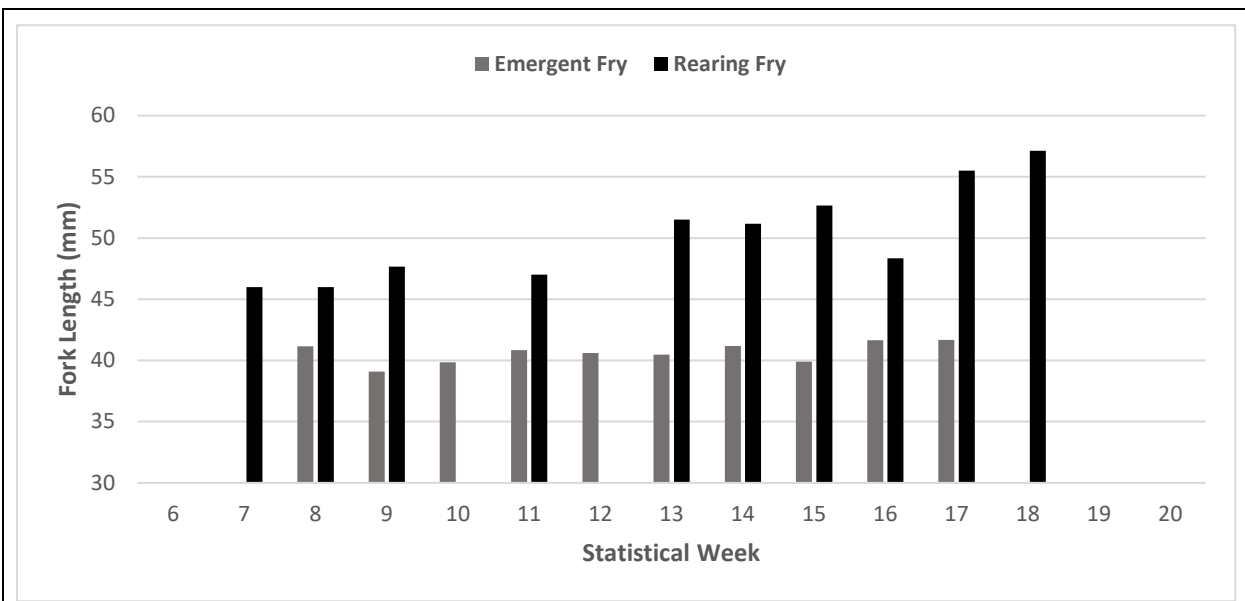


Figure A-19: Weekly mean fork lengths of Emergent Fry and Rearing Fry at SFN, 2020. Means for Emergent Fry in Weeks 6, 7 and 18 are omitted due to small sample sizes. All means for Rearing Fry are included regardless of sample sizes.

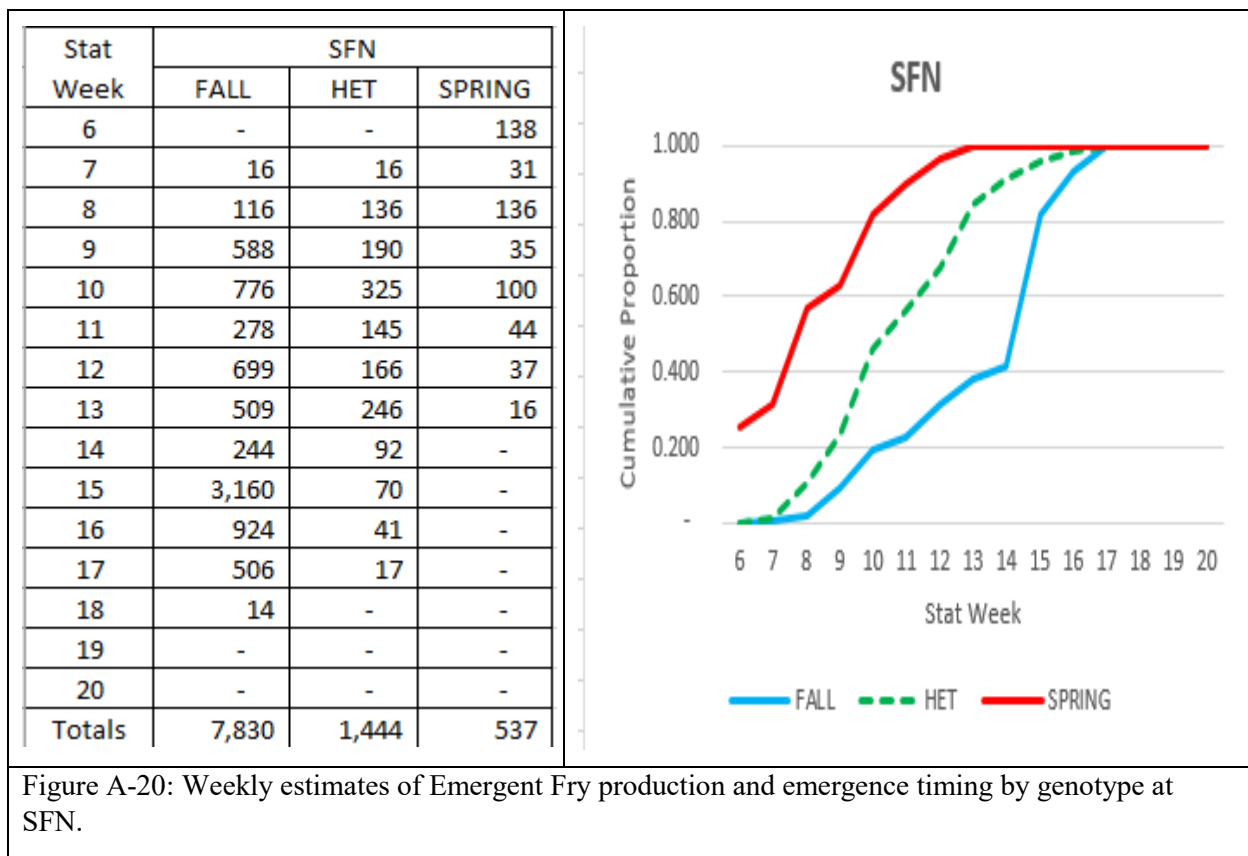


Table A-4: Chinook redd counts by run type and Emergent Fry genotype frequencies for SFN.									
2019 Run Type Classed Redds				Emergent Fry Genotypes					
Fall		Spring		FALL		HET		SPRING	
156	79.2%	41	20.8%	7,830	79.8%	1,444	14.7%	537	5.5%

North Fork Newaukum (NFN)

Trapping Period

The North Fork Newaukum River trap (NFN) was operated from February 10 (Week 7) through May 13 (Week 20) of the 2020 sampling season (Figure A-21). The trap was fished for 890 hours during the season.

Streamflow and Temperature Conditions

Weekly mean streamflow for the site decreased rapidly from 462 cfs in Week 6 to 99 cfs by Week 9 and then decreased gradually to 68 cfs by Week 13. The flows increased to 148 cfs in Week 14 and then varied around 64 cfs for the remainder of the season. The increased flows in Week 14 may have hindered trap effectiveness but, since the trap still caught Chinook fry on those days, the magnitude of effect is not clear.

Weekly mean stream temperatures at NFN ranged from 5.6 °C to 10.5° C. Stream temperatures fluctuated around 6.9° C during Weeks 7-14, increased gradually to 10.5 °C by Week 17 and then varied around 9.9° C for the remainder of the sampling season.

Chinook Fry Catches

Three hundred seven Chinook fry were captured at NFN of which 233 were processed for length measurements and tissue samples. No Chinook fry were captured in the initial seven weeks (Weeks 7-13) of sampling, so it is not likely the sampling missed any substantial early transport of fry past the site. The fry abundance gradually increased from low in Week 14 to peak abundance in Week 18. Catches abruptly declined to low abundance in Weeks 19 and 20, the final weeks of operation. Effects of the relatively high streamflow in Week 14 on Chinook fry transport are unknown.

Length Frequencies and Life History Stages

Chinook fry captured at NFN ranged from 35 mm to 60 mm and averaged 43.4 mm for the entire season. The distribution of measured lengths varied among weeks (Figure A-22). Mean lengths varied moderately around 40.8 mm during Weeks 15-17 and then increased progressively to 55.9 mm by Week 20 (Figure A-23). A partial explanation for the increased mean lengths in Weeks 18-20 is the shift from Emergent Fry to Rearing Fry dominance in the make-up of weekly catches.

Chinook catches at NFN were mostly Emergent Fry (73.6%) and their numbers dominated catch in Weeks 14-17 (Figure A-21). Rearing Fry contributed to catch in small numbers during Weeks 16 and 17 but dominated catches in Weeks 18-20. The measured lengths of Emergent Fry increased over time ($F_{1, 172} \text{ df} = 3.939$; $p < 0.05$; Figure A-24). Although statistically significant, the small increase in length through the season may not be important. The original measurements were to the nearest millimeter so a change in mean length of 1.0 mm over four weeks is not provoking and probable explanations are not obvious. Lengths of Rearing Fry also increased over weeks ($F_{1, 57} \text{ df} = 31.607$; $p < 0.0001$)

Emergent Fry Abundance and Emergence Timing

The abundance and genotype frequencies of Emergent Fry moving downstream past the NFN site were estimated from the sampling data (see Appendix B for estimating procedures). An estimated 3,412 Emergent Fry drifted through the NFN site during the trapping period made up of 97.5% FALL and 2.5% HET individuals (Figure A-25). No SPRING individuals were detected at NFN. The capture data at NFN indicates there was no emergence until after Week 13. FALL individuals appeared first and was present through Week 18. HET individuals were present in small numbers during Weeks 17 and 18. There were no captures of Chinook fry after Week 18 although trapping occurred during Weeks 19 and 20.

Comparison of Adult Redd and Fry Genotype Frequencies

Chinook redd counts in spawning locations upstream of NFN were classified to run types by WDFW and are compared with Emergent Fry genotype frequencies at NFN in Table A-5. Redds classified as spring run type made up 6.8% of the total redds upstream of NFN while none of the Emergent Fry population was SPRING. Although there is considerable uncertainty in this comparison (See the main report *Spawning Adult Genotype Frequencies* Section for explanation) it certainly suggests the actual abundance of individuals homozygous for the spring allele is much less than the redd survey results imply.

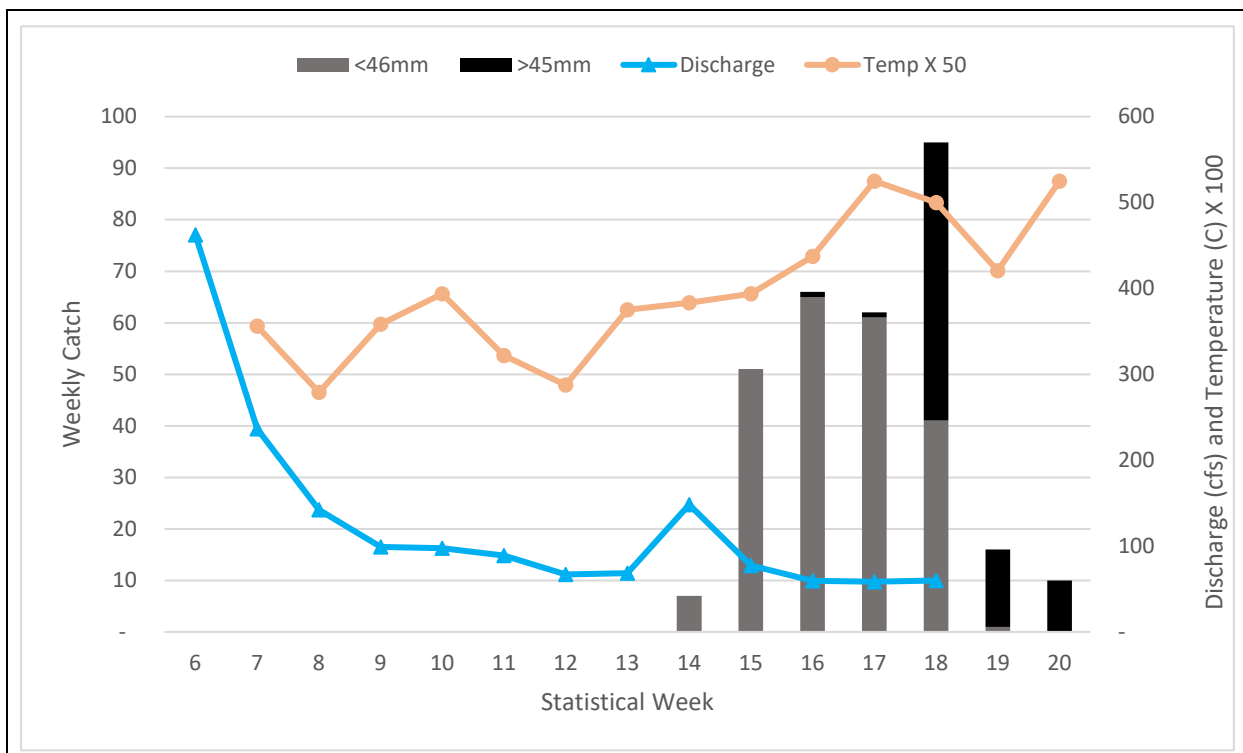


Figure A-21: Weekly Chinook catch, mean stream discharge and mean stream temperature at the NFN site, 2020. The weekly catches are partitioned into Emergent Fry and Rearing Fry based on lengths. Note the mean stream temperatures are multiplied by a constant (50) for scale (shared axis with discharge).

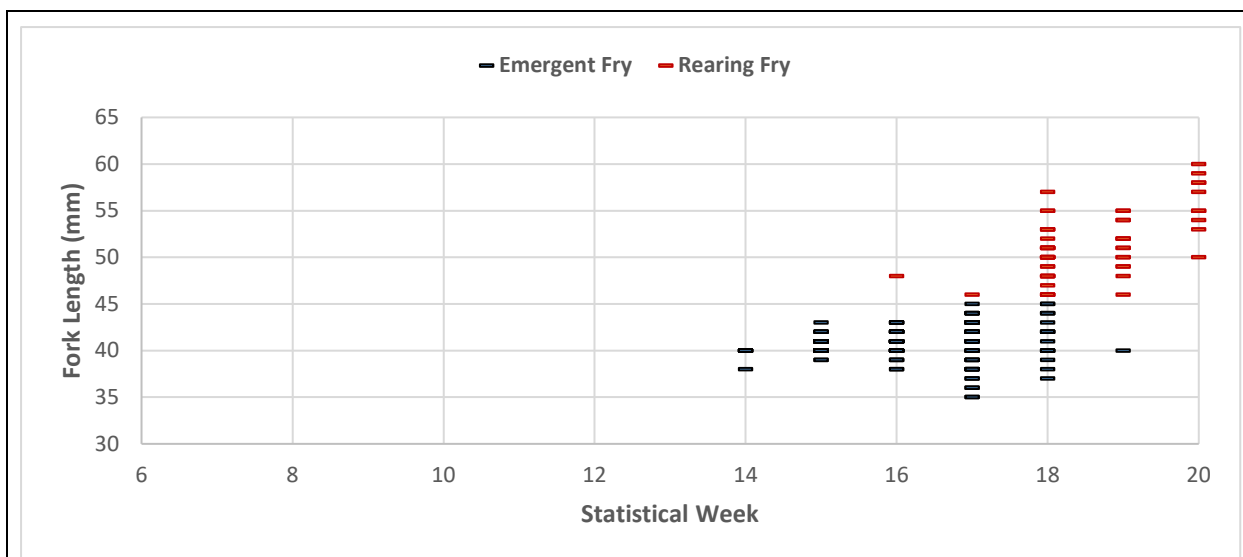


Figure A-22: Weekly fork lengths of Chinook fry measured at the NFN site, 2020.

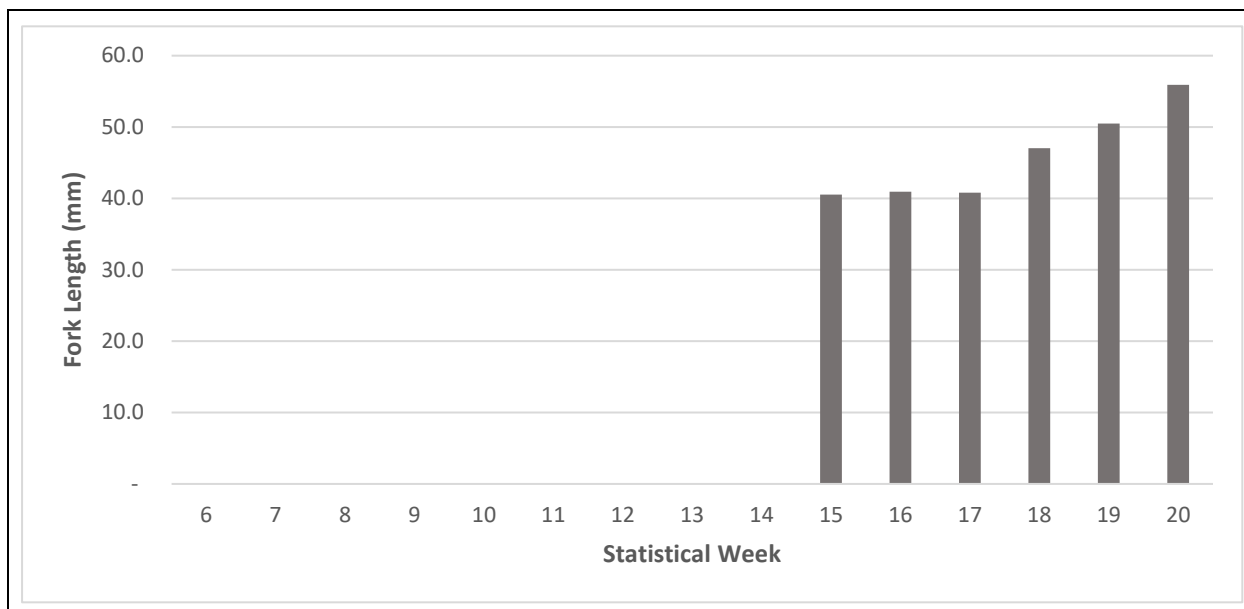


Figure A-23: Weekly mean fork lengths of Chinook fry measured at the NFN site, 2020. Note Week 14 was omitted due to a small sample size.

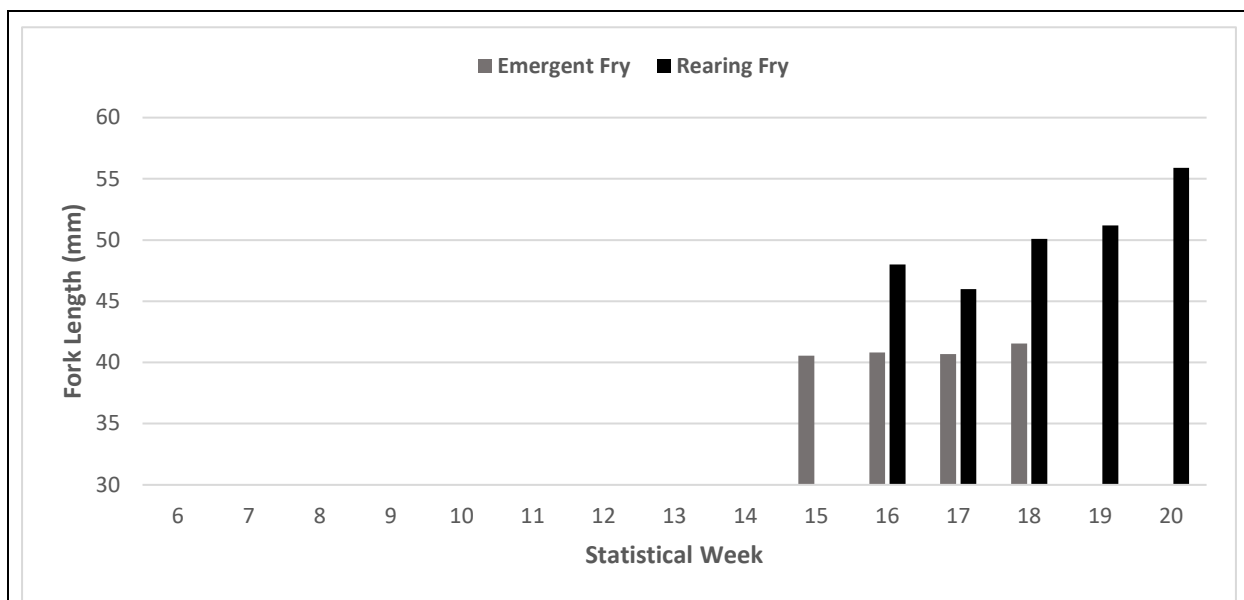
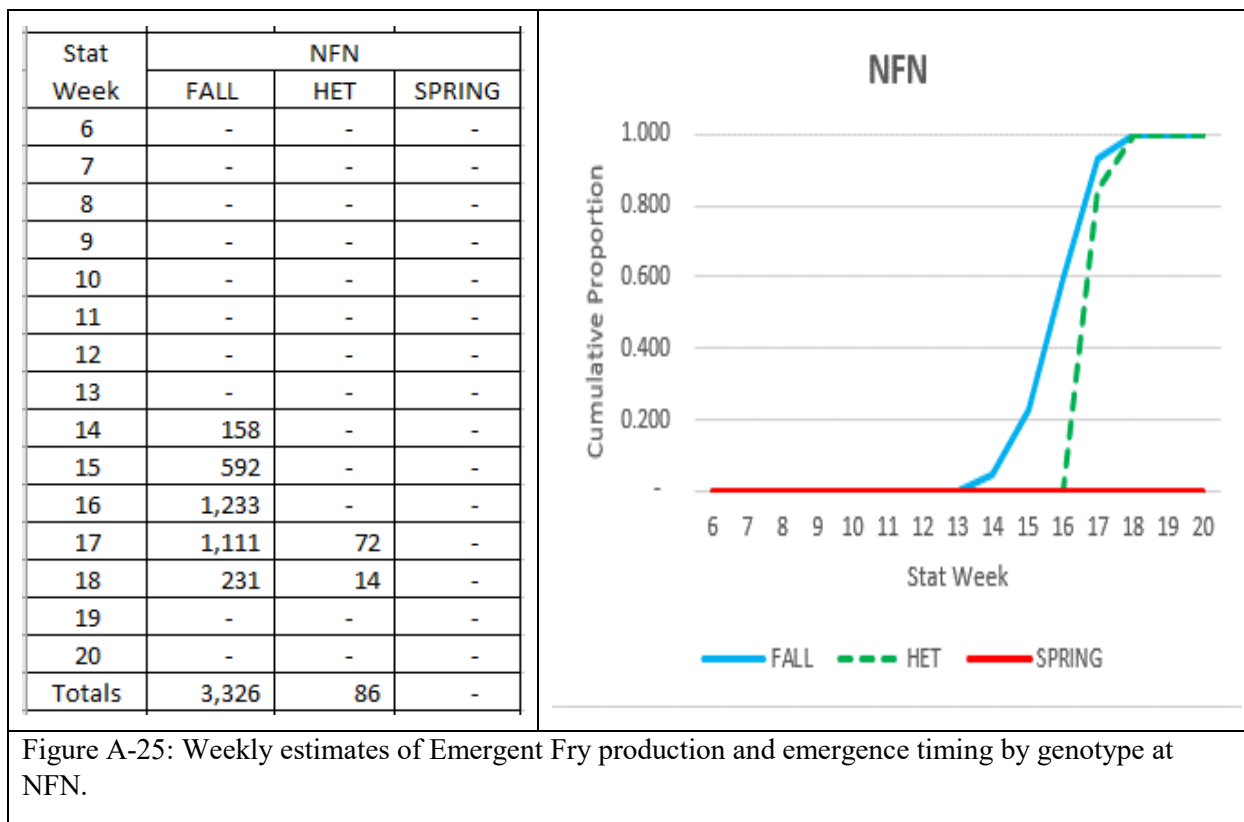


Figure A-24: Weekly mean fork lengths of Emergent Fry and Rearing Fry at NFN, 2020. Means for Emergent Fry in Weeks 14 and 19 are omitted due to small sample sizes. All means for Rearing Fry are included regardless of sample sizes.



2019 Run Type Classed Redds				Emergent Fry Genotypes					
Fall		Spring		FALL		HET		SPRING	
41	93.2%	3	6.8%	3,326	97.5%	86	2.5%	-	0.0%

Upper Skookumchuck (SKU)

Trapping Period

The Upper Skookumchuck River trap (SKU) was operated from March 18 (Week 12) through May 6 (Week 19) of the 2020 sampling season (Figure A-26). The trap was fished for 327 hours during the season.

Streamflow and Temperature Conditions

Stream flows at this site are influenced by the Skookumchuck Dam, a run-of-river facility approximately four miles upstream of the trap site. Weekly mean streamflow for SKU had declined from 1,019 cfs in Week 6 to 200 cfs by Week 12 when trap operations were initiated. Flows remained low (97-200 cfs) with moderate variation over the entire sampling period. Flows elevated slightly in Weeks 15 and 19 but without obvious effect on trap effectiveness.

Weekly mean stream temperatures increased gradually through the sampling period, from 8.5°C in Week 12 to 10.5°C in Week 19. Outflow water at the Skookumchuck Dam can be regulated to maintain temperatures to below 15°C but the observed values at the trap site were well below that limit.

Chinook Fry Catches

Two thousand two hundred two Chinook fry were captured at SKU of which 355 were processed for length measurements and tissue samples. Chinook fry were abundant at the site when sampling was initiated and for the next two weeks (Weeks 12-14) so a substantial amount of earlier fry transport past the site was likely missed. This conclusion is supported by catches at the SKO trap where 61% of its total seasonal catch occurred before Week 12. After relatively large catches in Weeks 12-14, the abundance declined abruptly in Week 15 and remained moderate-to-low for the remainder of the sampling period (Weeks 15-19).

Length Frequencies and Life History Stages

Chinook fry captured at SKU ranged from 35 mm to 68 mm and averaged 43.0 mm for the entire season (Figure A-27). The distribution of measured lengths varied among weeks. Mean lengths varied moderately around 40.8 mm during Weeks 12-16 and then increased to 51.6 mm in Week 18 (Figure A-28). A partial explanation for the increased mean lengths in Weeks 17 and 18 is the increased entry of Rearing Fry in catches after Week 16.

Chinook catches at SKU were mostly Emergent Fry (95.6%) and their numbers dominated catch in Weeks 12-16 (Figure A-26). Rearing Fry increased from a small proportion of the catch in Week 16 to 100-percent of the catch in Week 19. The measured lengths of Emergent Fry increased over time ($F_{1, 278} df = 4.863$; $p < 0.05$; Figure A-29). Although statistically significant, the small increase in length through the season may not be important. The original measurements were to the nearest millimeter so a change in mean length of 1.0 mm over five weeks is not provoking and probable explanations are not obvious. Lengths of Rearing Fry also increased over weeks ($F_{1, 73} df = 37.279$; $p < .0001$).

Emergent Fry Abundance and Emergence Timing

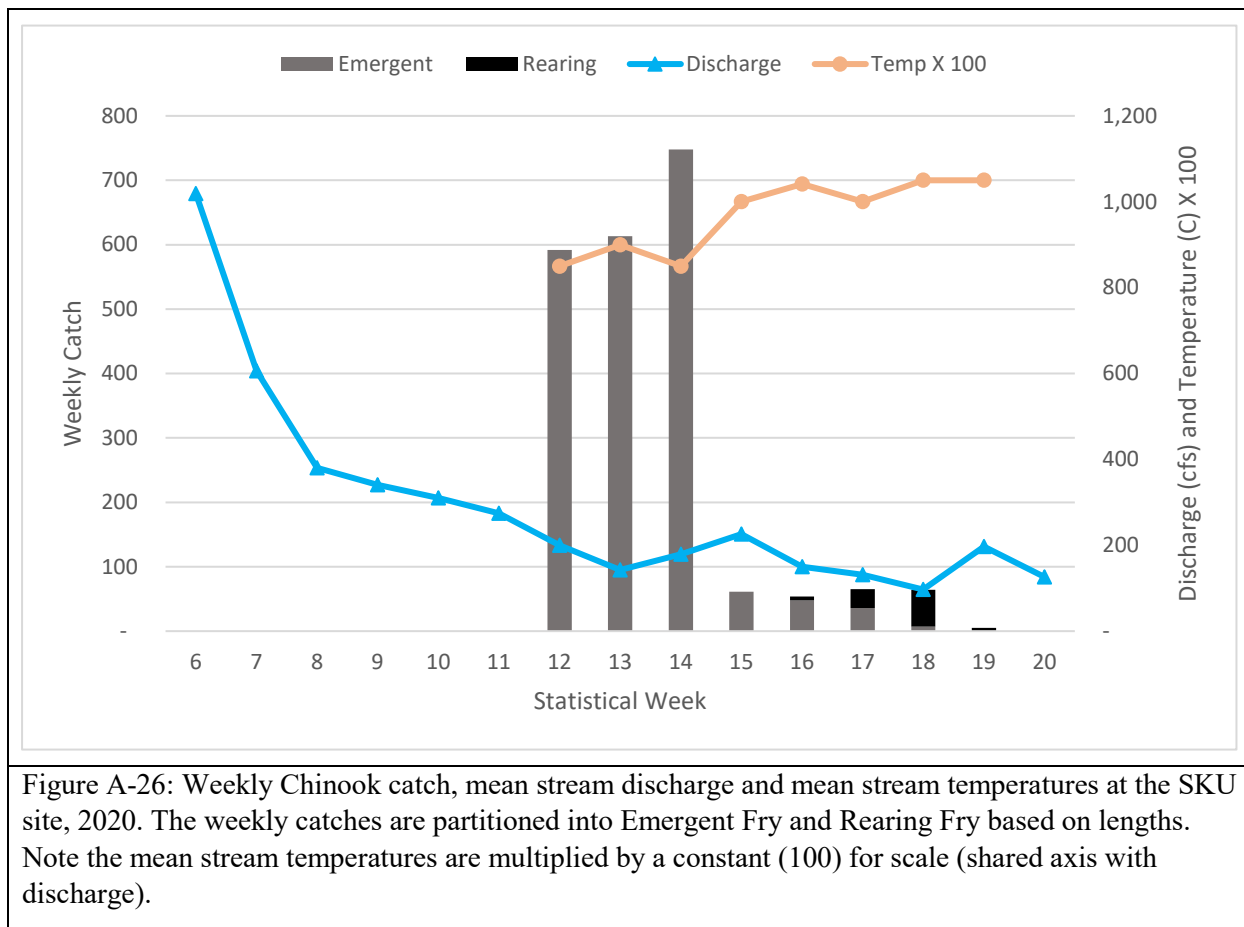
The abundance and genotype frequencies of Emergent Fry moving downstream past the SKU site were estimated from the sampling data (see Appendix B for estimating procedures). An estimated 245,519 Emergent Fry drifted through the SKU site during the trapping period made up of 95.6% FALL, 3.6% HET and 0.7% SPRING individuals (Figure A-30). All three genotypes were present Week 12, the first week of sampling. No SPRING individuals were captured after Week 12. FALL and HET timing was similar through the sampling period although FALL timing was a little later than HET.

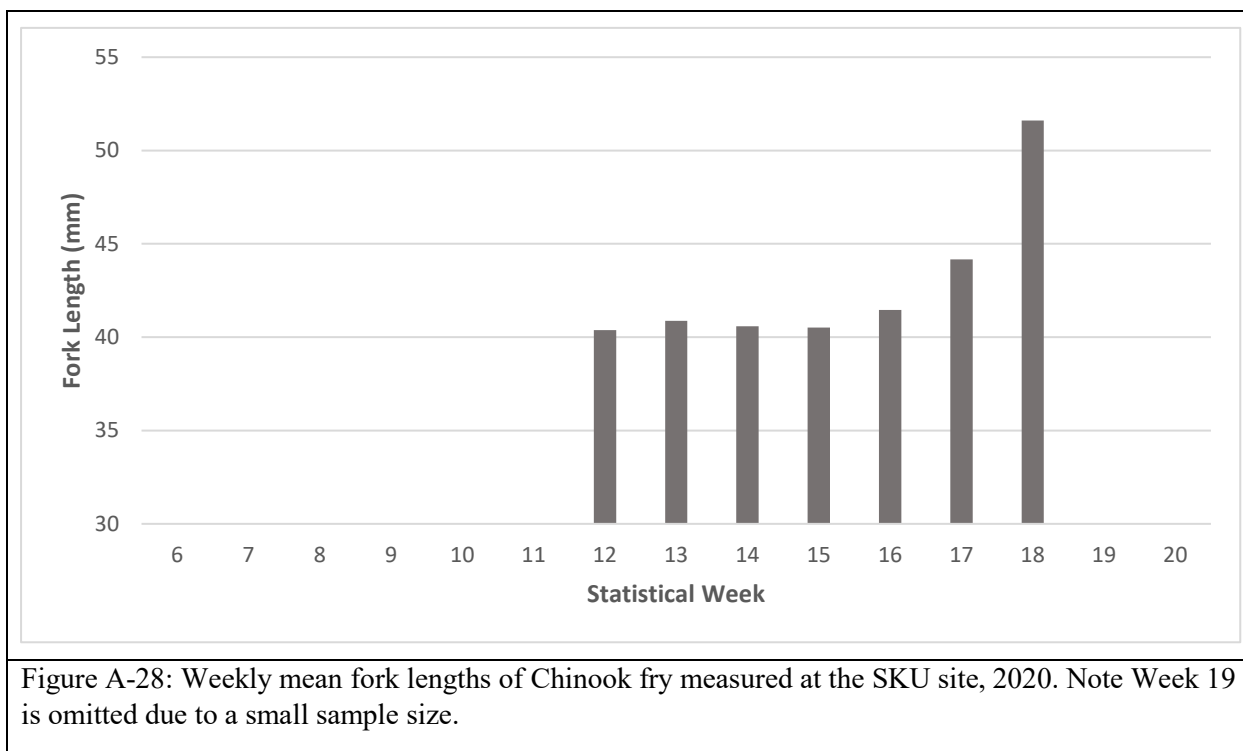
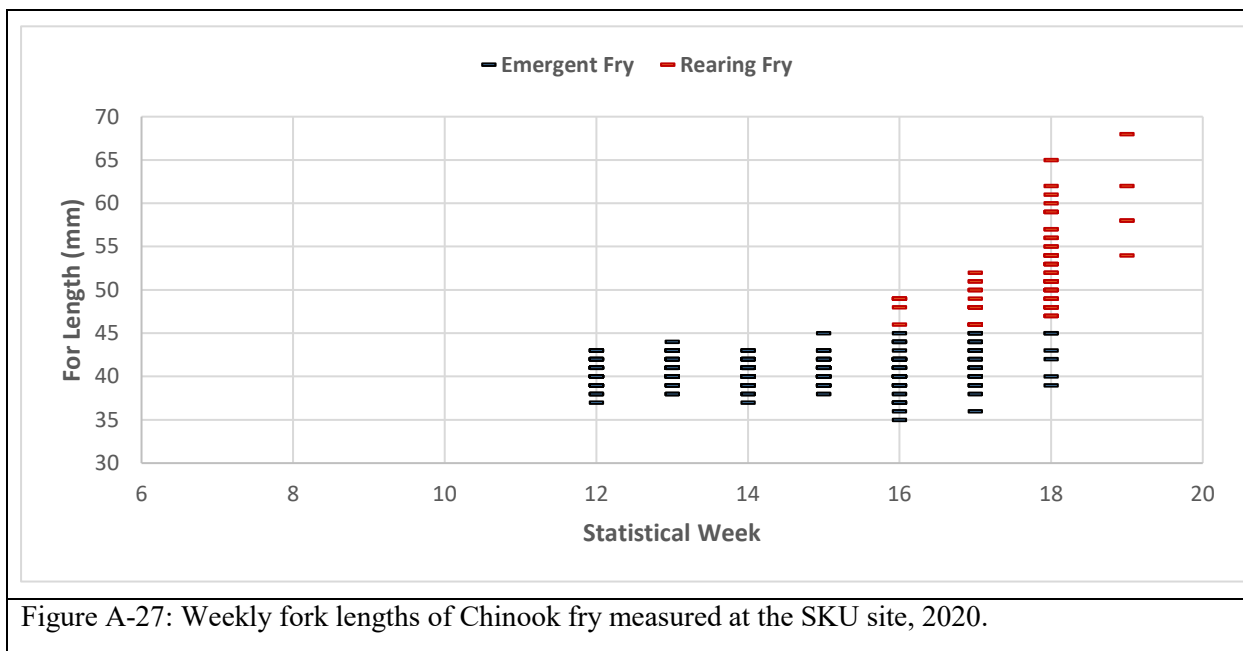
Trapping at the SKU site did not begin until Week 12 so, since the general timing pattern observed across all the other sites is for earlier emergence of SPRING individuals, the study data for SKU is biased toward underrepresentation of SPRING attributes and contributions. In addition, a large number of Emergent Fry were captured during the first week of sampling indicating a substantial portion of the population had already passed SKU. Consequently, use of the data from the SKU site could bias results toward underestimating the occurrence and influences of the SPRING genotype.

Comparison of Adult Redd and Fry Genotype Frequencies

Chinook redd counts in spawning locations upstream of SKU were classified to run types by WDFW and are compared with Emergent Fry genotype frequencies at SKU in Table A-6. Redds classified as spring run type made up 9.2% of the total redds upstream of SKU while 2.0% of the Emergent Fry population was SPRING. The bias in genotype frequency estimates for SKU might provide at least a partial explanation for this difference. Although there is considerable uncertainty in this comparison (See the

main report *Spawning Adult Genotype Frequencies* Section for explanation) it certainly suggests the actual abundance of individuals homozygous for the spring allele is much less than the redd survey results imply.





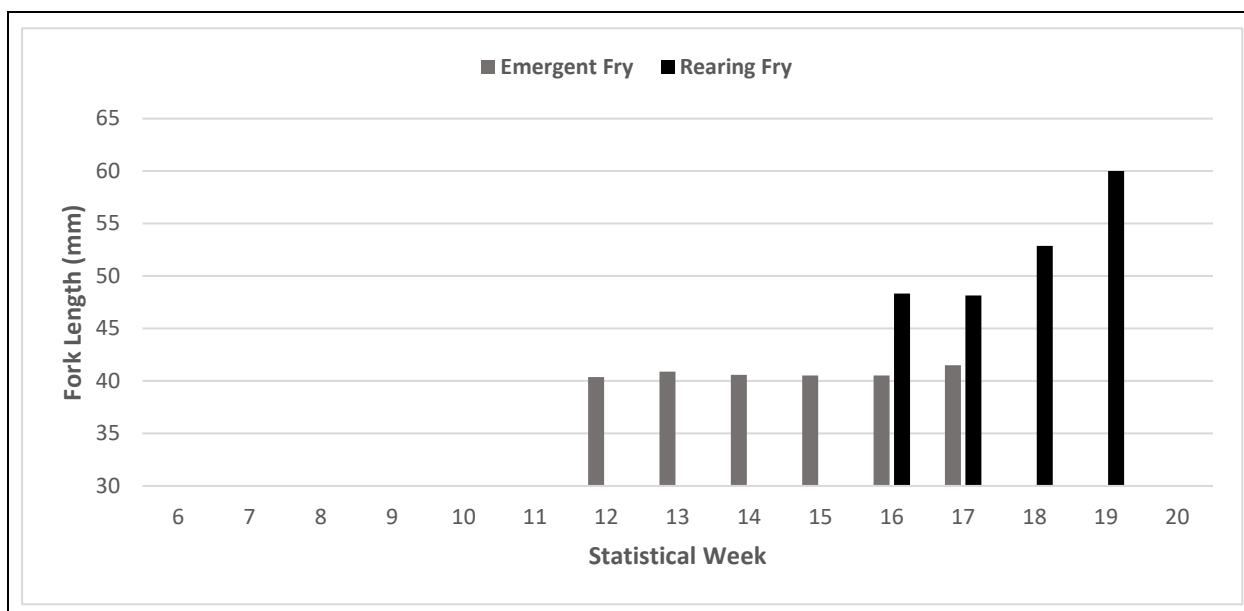


Figure A-29: Weekly mean fork lengths of Emergent Fry and Rearing Fry at SKU, 2020. Mean for Emergent Fry in Week 18 is omitted due to a small sample size. All means for Rearing Fry are included regardless of sample size

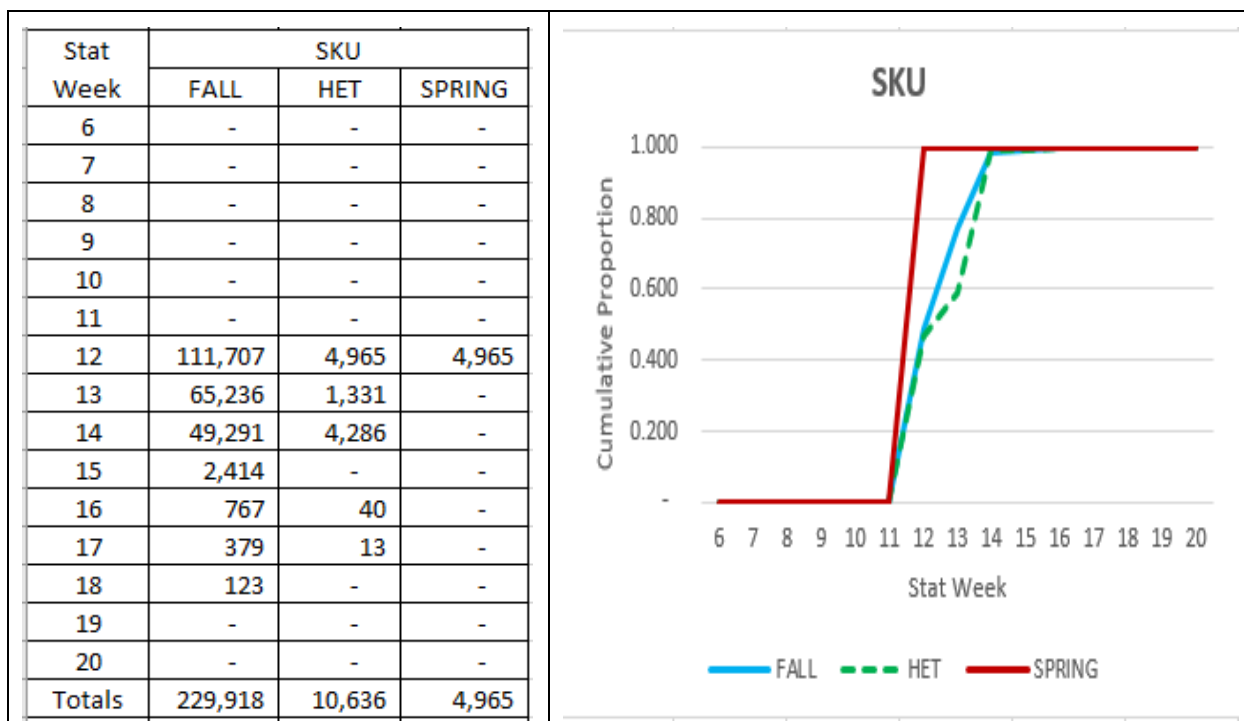


Figure A-30: Weekly estimates of Emergent Fry production and emergence timing by genotype at SKU.

Table A-6: Chinook redd counts by run type and Emergent Fry genotype frequencies for SKU.									
2019 Run Type Classed Redds				Emergent Fry Genotypes					
Fall		Spring		FALL		HET		SPRING	
326	90.8%	33	9.2%	229,918	93.6%	10,636	4.3%	4,965	2.0%

Middle Skookumchuck Site (SKO)

Trapping Period

The Middle Skookumchuck River trap (SKO) was operated from February 11 (Week 7) through May 13 (Week 20) of the 2020 sampling season (Figure A-31). The trap was fished for 421 hours during the season.

Streamflow and Temperature Conditions

Streamflows and water temperatures may be influenced by the Skookumchuck Dam but to a lesser extent than at the SKU site. Weekly mean streamflow for the site decreased rapidly from 1,538 cfs in Week 6 to 477 cfs in Week 8 and then decreased gradually to 162 cfs by Week 13. The flows increased abruptly to 327 cfs in Week 14 and the flow effects extended into Week 15. Flows fluctuated around 150 cfs for the remainder of the season. Effects of the relatively high streamflow in Weeks 14 and 15 on Chinook fry transport and catch are unknown.

Weekly mean stream temperatures at SKO ranged from 6.3°C to 11.3°C. Stream temperatures fluctuated around 7.2 °C during Weeks 7-12 and then gradually increased to 11.0 °C by Week 20.

Chinook Fry Catches

Two thousand four hundred seventy-five Chinook fry were captured of which 650 were processed for length measurements and tissue samples. Chinook fry were present at the site in moderate abundance when sampling was initiated in Week 7 so it is likely sampling missed some earlier fry emergence and transport. However, based on the relatively moderate catch in Week 7 and the ascending abundance trend in catches through Week 11, it is unlikely the sampling missed a substantial proportion of the total population that drifted through the site. Catches of Chinook fry increased substantially from Week 7 to Week 11, decreased gradually in Weeks 12 and 13 and then remained moderate-to-low for the remainder of the sampling season.

Length Frequencies and Life History Stages

Chinook fry captured at SKO ranged from 34 mm to 81 mm and averaged 42.9 mm for the entire season (Figure A-32). The distribution of measured lengths varied among weeks. Mean lengths varied moderately around 40.6 mm during Weeks 7-14 and then increased to 52.8 mm by Week 19 (Figure A-33). A partial explanation for the increased mean lengths in Weeks 15-19 is the increased entry of Rearing Fry in the catches after Week 14.

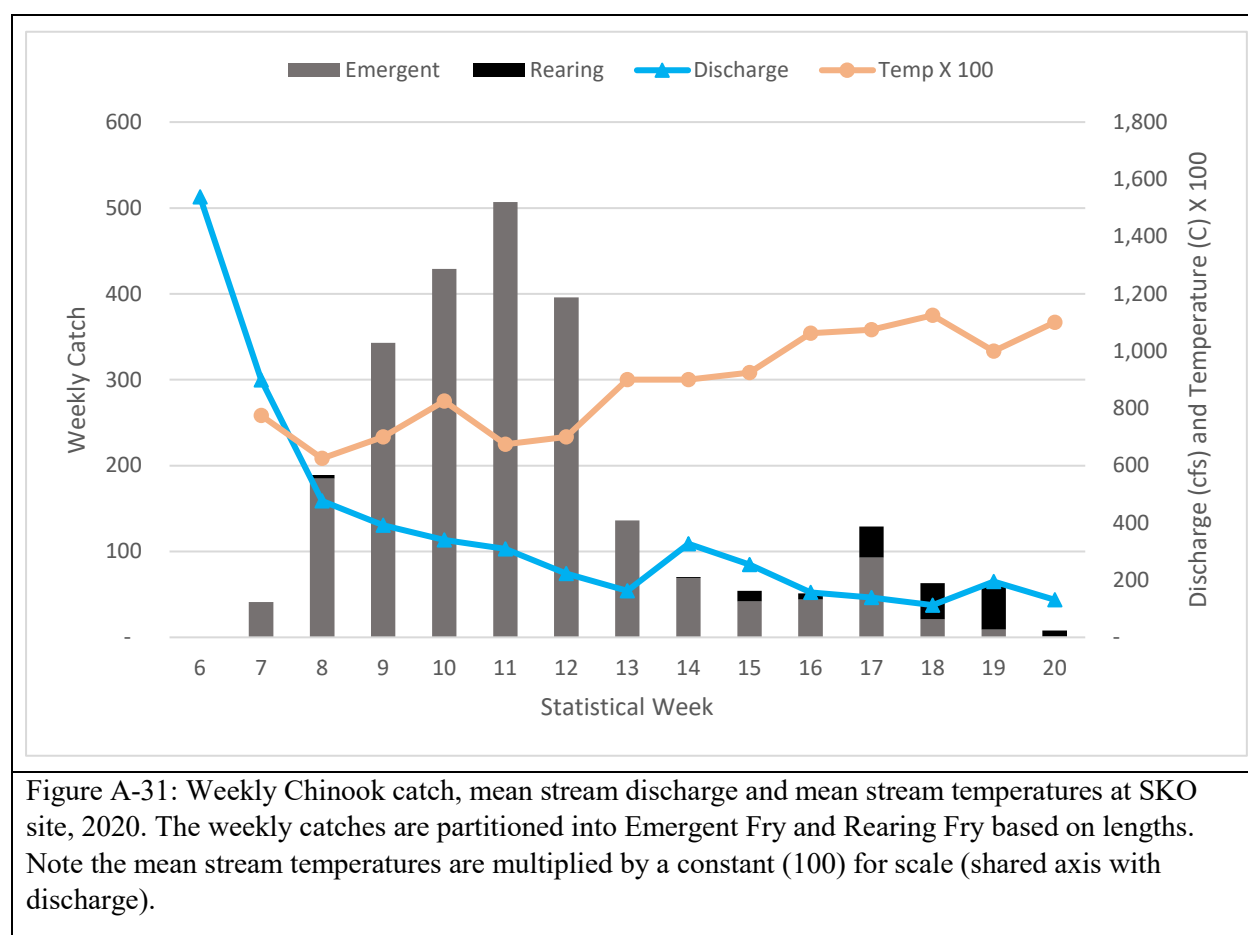
Chinook catches at SKO were mostly Emergent Fry (93.6%) and their numbers dominated catch in weeks 7-17 (Figure A-31). Rearing Fry contributed to catch in small numbers during Weeks 14-16 and then progressed to dominate catches in Weeks 18-20. The measured lengths of Emergent Fry increased over time ($F_{1, 531} df = 30.611$; $p < 0.0001$; Figure A-34). Although statistically significant, the small increase in length through the season may not be important. The original measurements were to the nearest millimeter so a change in mean length of 1.3 mm over ten weeks is not provoking and probable explanations are not obvious. Lengths of Rearing Fry also increased over weeks ($F_{1, 115} df = 6.969$; $p < 0.05$).

Emergent Fry Abundance and Emergence Timing

The abundance and genotype frequencies of Emergent Fry moving downstream past the SKO site were estimated from the sampling data (see Appendix B for estimating procedures). An estimated 215,753 Emergent Fry drifted through the SKO site during the trapping period made up of 79.5% FALL, 18.0% HET and 2.5% SPRING individuals (Figure A-35). All three genotypes were present in the first sampling on Week 7. Spring individuals had the earliest overall timing followed closely by HET. FALL individuals had the latest emergence timing.

Emergent Fry Abundance and Emergence Timing

Chinook redd counts in spawning locations upstream of SKO were classified to run types by WDFW and are compared with Emergent Fry genotype frequencies at SKO in Table A-7. Redds classified as spring run type made up 14.2% of the total redds upstream of SKO while 2.5% of the Emergent Fry population was SPRING. Although there is considerable uncertainty in this comparison (See the main report *Spawning Adult Genotype Frequencies* Section for explanation) it certainly suggests the actual abundance of individuals homozygous for the spring allele is much less than the redd survey results imply.



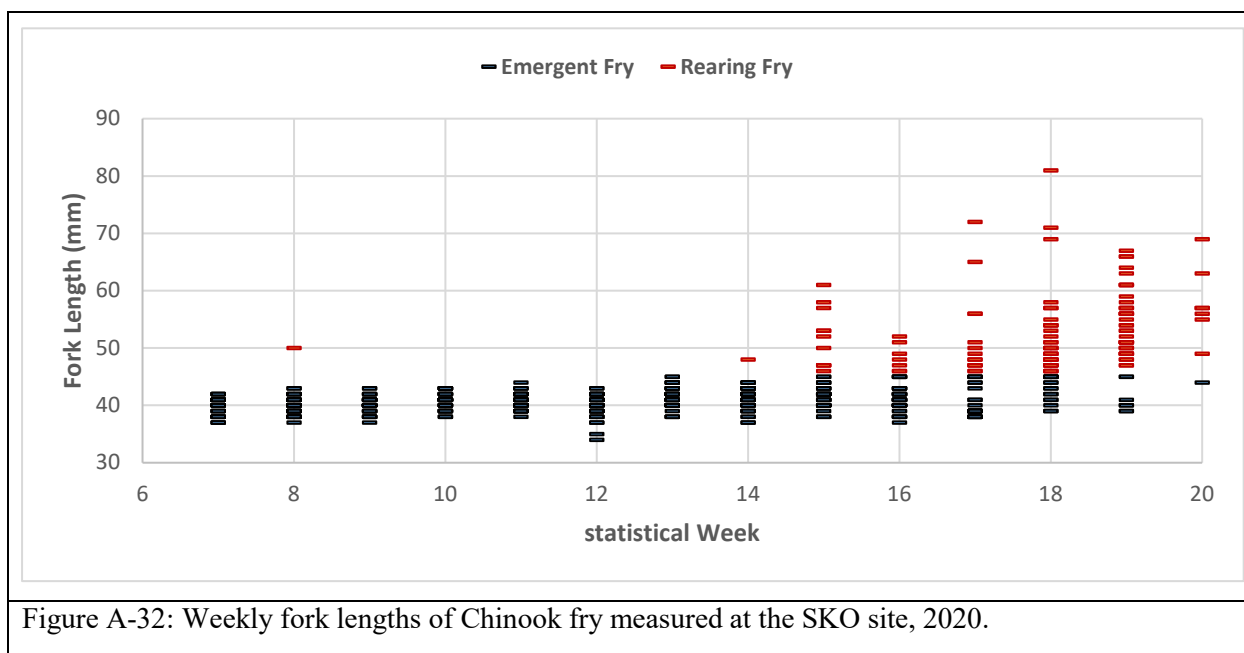


Figure A-32: Weekly fork lengths of Chinook fry measured at the SKO site, 2020.

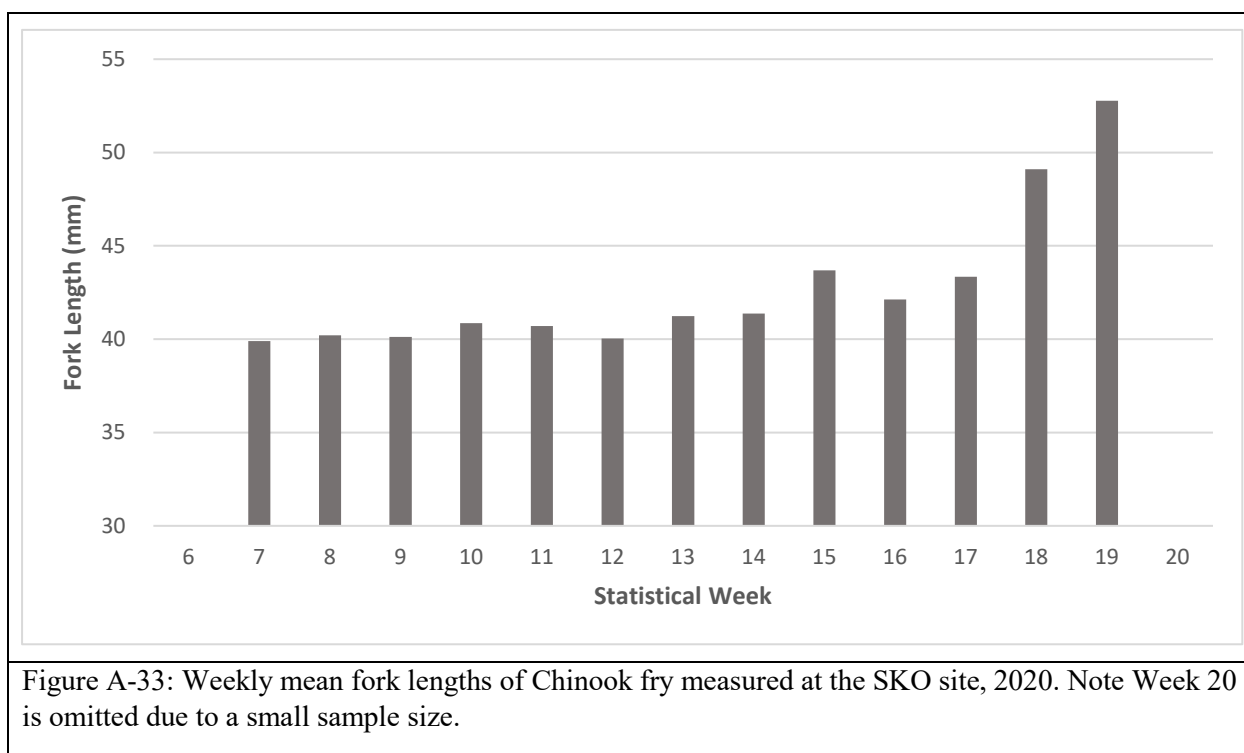


Figure A-33: Weekly mean fork lengths of Chinook fry measured at the SKO site, 2020. Note Week 20 is omitted due to a small sample size.

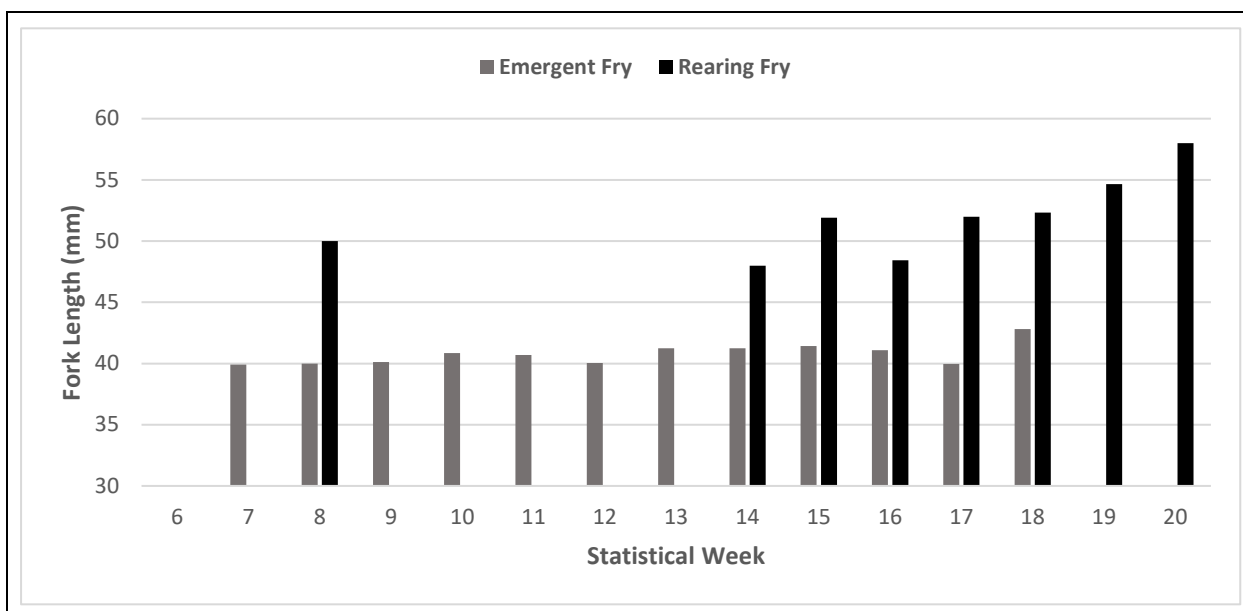


Figure A-34: Weekly fork lengths of Emergent Fry and Rearing Fry at SKO, 2020. Means for Emergent Fry in Weeks 19 and 20 are omitted due to small sample sizes. All means for Rearing Fry are included regardless of sample sizes.

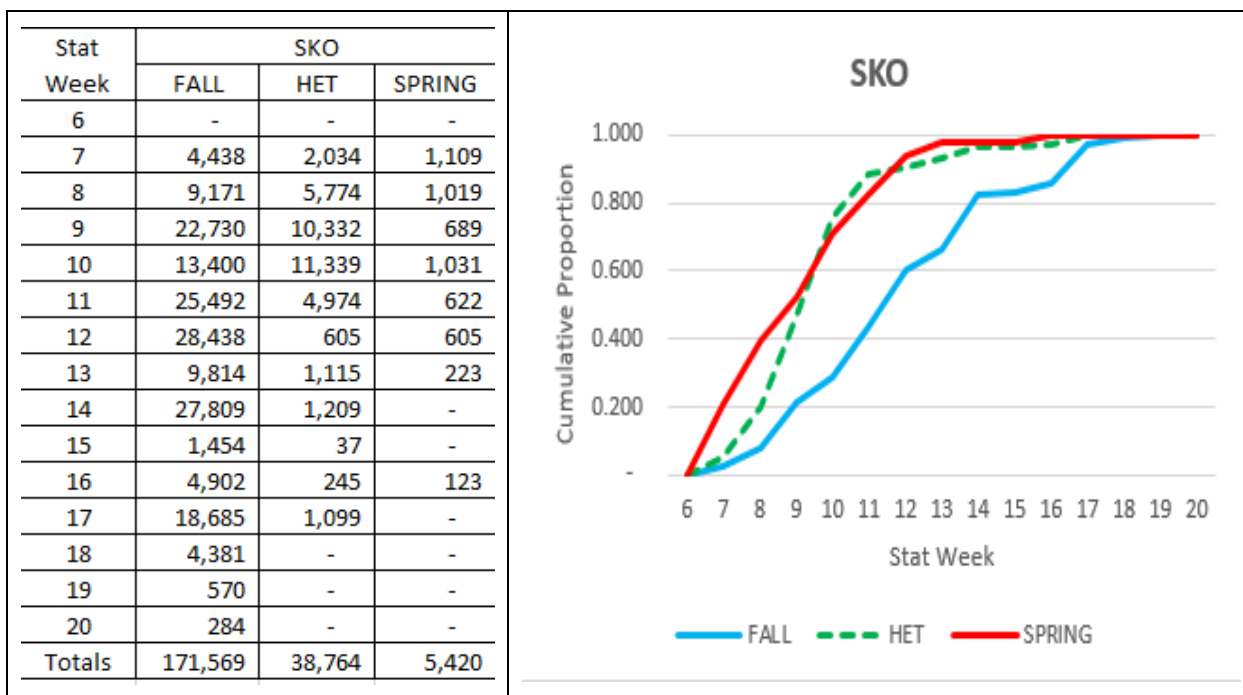


Figure A-35: Weekly estimates of Emergent Fry production and emergence timing by genotype at SKO.

Table A-7: Chinook redd counts by run type and Emergent Fry genotype frequencies for SKO.									
2019 Run Type Classed Redds				Emergent Fry Genotypes					
Fall		Spring		FALL		HET		SPRING	
876	85.8%	145	14.2%	171,569	79.5%	38,764	18.0%	5,420	2.5%



Run-Type Composition of Juvenile Chinook Salmon in the Upper Chehalis River Basin in 2020

APPENDIX B: Computational Steps From Tissue Sample Data To Estimates of Genotype Production

Quinault Indian Nation Department of Fisheries

July 2021

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Introduction

The 2020 pilot study was designed to capture and process samples of Chinook salmon fry during a majority of their period of emergence. The project fry traps were operated at seven selected locations (sites) from early February through mid-May and the data and information acquired were partitioned into weekly periods for processing and interpretation. The trapping sites selected were:

- Upper Skookumchuck River (SKU);
- Mainstem Skookumchuck River (SKO);
- Mainstem Newaukum River (MSN);
- North Fork Newaukum River (NFN);
- South Fork Newaukum River (SFN);
- South Fork Chehalis River (SFC); and,
- Upper Mainstem Chehalis River (MSC).

A core objective of this project is to improve the shared understanding of relative abundances of spring and fall Chinook run types in the upper Chehalis River Basin and to help provide a clearer perspective on threats to the status of the spring run type. Project procedures and methods were designed to meet this objective by estimating run-type genotype frequencies of Chinook juveniles originating from spawning locations utilized by spring Chinook. The project data sets used for this purpose were Chinook fry catch and trapping effort, fry fork length (FL) measurements and genotype identifications of tissue samples.

Appendix A of this report presents descriptive summaries of trapping effort and Chinook fry catches together with estimated relative abundance and emergence timing of the three run-type genotypes for each project site.

Appendix B describes the computational steps employed to process and analyze the project data to arrive at estimates of genotype frequencies of Emergent Fry from the fraction of the Chehalis Basin that supports spring Chinook spawning and production.

Genotype Frequency Estimates – Computational Steps

This APPENDIX B describes the computational steps and data analyses used to transform the raw project data into genotype frequency estimates for the targeted spawning areas.

The first step was to estimate genotype frequencies in all the tissue samples collected during the season and characterize their variation among sites. Chinook fry captured in this study were partitioned into two life history, behavioral categories based on size. Fry less than or equal to 45 mm fork length (FL) are termed Emergent Fry and those greater than 45 mm FL are termed Rearing Fry (see the main report, *Study Design* section for rationale; see APPENDIX A, *Length Frequencies and Life History Stages* sections for partitioning at each site). The genotype frequencies of tissue samples taken from only Emergent Fry were then estimated and compared for variation among sites. These steps are described in the next sections.

Genotype Frequencies in Tissue Samples

Genotype Frequencies for All Chinook Fry Tissue Samples

Six thousand two hundred sixty six (6,266) Chinook salmon fry were captured during the 2020 sampling season. Chinook fry were captured at all the trapping sites throughout the season. The sampling protocol was to measure the fork length and take a tissue sample (caudal fin snip) from all the Chinook fry captured up to 50 per week at each site. The tissue samples were sent to the University of California – Davis (UC-Davis) for genetic analysis (genotyping at the GREB1L locus). This study classified genotypes at the GREB1L locus as being homozygous for the spring allele (SPRING), homozygous for the fall allele (FALL), or spring-fall heterozygotes (HET). Two thousand two hundred sixty one (2,261) tissue samples were taken from Chinook fry and 2,244 were identified to genotype (Table B-1). All three genotypes were present at all trapping sites except NFN where no SPRING were detected.

Table B-1: Chinook fry tissue samples identified to genotype for each site in 2020.

Location	Samples Processed	Genotype Identified	FALL	HET	SPRING
MSC	324	322	207	98	17
SFC	124	124	97	23	4
MSN	128	128	65	52	11
SFN	447	441	309	90	42
NFN	233	232	227	5	-
SKU	355	354	339	13	2
SKO	650	643	519	105	19
TOTAL	2,261	2,244	1,763	386	95

The FALL genotype was the most abundant (78.6%), ranging from 50.8% at MSN to 97.8% at NFN (Table B-2). The HET genotype was second most common (17.2%) ranging from 2.2% at NFN to 40.6% at MSN. The SPRING genotype was the least abundant (4.2%) and ranged from nil at NFN to 9.5% at SFN.

The relative abundance of genotypes varied through the season (Figure B-1). FALL fry were most abundant throughout the season beginning at approximately 50% in Weeks 7 and 8, and then increasing gradually to 94% in Weeks 19 and 20. HET individuals were the next most abundant throughout the season starting from about 34% in Weeks 7-10 and then decreasing to approximately 6% during Weeks 15-20. SPRING fry were the least abundant throughout the season. SPRING individuals were approximately 15% of fry captured early in the season (Weeks 7 and 8) and their relative abundance declined gradually to about 1% by Week 15. No SPRING individuals were detected in the samples from Weeks 19 and 20.

Table B-2: Genotype frequencies of Chinook fry tissue samples collected in 2020.

Location	FALL	HET	SPRING
MSC	0.643	0.304	0.053
SFC	0.782	0.186	0.032
MSN	0.508	0.406	0.086
SFN	0.701	0.204	0.095
NFN	0.978	0.022	0.000
SKU	0.957	0.037	0.006
SKO	0.808	0.163	0.029
TOTAL	0.786	0.172	0.042

Genotype Frequency Variation for All Fry

Confidence intervals (95%) were calculated for observed genotype frequencies at each sampling site and then used to determine whether variation among sites was significant (Table B-3; Goodman 1965). Variation is determined to be significant if confidence intervals for paired genotype-site comparisons do not overlap. Site-specific genotype frequencies and confidence intervals from Table B-3 are presented in Figure B-2 to simplify comparisons. The results (significant vs non-significant variation) of all site-by-site comparisons for genotype frequencies are shown in Figure B-3.

Each row-by-column cell in the upper right portions of each section of Figure B-3 represent a comparison of genotype frequencies at two paired sites. The cells are blank for site comparisons that had overlapping CIs so the frequencies are not significantly different. The cells contain an asterisk if CIs did not overlap and thus the genotype frequencies are significantly different. The shaded area in the lower left portion of each section of Figure B-3 are cells that represent paired site comparisons that are included in the upper right portions. The Pooled Sites column is included to show results for comparisons of genotype frequencies at each site with the frequencies calculated for all tissue samples. There were seven trapping sites in this study so there are 21 possible site pairs for comparisons and, since there are three genotypes, there are 63 possible genotype-site paired comparisons.

There was substantial genotype frequency variation among sites as more than half (36 of 63 comparisons); 57.1% of the site-genotype paired comparisons were significant. Frequency variation among sites was most prevalent for the FALL genotype (15 of 21 comparisons; 71.4%) followed by HET (14 of 21 comparisons; 66.7%) and SPRING (7 of 21 comparisons; 33.3%).

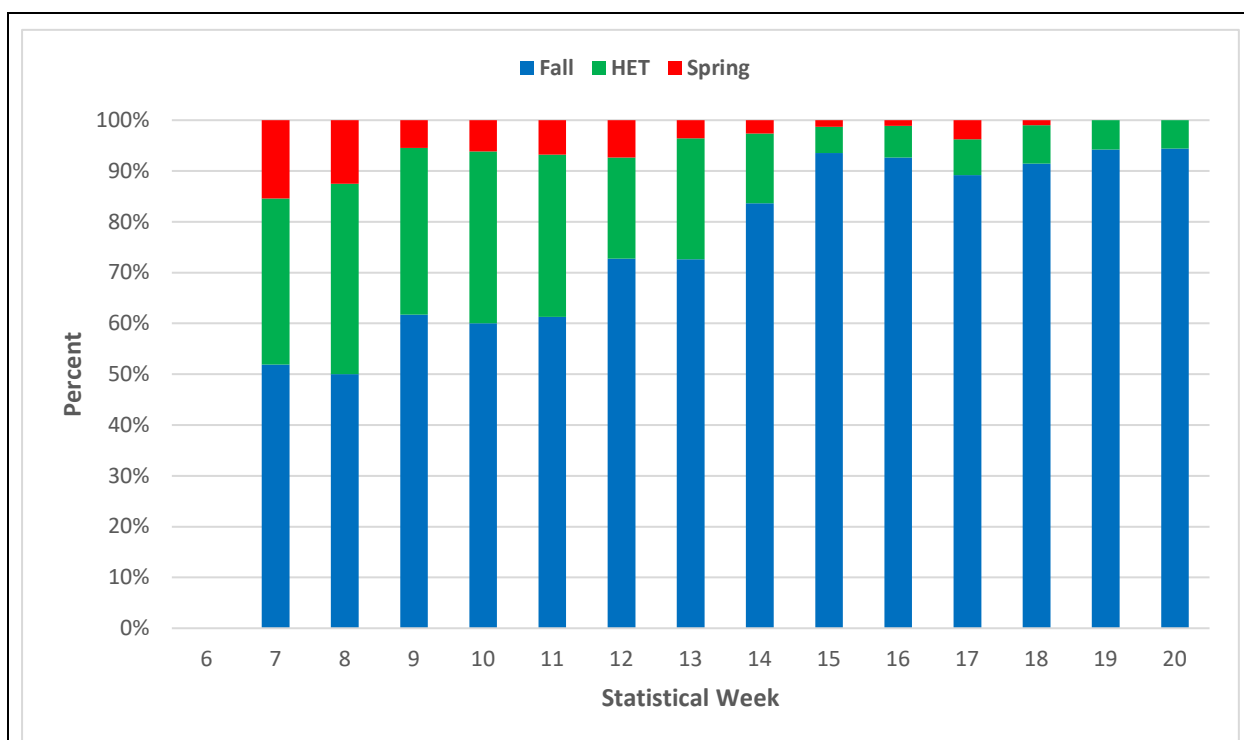


Figure B-1: Weekly relative abundance of Chinook fry genotypes at all sites in 2020¹.

Table B-3: Genotype frequencies and 95% confidence intervals (CI) for all Chinook fry from each site in 2020.

	FALL			HET			SPRING		
Location	Mean	Lower CI	Upper CI	Mean	Lower CI	Upper CI	Mean	Lower CI	Upper CI
MSC	0.643	0.577	0.704	0.304	0.247	0.369	0.053	0.030	0.091
SFC	0.782	0.682	0.857	0.185	0.117	0.282	0.032	0.010	0.095
MSN	0.508	0.404	0.611	0.406	0.309	0.512	0.086	0.043	0.164
SFN	0.701	0.646	0.750	0.204	0.162	0.254	0.095	0.067	0.134
NFN	0.978	0.942	0.992	0.022	0.008	0.058	-	-	0.024
SKU	0.958	0.924	0.977	0.037	0.019	0.069	0.006	0.001	0.026
SKO	0.808	0.767	0.842	0.164	0.131	0.201	0.030	0.017	0.050
ALL SITES	0.786	0.764	0.806	0.172	0.154	0.192	0.042	0.033	0.054

¹ One tissue sample was collected in Week 6 and scored as SPRING but is omitted here due to sample size.

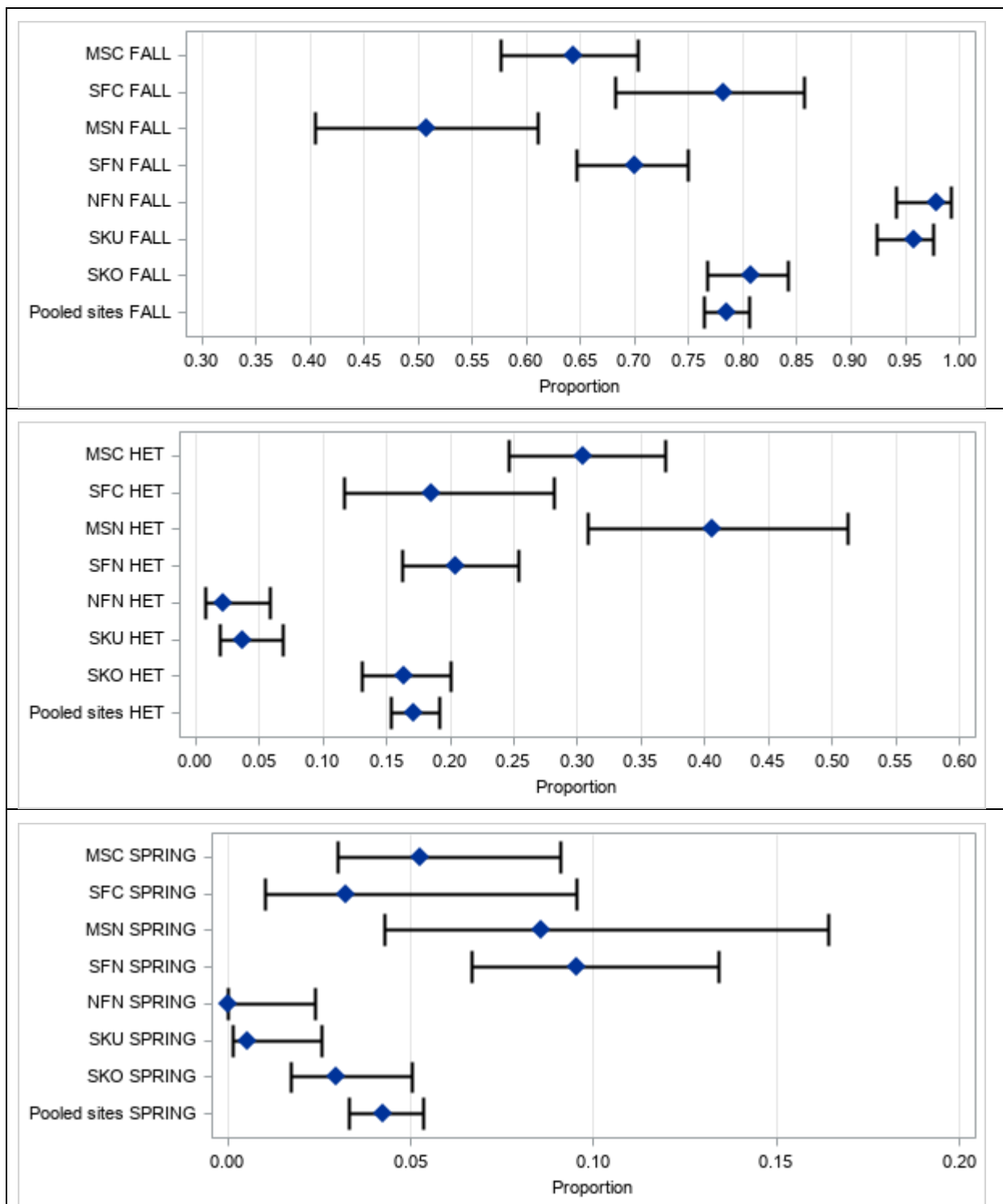


Figure B-2: Chinook fry genotype frequencies (blue diamond) and 95% confidence intervals at each sampling site in 2020.

FALL	MSC	SFC	MSN	SFN	NFN	SKU	SKO	Pooled Sites
MSC					*	*	*	*
SFC			*		*	*		
MSN				*	*	*	*	*
SFN					*	*	*	*
NFN							*	*
SKU							*	*
SKO								
HET	MSC	SFC	MSN	SFN	NFN	SKU	SKO	Pooled Sites
MSC					*	*	*	*
SFC			*		*	*		
MSN				*	*	*	*	*
SFN					*	*		
NFN							*	*
SKU							*	*
SKO								
SPRING	MSC	SFC	MSN	SFN	NFN	SKU	SKO	Pooled Sites
MSC					*	*		
SFC								
MSN					*	*		
SFN					*	*	*	*
NFN								*
SKU								*
SKO								

Figure B-3: Paired comparisons of Chinook fry genotype frequencies among sites. Significant differences in frequencies are identified by an asterisk (*).

Genotype Frequencies of Emergent Fry Tissue Samples

One thousand eight hundred eighty nine (1,889) of the tissue samples processed were Emergent Fry and 1,871 of them were identified as to genotype (Table B-4). All three genotypes were found at all sites except NFN where only FALL and HET individuals were detected. The FALL genotype was the most abundant (78.6%) in samples from all sites, ranging from 50.9% at MSN to 97.7% at NFN (Table B-5). The HET genotype was the second most common (17.6%) ranging from 2.3% at NFN to 40.2% at MSN. The SPRING genotype was the least abundant (3.8%) ranging from nil at NFN to 8.9% at MSN. Genotype frequencies in Emergent Fry were nearly identical to frequencies in the overall fry samples. This is not surprising since the Emergent Fry in Table B-5 made up 83.4% of the overall data set shown in Table B-2.

The genotype frequencies of Emergent Fry varied through the sampling season (Figure B-4). FALL Emergent Fry were the most abundant throughout the season beginning at approximately 50% in Weeks 7 and 8, and then increasing gradually to 98% in Weeks 18 and 19. HET individuals were the next most abundant ranging from about 34% early in the season (Weeks 7-10) to approximately 3% in Weeks 15-

Table B-4: Chinook Emergent Fry tissue samples identified to genotype for each site in 2020.

Location	Samples Processed	Genotype Identified	FALL	HET	SPRING
MSC	308	306	199	91	16
SFC	84	84	63	17	4
MSN	112	112	57	45	10
SFN	399	393	294	76	23
NFN	174	173	169	4	-
SKU	276	275	263	10	2
SKO	536	528	426	86	16
TOTAL	1,889	1,871	1,471	329	71

Table B-5: Genotype frequencies of Chinook Emergent Fry tissue samples collected in 2020.

Location	FALL	HET	SPRING
MSC	0.650	0.298	0.052
SFC	0.750	0.202	0.048
MSN	0.509	0.402	0.089
SFN	0.748	0.193	0.059
NFN	0.977	0.023	-
SKU	0.957	0.036	0.007
SKO	0.807	0.163	0.030
TOTAL	0.786	0.176	0.038

18. No HET individuals were detected in Weeks 19 and 20. SPRING fry were the least abundant throughout the season. SPRING individuals were approximately 14% of the fry captured early in the season (Weeks 7 and 8) and their frequency declined gradually to less than 1% by Week 15. Only one SPRING individual was detected in samples after Week 13.

Genotype Frequency Variation for Emergent Fry

Confidence intervals (95%) were calculated for the observed Emergent Fry genotype frequencies at each sampling site then and used to determine whether variation among sites was significant (Table B-6).

Variation is determined to be significant if confidence intervals for paired genotype-site comparisons do not overlap. Site-specific genotype frequencies and confidence intervals from Table B-6 are presented in Figure B-5 to simplify comparisons. The results of all site-by-site comparisons for genotype frequencies

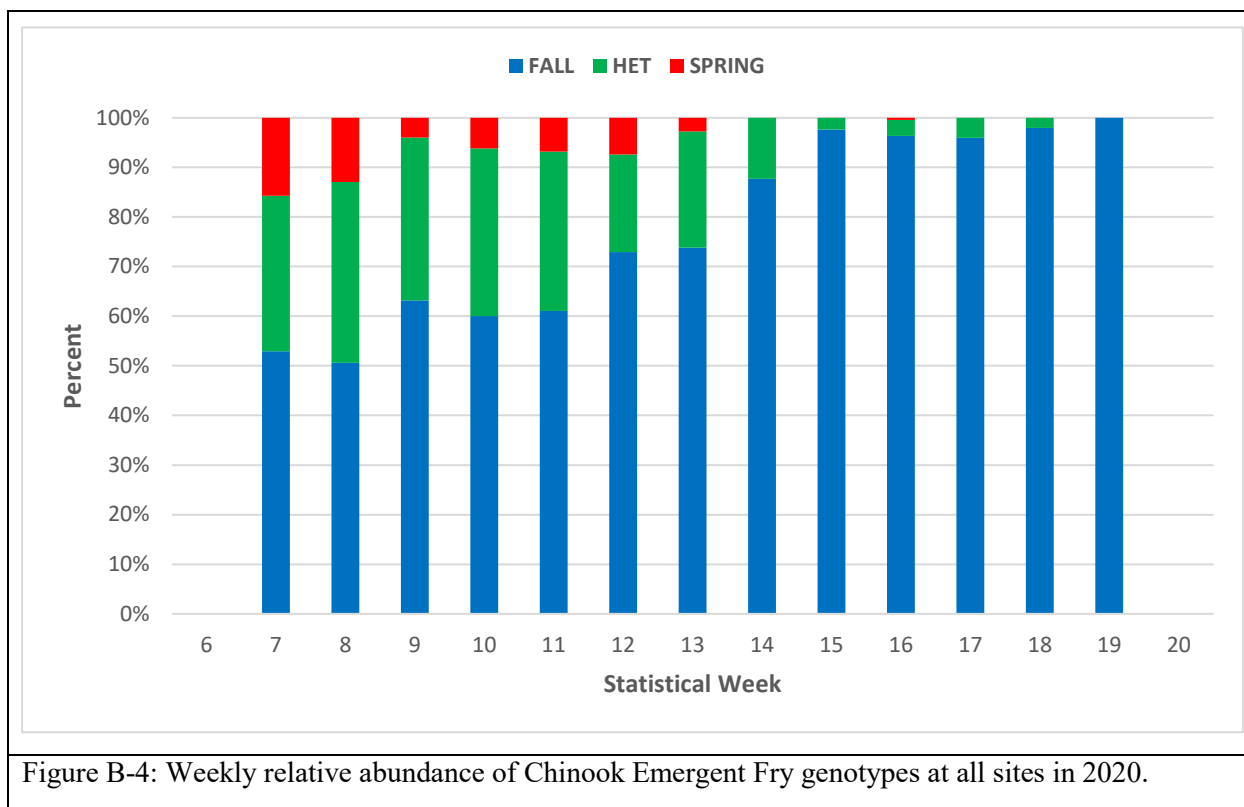
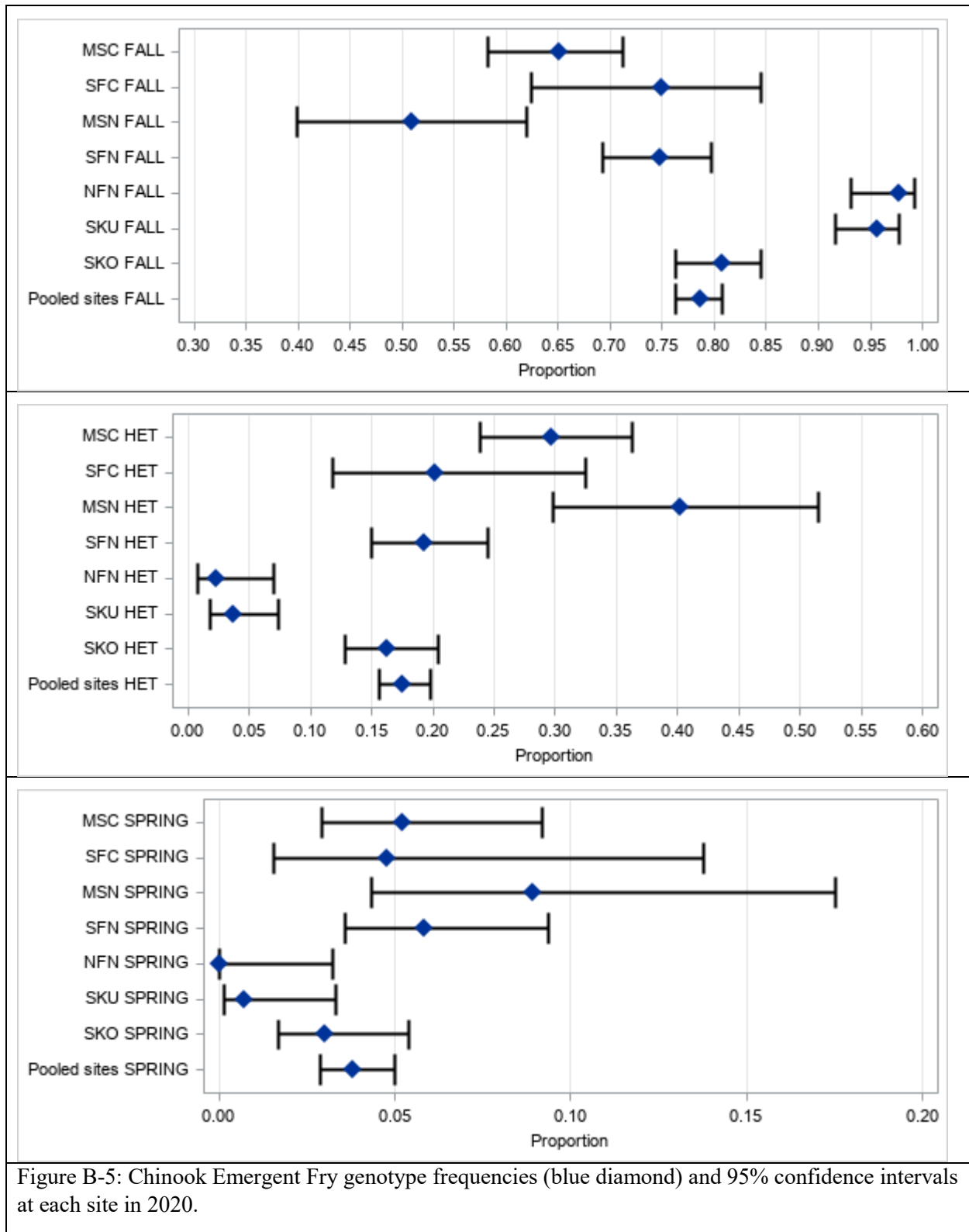


Table B-6: Genotype frequencies and 95% confidence intervals (CI) for Chinook Emergent Fry from each site in 2020.

	FALL			HET			SPRING		
Location	Mean	Lower CI	Upper CI	Mean	Lower CI	Upper CI	Mean	Lower CI	Upper CI
MSC	0.650	0.583	0.712	0.297	0.239	0.363	0.052	0.029	0.092
SFC	0.750	0.623	0.845	0.202	0.118	0.325	0.048	0.015	0.138
MSN	0.509	0.398	0.619	0.402	0.298	0.515	0.089	0.043	0.175
SFN	0.748	0.692	0.797	0.193	0.150	0.245	0.059	0.036	0.094
NFN	0.977	0.931	0.993	0.023	0.008	0.069	-	-	0.032
SKU	0.956	0.916	0.978	0.036	0.018	0.074	0.007	0.002	0.033
SKO	0.807	0.763	0.845	0.163	0.128	0.205	0.030	0.017	0.054
ALL SITES	0.786	0.763	0.808	0.176	0.156	0.198	0.038	0.029	0.050

are shown in Figure B-6. The description of how to interpret Figure B-3 in the prior section also applies to interpretation of Figure B-6.

There was substantial variation of genotype frequencies among sites for the Emergent Fry tissue samples although less than for all fry samples. Thirty one (49.2%) of the paired genotype-site comparisons were significant. Variation among sites was again most prevalent for the FALL genotype (14 of 21 comparisons; 66.7%) followed by HET (13 of 21 comparisons; 61.9%) and SPRING (4 of 21 comparisons; 19.0% of site pairs).



FALL	MSC	SFC	MSN	SFN	NFN	SKU	SKO	Pooled Sites
MSC					*	*	*	*
SFC			*		*	*		
MSN				*	*	*	*	*
SFN					*	*		
NFN							*	*
SKU							*	*
SKO								
HET	MSC	SFC	MSN	SFN	NFN	SKU	SKO	Pooled Sites
MSC					*	*	*	*
SFC					*	*		
MSN				*	*	*	*	*
SFN					*	*		
NFN							*	*
SKU							*	*
SKO								
SPRING	MSC	SFC	MSN	SFN	NFN	SKU	SKO	Pooled Sites
MSC								
SFC								
MSN					*	*		
SFN					*	*		
NFN								
SKU								
SKO								

Figure B-6: Paired comparisons of Chinook Emergent Fry genotype frequencies among sites. Significant differences in frequencies are identified by an asterisk (*).

Tissue Sample Genotype Frequency Application

The raw genotype frequency data from the tissue samples are useful for describing general attributes and trends. These data show the FALL genotype was the most common at all sites and times, the HET genotype was also relatively abundant at some sites and that SPRING individuals were the least abundant at all sites and times. However, these results must be used with some caution. These data show substantial variation of genotype frequencies over time and among sites and some of this variation is likely due to differences in site-specific variation in trapping effort and catch efficiencies. The following sections of this appendix describe the steps taken to account for variation in trapping effort and efficiency and normalize genotype frequency estimates among sites.

Emergent Fry Catches

The daily catches of Chinook fry were partitioned into Emergent Fry and Rearing Fry using daily total fry catches and daily length measurements at each site. Daily length measurements at each site were processed to determine the proportions of lengths ≤ 45 mm FL. These proportions were used to

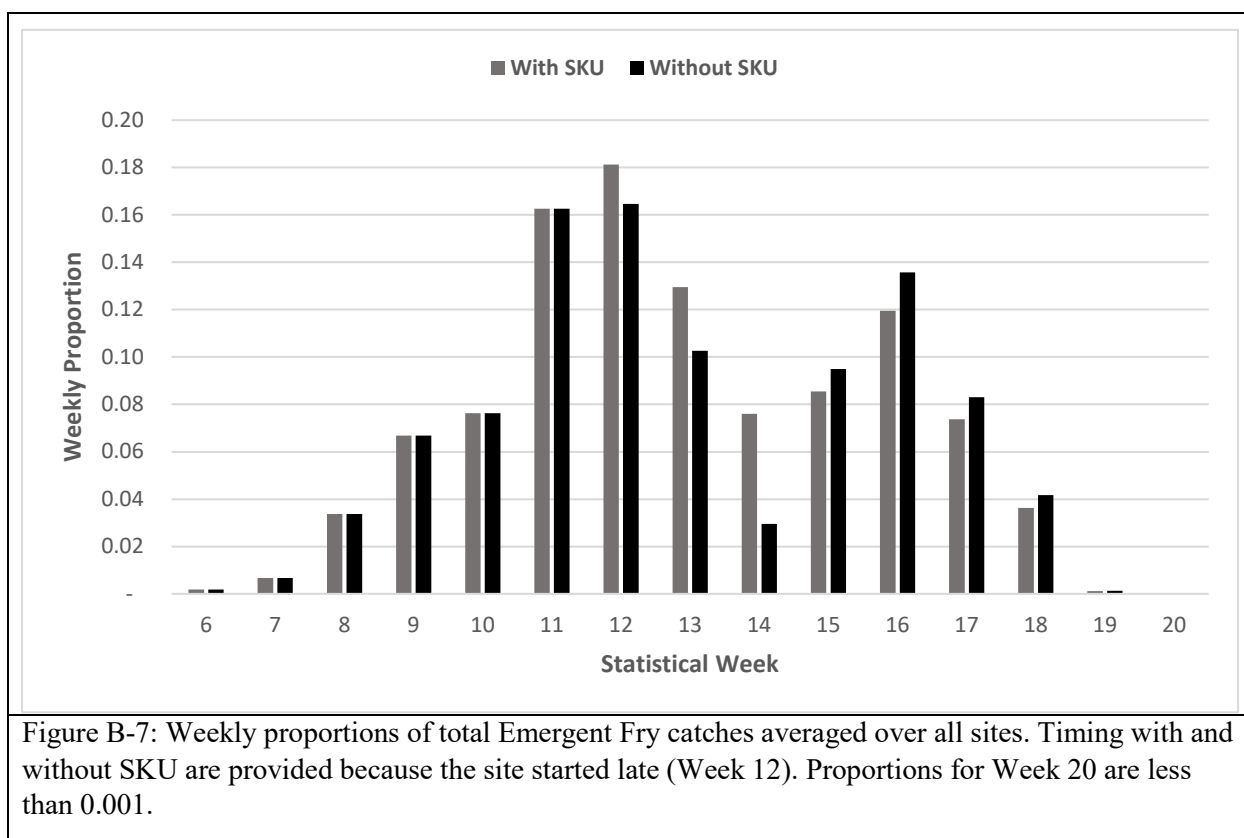
represent the frequency of Emergent in the corresponding daily catches. Daily catches of Emergent Fry were estimated by multiplying the proportion of lengths that were ≤ 45 mm FL times the daily total catch of Chinook fry. The estimated daily catches were then summed within each statistical week to estimate weekly Emergent Fry catches (Table B-7).

Table B-7: Estimated weekly catches of Emergent Fry at each trap site, 2020.							
Statistical Week	Locations						
	MSC	SFC	MSN	SFN	NFN	SKU	SKO
6				1			
7	6	-		4	-		41
8	13	-	6	20	-		185
9	19	1	10	57	-		343
10	21	2	9	64	-		429
11	69	21	29	42	-		507
12	79	24	24	66	-	592	396
13	69	8	14	91	-	613	136
14	16	1	5	11	7	748	69
15	16	8	4	85	51	61	42
16	72	13	4	76	65	48	44
17	21	5	2	32	61	36	93
18	1	1	5	1	41	7	21
19	-	-	-	-	1	-	9
20					-		1
TOTAL	402	84	112	550	225	2,105	2,316

Emergent Fry were present on the first and last weeks (Weeks 6 and 7, and Weeks 19 and 20, respectively) of trapping operations but at low abundance (Figure B-7). This suggests the sampling period chosen for this pilot study covered essentially all of the period fry were emerging from locations upstream of the traps. The data show peak emergence occurred around mid-March (Week 12) and then declined gradually to mid-May (Week 20). There is some evidence of a secondary peak emergence in mid-April (Week 16) but this is difficult to interpret because of a high flow event in Week 14 at all sites and its effects on fry behavior and trap effectiveness are uncertain.

Normalization of Trapping Effort

Catches of Emergent Fry varied among sites and weeks. Most of this variation was likely due to differences in local fry abundance but some was also due to variation among sites and time in fishing effort. Causes for the variation of effort included differences in actual hours each trap was fished during each week and differences in the width of stream channel actually sampled each trapping day. Auxiliary panels were occasionally installed at some sites to sample a greater portion of the channel width and divert more fry toward the trap to increase catches (see the main report, *Fry Trap Design* for a description of the auxiliary panels). The weekly catch estimates needed to be normalized among sites and weeks to a standard catch-per-unit effort (CPUE) to allow comparisons over area and time.



The base unit of effort that could be applied to all sites in all weeks was trapping by one trap width (3-feet) for one hour. This unit of effort will be referred to as a trap-hour in the remainder of the document. The amount of effort exerted each day of operation at each site was calculated by:

$$E_d = \frac{w_d}{3} \times h_d$$

where E_d is the total trap-hours of effort on day (d), w_d is the measured channel width fished in feet (including lead panels) on day (d) (note each trap opening is 3-feet wide and is the minimum channel width fished) and h_d is the total hours fished on day (d). Weekly effort is simply the sum of daily values within each statistical week.

Effort varied among sites and weeks ranging from 724 trap-hours at SKO to 3,120 trap-hours at SFC (Table B-8). Effort at SKO was low because fry abundance was high and, in most weeks, it only took a single day to reach the 50-fish-per-week sampling objective. Effort at SFC was relatively high because fry abundance was low, so the trap was operated several days each week and auxiliary panels were added to increase the effective fishing area on most sampling days. The combined effort at all sites gradually increased early in the season from Week 6 through Week 13, was much reduced by the Week 14 streamflow event, and then stabilized around 1,100 trap-hours per week for the remainder of the season (Weeks 15-19). The gradual increase of effort early in the season was a trend similar to observed increases of Emergent Fry catches during the same period (Figure B-8). This does not mean the increasing catches of Emergent Fry were simple due to increased effort but it does support the rationale for transforming the data to CPUE and account for variation in both effort and fry abundance. There was little similarity in trends of catch and effort later in the season as catch gradually declined while effort remained stable at a relatively high level.

Table B-8: Weekly effort (trap-hours) at each site during the 2020 season.

	Location							
Statistical Week	MSC	SFC	MSN	SFN	NFN	SKU	SKO	Total
6	-	-	-	19	-	-	-	19
7	46	65		162	93	-	25	391
8	148	91	19	137	243	-	53	691
9	140	164	76	188	520	-	47	1,134
10	98	136	74	142	90	-	76	616
11	141	337	183	235	193	-	75	1,165
12	69	469	513	189	289	23	61	1,612
13	71	534	441	305	287	44	56	1,738
14	77	67	126	89	98	43	12	514
15	146	274	245	66	154	123	140	1,149
16	44	269	350	192	94	197	48	1,195
17	159	210	189	148	92	208	22	1,028
18	70	336	164	170	308	171	23	1,242
19	41	169	146	169	274	191	68	1,058
20	-	-	-	-	93	-	16	109
TOTAL	1,249	3,120	2,527	2,211	2,829	1,000	724	13,661

Emergent Fry Catch-per-Unit-Effort

The catch and effort data were converted to CPUE by simply dividing the weekly catch of Emergent Fry (Table B-7) by the corresponding total weekly effort at each site (Table B-8). Since the CPUE values are based on a standardized unit of effort applied to all sites, they provide better representation of relative fry abundance in each week at each site than the actual catches alone. CPUE varied among sites ranging from a mean of 0.02 fry per trap-hour at SFC to a mean of 7.24 fry per trap-hour at SKU (Table B-9). CPUE was also relatively high at SKO. Overall, the Skookumchuck River sites had CPUEs nearly an order of magnitude higher than the other sites.

The CPUE values were used to show relative timing of Emergent Fry movement through the trap sites. Weekly CPUEs were summed to calculate a total for each site and each weekly CPUE was then divided by the total to estimate the proportion of total CPUE contributed within each week. A mean proportion for all sites was then calculated to represent the overall timing of fry emergence (Figure B-9). The weekly proportions of CPUE show a pattern of timing and abundance similar to the catches alone in Figure B-7. Emergent Fry were present but at low abundance in the first and final weeks of trap operations (Weeks 6-7 and 19-20, respectively). The CPUE values support the conclusion that the sampling period selected for this pilot study covered essentially all the time fry were emerging from locations upstream of the traps. CPUE shows the same bimodal peaks of abundance the catches in Weeks 12 and 16 but the relative magnitudes are switched; CPUE values in Week 16 are greater than Week 12. It is still uncertain whether the high flow event in Week 14 influenced the abundance of Emergent Fry drifting through the trap sites, the effectiveness of the traps at capturing the fry, or neither or both of these possible effects.

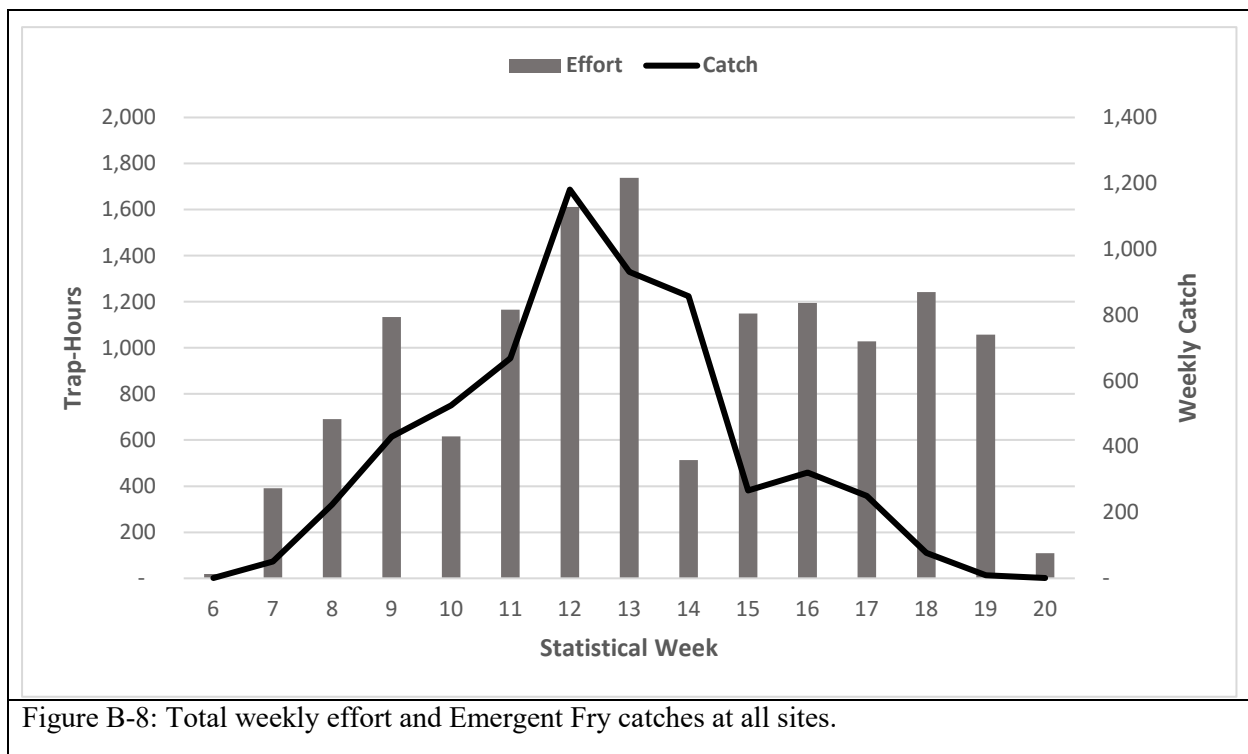


Table B-9: CPUE of Emergent Fry at each site in 2020.							
	Location						
Statistical Week	MSC	SFC	MSN	SFN	NFN	SKU	SKO
6				0.05			
7	0.13	-		0.02	-		1.65
8	0.09	-	0.32	0.15	-		3.48
9	0.14	0.01	0.13	0.31	-		7.35
10	0.21	0.01	0.12	0.45	-		5.61
11	0.49	0.06	0.16	0.18	-		6.77
12	1.15	0.05	0.05	0.35	-	25.55	6.46
13	0.97	0.01	0.03	0.30	-	13.98	2.43
14	0.21	0.01	0.04	0.12	0.07	17.40	5.51
15	0.11	0.03	0.02	1.28	0.33	0.50	0.30
16	1.65	0.05	0.01	0.39	0.69	0.24	0.90
17	0.13	0.02	0.01	0.22	0.66	0.18	4.21
18	0.01	0.00	0.03	0.01	0.13	0.04	0.93
19	-	-	-	-	0.00	-	0.13
20					-		0.06
Mean CPUE	0.41	0.02	0.08	0.27	0.13	7.24	3.27
Sum CPUE	5.28	0.27	0.92	3.83	1.89	57.89	45.79

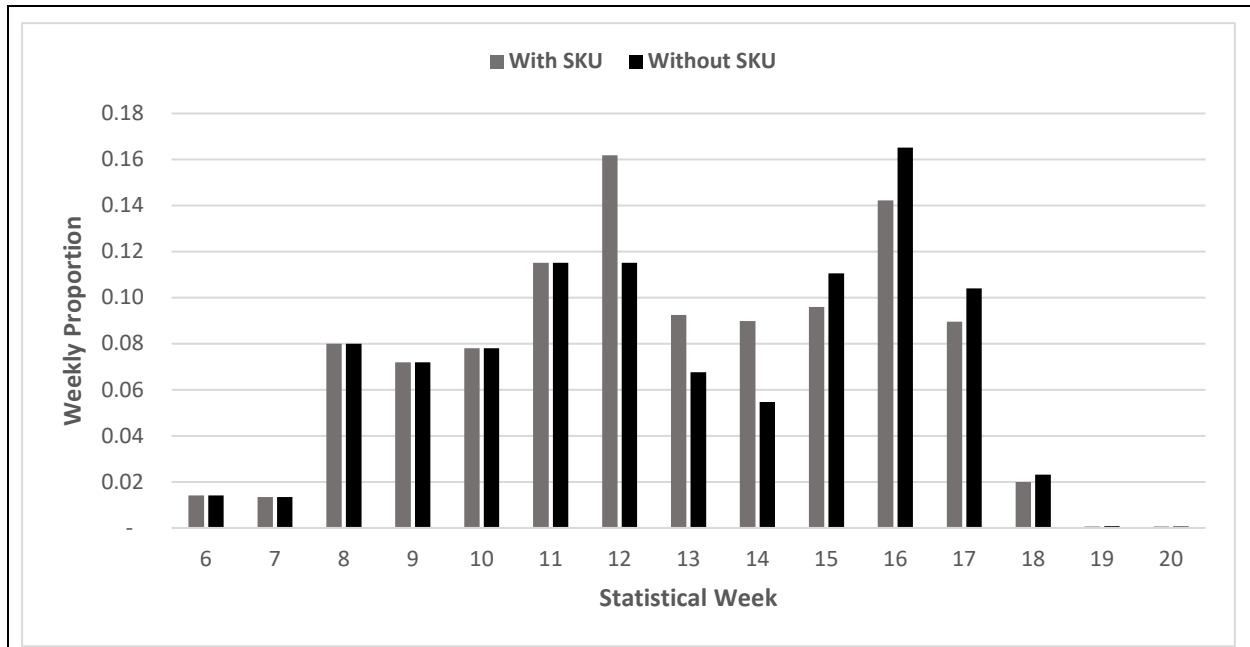


Figure B-9: Weekly proportions of total CPUE averaged over all sites. Timing with and without SKU are provided because the site started late (Week 12). Proportions for Weeks 19 and 20 are less than 0.001.

Emergent Fry Catch and CPUE by Genotype

Weekly catches of Emergent Fry at each site were partitioned into the three subject genotypes (FALL, HET, and SPRING). Partitioning was based on the genotype frequencies of tissue samples taken each week at each site and processed by UC-Davis. The weekly catches by genotype were estimated by:

$$WC_g = \sum_{d=1}^n (C_d * F_{gd})$$

where, for each site, WC_g is the estimated weekly catch of Emergent Fry of genotype g , C_d is the total catch of Emergent Fry on day d , and F_{gd} is the frequency of genotype g in the tissue samples collected on day d (Table B-10). The estimated weekly catches by genotype in Table B-10 do not lend themselves to direct comparisons among sites and weeks because of variation among sites and weeks for effort (the number of hours fished each week) and trap efficiencies (fraction of the Emergent Fry population trapped). Two steps were taken to transform the data from estimated weekly catches by genotype to comparable catch estimates that account for variations in effort and trap efficiencies. First, the partitioned actual catches by genotype were converted to CPUE to standardize all sites and weeks to catches per standard unit of effort (trap-hour).

The estimated weekly catches of each genotype at each site were converted to standard CPUEs using the weekly Emergent Fry CPUE values in Table B-9. This step was necessary to normalize catches of each genotype for comparisons and compilations of trapping results among sites and weeks (see section *Normalization of Trapping Effort*) For each site, the weekly CPUE for each genotype was estimated by:

$$CPUE_g = CPUE_{EF} * F_g$$

Table B-10: Weekly catches of Emergent Fry at each site by genotype.

Statistical Week	MSC			SFC			MSN			SFN			NFN			SKU			SKO		
	FALL	HET	SPRING	FALL	HET	SPRING	FALL	HET	SPRING	FALL	HET	SPRING	FALL	HET	SPRING	FALL	HET	SPRING	FALL	HET	SPRING
6	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-
7	2	4	-	-	-	-	-	-	-	1	1	2	-	-	-	-	-	-	24	11	6
8	10	3	-	-	-	-	1	4	1	6	7	7	-	-	-	-	-	-	106	67	12
9	11	8	-	-	1	-	2	6	2	41	13	2	-	-	-	-	-	-	231	105	7
10	13	7	1	2	-	-	6	2	1	41	17	5	-	-	-	-	-	-	223	189	17
11	46	18	5	13	8	-	12	14	3	25	13	4	-	-	-	-	-	-	416	81	10
12	19	36	23	15	6	3	13	9	2	51	12	3	-	-	-	544	24	24	380	8	8
13	32	34	3	5	2	1	9	4	1	60	29	2	-	-	-	601	12	-	120	14	3
14	11	5	-	1	-	-	2	3	-	8	3	-	7	-	-	688	60	-	66	3	-
15	16	-	-	8	-	-	1	3	-	83	2	-	51	-	-	61	-	-	41	1	-
16	70	2	-	13	-	-	4	-	-	73	3	-	65	-	-	45	2	-	41	2	1
17	21	-	-	5	-	-	2	-	-	31	1	-	57	4	-	35	1	-	88	5	-
18	1	-	-	1	-	-	5	-	-	1	-	-	38	2	-	7	-	-	21	-	-
19	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	9	-	-
20	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-
Totals	252	117	32	63	17	4	57	45	10	422	102	26	218	6	-	1,981	100	24	1,767	486	64

where, for each site in each week, $CPUE_g$ is the estimated catch per trap-hour of genotype g , $CPUE_{EF}$ is the weekly estimated catch per trap-hour for all Emergent Fry (Table B-9), and F_g is the weekly estimated frequency of genotype g in Emergent Fry catches (Table B-11).

Table B-11: CPUE of Emergent Fry for each genotype and week at each site.

Statistical Week	MSC			SFC			MSN			SFN			NFN			SKU			SKO		
	FALL	HET	SPRING	FALL	HET	SPRING	FALL	HET	SPRING	FALL	HET	SPRING	FALL	HET	SPRING	FALL	HET	SPRING	FALL	HET	SPRING
6	-	-	-	-	-	-	-	-	-	-	-	0.05	-	-	-	-	-	-	0.97	0.44	0.24
7	0.04	0.09	-	-	-	-	-	-	-	0.01	0.01	0.01	-	-	-	-	-	-	2.00	1.26	0.22
8	0.07	0.02	-	-	-	-	0.05	0.21	0.05	0.04	0.05	0.05	-	-	-	-	-	-	4.95	2.25	0.15
9	0.08	0.06	-	-	0.01	-	0.03	0.08	0.03	0.22	0.07	0.01	-	-	-	-	-	-	2.92	2.47	0.22
10	0.13	0.07	0.01	0.01	-	-	0.08	0.03	0.01	0.29	0.12	0.04	-	-	-	-	-	-	5.55	1.08	0.14
11	0.33	0.13	0.04	0.04	0.02	-	0.07	0.08	0.02	0.11	0.06	0.02	-	-	-	-	-	-	6.19	0.13	0.13
12	0.28	0.53	0.34	0.03	0.01	0.01	0.03	0.02	0.00	0.27	0.06	0.01	-	-	-	23.47	1.04	1.04	2.14	0.24	0.05
13	0.45	0.48	0.04	0.01	0.00	0.00	0.02	0.01	0.00	0.20	0.10	0.01	-	-	-	13.71	0.28	-	5.28	0.23	-
14	0.14	0.06	-	0.01	-	-	0.02	0.02	-	0.09	0.03	-	0.07	-	-	16.00	1.39	-	0.29	0.01	-
15	0.11	-	-	0.03	-	-	0.00	0.01	-	1.25	0.03	-	0.33	-	-	0.50	-	-	0.84	0.04	0.02
16	1.60	0.05	-	0.05	-	-	0.01	-	-	0.38	0.02	-	0.69	-	-	0.23	0.01	-	3.97	0.23	-
17	0.13	-	-	0.02	-	-	0.01	-	-	0.21	0.01	-	0.62	0.04	-	0.17	0.01	-	0.93	-	-
18	0.01	-	-	0.00	-	-	0.03	-	-	0.01	-	-	0.12	0.01	-	0.04	-	-	0.13	-	-
19	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.06	-	-
20	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

The CPUE values in Table B-11 provide comparable estimates, for each week, of the densities of Emergent Fry of each genotype that passed through a three-foot wide section of each site during a single hour. Whereas these CPUE values can be compared among sites and weeks to represent relative densities of Emergent Fry of each genotype more informative and useful statistics are estimates of the actual numbers of fry drifting through each site. The weekly estimates of CPUE for each genotype in each week at each site were multiplied by 168 (the number of hours in a week) to estimate the number of Emergent Fry of each genotype that would have been caught had the traps been operated all week (Table B-12). This assumes the weekly CPUE values in Table B-11 represent the average CPUEs for each entire week.

Emergent Fry Abundance and Genotype Frequencies

The abundance estimates in Table B-12 represent the numbers of Emergent Fry of each genotype that drifted through a 3-foot section of each site during each week. Although the values represent relative densities well, they do not provide good comparisons among sites or weeks for overall population abundances at each site each week. Steam widths varied among sites and weeks within sites so the values in Table B-12 represent variable fractions of total abundances. Since the fraction of channel coverage varied among sites and weeks, the catch estimates in Table B-12 must be expanded to account for trap efficiency (proportion of the total population sampled) to estimate the total numbers of Emergent Fry drifting past the trap site each week.

Table B-12: Estimated weekly catch of Emergent Fry by one trap unit operated for the entire week.

Statistical Week	MSC			SFC			MSN			SFN			NFN			SKU			SKO		
	FALL	HET	SPRING	FALL	HET	SPRING	FALL	HET	SPRING	FALL	HET	SPRING	FALL	HET	SPRING	FALL	HET	SPRING	FALL	HET	SPRING
6																					
7	7	15	-	-	-	-				1	1	2	-	-	-				162	74	41
8	11	3	-	-	-	-	9	36	9	7	9	9	-	-	-				336	211	37
9	13	10	-	-	1	-	4	13	4	37	12	2	-	-	-				832	378	25
10	22	12	2	2	-	-	14	5	2	49	21	6	-	-	-				490	415	38
11	55	21	6	6	4	-	11	13	3	18	9	3	-	-	-				933	182	23
12	47	89	57	5	2	1	4	3	1	46	11	2	-	-	-	3,943	175	175	1,040	22	22
13	76	80	7	2	1	0	3	2	0	33	16	1	-	-	-	2,302	47	-	359	41	8
14	24	11	-	3	-	-	3	4	-	15	6	-	12	-	-	2,689	234	-	888	39	-
15	18	-	-	5	-	-	1	2	-	211	5	-	55	-	-	83	-	-	49	1	-
16	269	8	-	8	-	-	2	-	-	63	3	-	116	-	-	39	2	-	141	7	4
17	22	-	-	4	-	-	2	-	-	35	1	-	104	7	-	28	1	-	667	39	-
18	2	-	-	1	-	-	5	-	-	1	-	-	21	1	-	7	-	-	156	-	-
19	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	21	-	-
20																			10	-	-
Totals	567	249	72	36	8	1	58	77	19	517	93	35	308	8	-	9,091	459	175	6,085	1,410	197

The measure of trap efficiency selected for this report is the fraction of total channel width covered by a single trap unit; i.e., three feet divided by the wetted-channel width. Trap efficiency was estimated for each sampling day at each site and the estimates of weekly trap efficiencies used in the remainder of this Appendix are simply the means of daily efficiency estimates within each week. This approach assumes densities of Emergent Fry drifting past the sites are uniform across the entire wetted width or that the traps are capturing at a rate near the average of variable densities. A series of mark-recapture trials were performed to provide information on trap efficiencies. One hundred forty seven (147) Chinook Fry were marked with a caudal fin snip at six of the trapping sites and released upstream of each respective trap (Table B-13). Twenty-nine (29) marked fish were recaptured at five of the sites. The ratios of total fry recaptured to total fry released at each site was used to estimate trap efficiencies when the trials were performed. Mean ratios of effective trap width to wetted stream width were calculated for the trial dates at each respective site for comparisons with the mark-recapture results.

Table B-13: Trap efficiency estimates using mark-recapture and trap width to wetted width methods.

Location	Mark-Recapture Trials			Efficiency Estimates	
	Release Date	Fry Released	Fry Recapture per Trap-Hour	Mark-Recapture Method	Trap: Wetted-Width Method
MSC	4/21	13	0.43	0.033	0.021
SFC	4/29	10	1.00	0.100	0.091
SFN	4/21	21	1.29	0.061	0.070
NFN	4/7	20	2.70	0.135	0.094
NFN	4/29	15	1.07	0.071	0.088
NFN	5/6	9	-	-	0.094
SKU	4/9	15	-	-	0.034
SKU	4/29	17	2.10	0.124	0.059
SKO	4/14	27	-	-	0.029

Trap efficiency estimates using the mark-recapture and trap-width per wetted-width methods are compared in Figure B-10. There is substantial agreement in the two methods ($R^2=0.6953$) and these results support use of the trap-width/wetted-width estimate of trap efficiency used in this report.

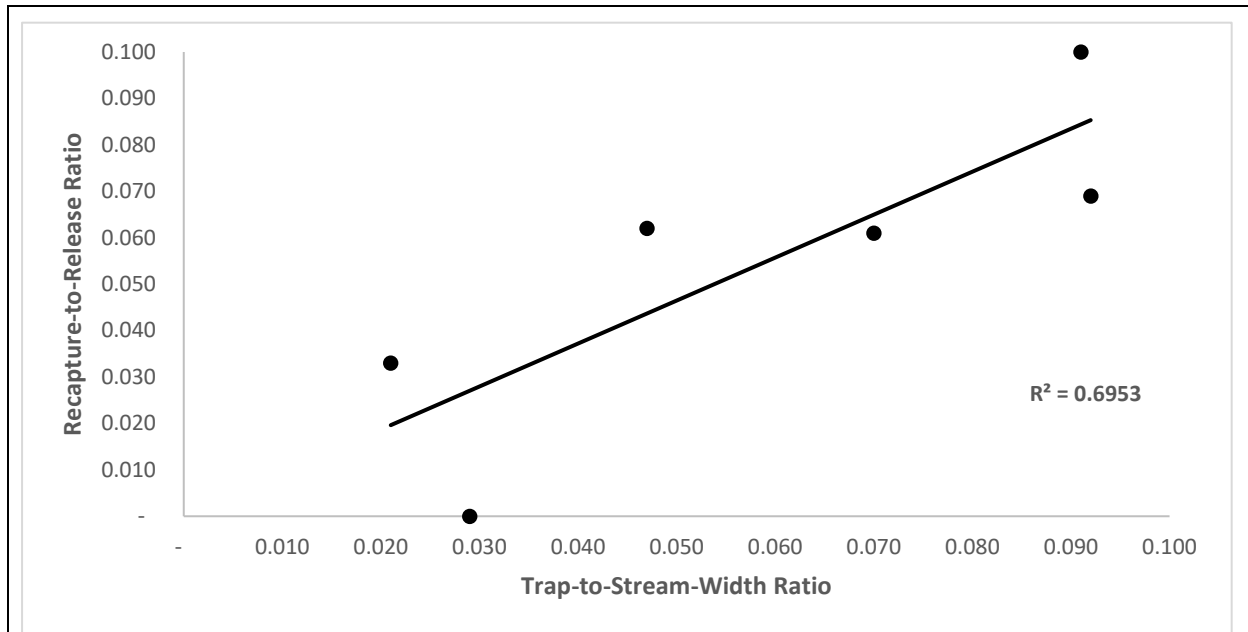


Figure B-10: Comparisons of trap efficiency estimates using mark-recapture and trap-width/wetted-width methods.

Estimates of trap efficiency were calculated for each day of trap operation at each site by dividing the standard trap width (3 feet) by the measured wetted channel width measured at each respective site on each day. Weekly estimates of trap efficiencies were calculated as the mean daily efficiency of the daily estimates in each week (Table B-14).

Table B-14: Weekly estimates of trap efficiency at each site.

Statistical Week	Locations						
	MSC	SFC	MSN	SFN	NFN	SKU	SKO
6				0.066			
7	0.013	0.046		0.066	0.041		0.037
8	0.013	0.054	0.060	0.063	0.041		0.037
9	0.013	0.058	0.060	0.063	0.053		0.037
10	0.013	0.061	0.058	0.063	0.079		0.037
11	0.013	0.064	0.058	0.064	0.080		0.037
12	0.014	0.068	0.068	0.065	0.086	0.035	0.037
13	0.019	0.070	0.058	0.065	0.086	0.035	0.037
14	0.013	0.036	0.046	0.062	0.076	0.055	0.032
15	0.013	0.051	0.063	0.067	0.094	0.034	0.034
16	0.021	0.075	0.056	0.069	0.094	0.050	0.029
17	0.021	0.094	0.064	0.070	0.094	0.075	0.036
18	0.045	0.091	0.071	0.071	0.090	0.059	0.036
19	0.021	0.097	0.071	0.071	0.094	0.050	0.037
20					0.094		0.037

The estimated numbers of Emergent Fry drifting through a 3-foot section of each trap site each week (Table B-12) were expanded to estimate the total number of Emergent Fry drifting through the entire stream width at each trap site in each week. The weekly populations, by genotype, at each site were estimated by:

$$N_{gw} = \frac{C_{gw}}{E_w}$$

where N_{gw} is the total Emergent Fry population of genotype g drifting past the trap site in week w , C_{gw} is the estimated catch of genotype g in week w of a trap unit operating continuously for the entire week (Table B-12), and E_w is the estimated weekly trap efficiency at each respective site (Table B-14). The largest estimated populations of Emergent Fry were at the Skookumchuck River sites and the smallest population was at SFC (Table B-15). SPRING was the least abundant genotype at all sites ranging from none at NFN to 5,420 at SKO. FALL was the most abundant genotype at all sites except MSN where HET was more abundant. An important finding based on these estimates is the abundance of HET individuals relative to SPRING abundance. HET were over four times more abundant than SPRING individuals were. The weekly estimates also show a tendency for SPRING fry to appear early in the season, FALL to appear later and HET fry to be somewhat intermediate but more similar to SPRING timing (Figure B-11).

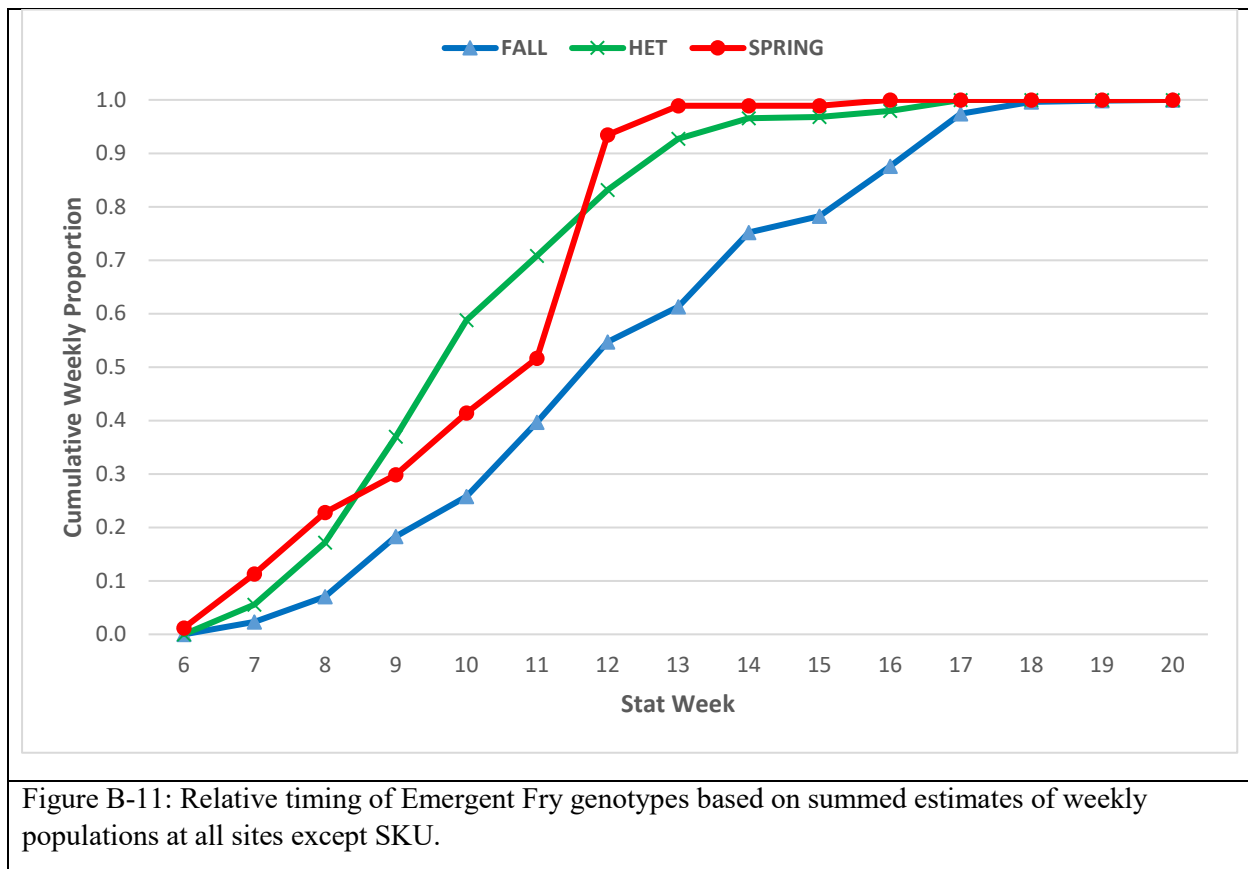
Table B-15: Estimated weekly Emergent Fry populations by genotype present at each site.

Statistical Week	MSC			SFC			MSN			SFN			NFN			SKU			SKO		
	FALL	HET	SPRING	FALL	HET	SPRING	FALL	HET	SPRING	FALL	HET	SPRING	FALL	HET	SPRING	FALL	HET	SPRING	FALL	HET	SPRING
6										-	-	138									
7	586	1,173	-	-	-	-				16	16	31	-	-	-				4,438	2,034	1,109
8	910	273	-	-	-	-	150	600	150	116	136	136	-	-	-				9,171	5,774	1,019
9	1,055	767	-	-	-	18	-	74	221	74	588	190	35	-	-	-			22,730	10,332	689
10	1,777	957	137	41	-	-	237	79	40	776	325	100	-	-	-				13,400	11,339	1,031
11	4,118	1,611	448	101	62	-	191	222	48	278	145	44	-	-	-				25,492	4,974	622
12	3,360	6,365	4,067	79	32	16	62	43	10	699	166	37	-	-	-	111,707	4,965	4,965	28,438	605	605
13	3,912	4,157	367	22	9	4	60	26	7	509	246	16	-	-	-	65,236	1,331	-	9,814	1,115	223
14	1,896	862	-	70	-	-	57	86	-	244	92	-	158	-	-	49,291	4,286	-	27,809	1,209	-
15	1,385	-	-	95	-	-	11	33	-	3,160	70	-	592	-	-	2,414	-	-	1,454	37	-
16	12,997	366	-	108	-	-	34	-	-	924	41	-	1,233	-	-	767	40	-	4,902	245	123
17	1,063	-	-	43	-	-	28	-	-	506	17	-	1,111	72	-	379	13	-	18,685	1,099	-
18	54	-	-	6	-	-	72	-	-	14	-	-	231	14	-	123	-	-	4,381	-	-
19	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	570	-	-
20																			284	-	-
Totals	33,113	16,531	5,018	565	120	20	976	1,311	327	7,830	1,444	537	3,326	86	-	229,918	10,636	4,965	171,569	38,764	5,420

The total abundances in Table B-15 were used to estimate genotype frequencies for Emergent Fry at all sites (Table B-16). These estimates were made to characterize and compare genotype frequency variation among sites and, ultimately, to estimate overall genotype frequencies for watershed areas upstream of the trap sites.

The estimates of genotype frequencies at each site (Table B-16) provide informative perspectives regarding spatial variation of the trait. These individual, site-specific values can also be used to estimate frequencies for the combined areas upstream of the trap locations. However, there was considerable genotype frequency variation among sites (see section *Genotype Frequency Variation for Emergent Fry*) and the sites show a wide range of juvenile abundances which suggests the method for estimating the genotype frequencies for all Emergent Fry originating from above all the trap sites should use a weighting factor to scale the effects of each site. Several weighting factors were considered and, for purposes of this report, the relative abundance at each site was chosen. In order to weight frequency estimates by abundance the method adopted here simply sums the abundance estimates for each genotype (Table B-15) across all sites and these sums were used to calculate the overall genotype frequency estimates (Table B-17).

The estimates of overall genotype frequencies show FALL individuals are the most common and SPRING individuals are uncommon. HET individuals make up 25.8% of the overall population and outnumber the SPRING individuals by a factor of greater than 4.



Location	FALL	HET	SPRING
MSC	0.606	0.302	0.092
SFC	0.801	0.171	0.028
MSN	0.373	0.502	0.125
SFN	0.798	0.147	0.055
NFN	0.975	0.025	-
SKU	0.937	0.043	0.020
SKO	0.795	0.180	0.025

Table B-17: Estimated Emergent Fry genotype frequencies from areas upstream of the trapping sites.			
	FALL	HET	SPRING
	0.840	0.129	0.031



Run-Type Composition of Juvenile Chinook Salmon in the Upper Chehalis River Basin in 2020

APPENDIX C: Fry Trapping Database Schema

Quinault Indian Nation Department of Fisheries

July 2021

West Fork Environmental Fry Trapping data				UC-Davis Genotyping Results	
Col	Description	Col	Description	Col	Description
A	Year	AA	Rainbow morts	A	Year
B	River	AB	Lamprey	B	Date
C	Site ID	AC	Lamprey morts	C	River
D	Date Set	AD	Sculpin	D	Site ID
E	Date Check	AE	Sculpin morts	E	Length
F	Time Set	AF	Dace	F	Sample ID
G	Time Check	AG	Dace morts	G	Photo
H	DateTime Set	AH	Shiner	H	Note
I	DateTime Check	AI	Shiner morts	I	QIN spreadsheet order
J	Hours Fished	AJ	Pikeminnow	J	SO notes
K	Minutes Fished	AK	Pikeminnow morts	K	DNA plate
L	Discharge Set	AL	Rock Bass	L	Plate well
M	Discharge Check	AM	Rock Bass morts	M	ID
N	Temp Set	AN	Largemouth Bass	N	Added to well
O	Temp Check	AO	Largemouth morts	O	Plating notes
P	Clarity Set	AP	Stickleback	P	Miller lab order
Q	Clarity Check	AQ	Stickleback morts	Q	qPCR well
R	Wetted Width	AR	Sucker	R	Genotype
S	Area Fished	AS	Sucker morts	S	IDs match
T	Chinook	AT	Comments	T	Zygosity
U	Chinook morts			U	Allele
V	Coho			V	Run Type
W	Coho morts				
X	Cutthroat				
Y	Cutthroat morts				
Z	Rainbow				



Run-Type Composition of Juvenile Chinook Salmon in the Upper Chehalis River Basin in 2020

APPENDIX D: Table of Statistical Weeks

Quinault Indian Nation Department of Fisheries
July 2021

The system of statistical weeks adopted by the Quinault Department of Fisheries is sequential beginning at midnight on Sunday mornings and ending at midnight on Saturday nights of each calendar week. Week 1 of each year is the week that includes January 1 even if the week begins on a Sunday in December.

Statistical Week	Beginning Date		
	2020	2021	2022
1	Dec 29	Dec 27	Dec 26
2	Jan 5	Jan 3	Jan 2
3	Jan 12	Jan 10	Jan 9
4	Jan 19	Jan 17	Jan 16
5	Jan 26	Jan 24	Jan 23
6	Feb 2	Jan 31	Jan 30
7	Feb 9	Feb 7	Feb 6
8	Feb 16	Feb 14	Feb 13
9	Feb 23	Feb 21	Feb 20
10	Mar 1	Feb 28	Feb 27
11	Mar 8	Mar 7	Mar 6
12	Mar 15	Mar 14	Mar 13
13	Mar 22	Mar 21	Mar 20
14	Mar 29	Mar 28	Mar 27
15	Apr 5	Apr 4	Apr 3
16	Apr 12	Apr 11	Apr 10
17	Apr 19	Apr 18	Apr 17
18	Apr 26	Apr 25	Apr 24
19	May 3	May 2	May 1
20	May 10	May 9	May 8
21	May 17	May 16	May 15
22	May 24	May 23	May 22
23	May 31	May 30	May 29